

Poster session

Tick-borne diseases

Incidence and trend of disseminated stages of Lyme Borreliosis in the province of Brabant Wallon (Belgium): retrospective data of a reference center

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Background: Lyme Borreliosis (LB) is the most common vector-borne disease in Europe (Bacon 2008). Epidemiological serological data in Belgium show an increasing incidence since 1993 (Ducoffre 2008). However, this serological data does not allow measuring the rate of disseminated forms, which constitute the burden of the disease. Using the annual incidence of disseminated stages of LB from a reference hospital (Clinique Saint-Pierre, Ottignies-Louvain-la-Neuve), we aimed at describing the clinical and epidemiological features of cases over an 11-years period (1997-2007) Methods: Hospital cases records of all LB diagnosed from January 1st, 1997 to December 31th, 2007 were collected. To estimate the incidence of disseminated LB in Brabant Wallon, we reported annual cases to the number of people living in the catchment area of the hospital. Results: Ninety-eight (98) cases of LB were registered, representing a mean of 8.9 cases per year. All cases were diagnosed at second or later stages of the disease. No linear trend in incident cases was observed. Most of the cases (75%) were recorded between June and November. Neuroborreliosis was the dominant clinical form every year, with a mean of 76.5%, similarly to a previous report in 1989 (Bigaignon 1989). Considering the catchment population during the 11 years of our study, the cumulative estimated incidence of disseminated LB was 19.5/100,000 person-years with an annual mean rate of 1.8/100,000 persons. The minimum estimated incidence was observed in 2002 (n=2, 0.48/100,000 person-years) and the maximum in 2004 (n=14, 3.37/100,000 person-years). In 2008, the Belgian Scientific Institute of Public Health reported a LB seropositivity rate of 12.8/100,000 person-years for Belgium and 6.4/100,000 person-years for the province of Brabant Wallon (Ducoffre 2009). Our results probably underestimated the actual incidence because patients may choose to be treated elsewhere. Nevertheless, compared with this seropositivity rate, our clinical LB disseminated rate indicated 1 LB clinical case requiring hospitalization for 3 positive LB sera. Conclusion Our study does not show an increase over time in clinical LB cases hospitalized in the province of Brabant Wallon between 1997 and 2007. Clinical cases of LB that required hospitalization represented one-third of seropositivity to LB, keeping in mind that many cases of Lyme arthritis do not require hospitalization. To estimate more accurately the trend of LB infection and the rate of complications, we need a sentinel surveillance of clinically defined LB cases and a follow-up of individuals exposed to tick bites such as servicemen, forest workers or scouts.

Hyalomma lusitanicum phenology in a Mesomediterranean environment of Central Spain

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The study was carried out in a private property located in Central Spain (37°24'N; 42°59'E; HT669m). The place of study covers an area of 13,000 Ha of Mesomediterranean bioclimatic environments. The present study was carried out monthly for a three-year period (2007-2009) collecting 2751 ticks from vegetation by the blanket dragging technique for 5220; in six representative points, and 8285 semi-engorged ticks recovered directly from 180 captured wild animals (red deer: 29, wild boar 31, rabbit: 112, hare: 2, roe deer: 1 and partridge: 5).

Global tick abundance rate (TAR) was 52.7, although some differences were observed depending on the year (40.4 in 2007 and 62.8 in 2008) and the month, the minimum being in winter (Jan-Feb) and the maximum in spring (May-Jun). [f1] The mean tick burden was 46.03 ticks per animal with hare, red deer, rabbit and wild boar being the most parasitized.

H. lusitanicum was the most abundant tick in both samples in vegetation (95.8%) and on hosts (71.90 %). This was the main species parasitizing red deer, roe deer, wild boar (over 90% of tick burden) as adult while immature stages were more frequent in rabbit (66%). Host seeking *H. lusitanicum* were mainly adults, and nymphs were only sporadically collected.

Some adults of *H. lusitanicum* were collected feeding even in the cold period, but the low number clearly increased from February and was highest in July. Adults were abundant in vegetation, increasing from March to June, then decreasing to October, and were sporadically collected from November to February. Larvae were never collected from vegetation but they became abundant on rabbit from March to April, and in a second peak in July. Nymph phenology was delayed in comparison with larvae, having peaks of feeding in May and August. Host seeking nymphs were only collected in July and August. Other species of ticks were also collected in low numbers and different rates depending on the sample. [f2]

Our data suggest that there are 2 generations of *H. lusitanicum* a year. Early larvae start their activity in March-April, feeding on rabbits. Engorged larvae molt probably in burrows, and feed as nymphs in May. Emerging adults feed mainly on red deer in June-July. Engorged females lay eggs and larvae emerge and feed in July, molt and feed as nymphs in August. After molting, most of the adults will remain in the soil waiting for better conditions while some overwinter on hosts.

Acknowledgments:

Thanks are extended to the property "La Garganta"; for financial and personal support. Silvia Cota was granted by Conacyt, Mexico

Borrelia bissettii – the potential causative agent of Lyme disease

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Borrelia burgdorferi sensu lato is a diverse group of bacteria widely distributed around the world. However, out of 17 recognized to date genospecies most are considered to have a limited distribution, except for *B. burgdorferi sensu stricto* and *B. bissettii*, which are encountered both in Europe and in North America. *B. burgdorferi sensu stricto* is the primary pathogenic genospecies that causes Lyme disease around the world. For a long time the ideas about pathogenicity of *B. bissettii* were based on the analysis of strain 25015 isolated from a fully fed *I. scapularis* larva (formerly *I. dammini*) removed from the white-footed mouse captured in Millbrook, NY. Infectivity but not pathogenicity of strain 25015 was confirmed years ago and persisted until seemingly incongruous reports that linked European isolates of *B. bissettii* with human Lyme disease. Confessions that the members of *B. bissettii* group may not be pathogenic to human because they

had been originally isolated from Ixodid ticks and small reservoir hosts only, failed when a Slovenian research group presented clinical findings for patients with Lyme disease caused by species with genotypic and phenotypic similarity to strain 25015. Re-evaluation of the potential of *B. bissettii* to induce disease conducted by a group of American scientists employed a murine model of disease studying the geographically disparate *B. bissettii* strains isolated from hard ticks (vectors) and from rodents (reservoir hosts). It was shown that rodent-originated strains of *B. bissettii* were capable of inducing disease, causing pathology within the bladder, heart, and femorotibial joint, whereas tick-originated strains did not disseminate or induce pathology to the above tissues.

Using the tools of molecular biology we detected *Borrelia bissettii* DNA in cardiac valve tissue of the patient with endocarditis and aortic valve stenosis in the Czech Republic and in the serum samples of patients with symptomatic borreliosis or chronic borreliosis infection. Our results strongly supports the fact that *B. bissettii* might be a causative agent of Lyme disease in Europe. However, the small number of patients confirmed to be infected with *B. bissettii* does not allow any conclusions to be made yet on the association of this species with organotropism or species-specific clinical manifestations. The detection of *B. bissettii* in cardiac valve tissue and in serum samples of Lyme disease patients in the Czech Republic raised questions of whether the issue of pathogenic potential of *B. bissettii* was adequately addressed. The fact that *B. bissettii* shares with *B. burgdorferi* the distinction of being present in both Old and New Worlds might mean the involvement of this species in Lyme disease worldwide. The potential for *B. bissettii* to cause disease is of considerable importance given the extensive geographic range in which it has been recently recognized.

Biotic and abiotic factors affecting phenology of *Ixodes ricinus* (Acari Ixodidae) in Italy.

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Understanding the factors affecting the seasonal population dynamics of the tick *Ixodes ricinus* is fundamental to better quantify the transmission rates of the many pathogens they transmit and to allow preventive medical actions. The aim of this study was to determine the relative contribution of climatic variables and deer population density (roe deer, *Capreolus capreolus* and red deer, *Cervus elaphus*) in affecting the abundance and seasonal pattern of *I. ricinus* at eleven sites of Italy where this species is endemic and recognized as a public health problem. Ticks data were obtained from a longitudinal three-year survey on the ecoepidemiology of tick-borne diseases as a part of the European project EDEN (<http://www.eden-fp6project.net>). We collected 25 447 individuals belonging to the species *I. ricinus* from all the monitored sites. These included 10 373 larvae (40.8%), 13 310 nymphs (52.3%) and 1 764 adults (6.9%), of which 927 males and 837 females. Larvae, nymph and adult stages usually quested from February-March to October-early November, with a unimodal pattern for adults (peak in May) and larvae (peak in August) and a bimodal pattern with a secondary peak in autumn for nymphs (maximum peak in May and secondary peak in October). The tick abundance (nymphs) was statistically different between sites and years, and also climatic variables showed spatio-temporal differences. While tick emergence and general seasonal pattern of activity were similar among the study sites, absolute host-seeking nymph abundance was influenced by local climatic conditions, and availability of hosts (red deer). The saturation deficit, i.e. an index including temperature and relative humidity, was the best predictor of nymph abundance, with both first and second order effects. Therefore, the effect of this index was positive until a threshold value (7.39 mmHg), after which nymph abundance decreased. Also the density of red deer had a negative effect on the abundance of questing nymphs for deer density values higher than 1.71 animals/km². On the other hand, seasonality of nymphs increment was independent by host density, and conditioned by limiting climatic factors (negative effect of saturation deficit). Finally, for the timing of seasonal emergence, we found, confirming results of previous studies, that the threshold temperature was different for larvae (average max T 10°C) and for nymphs (8°C).

Spatial heterogeneity and dynamics of pathogen transmission in tick-borne disease system

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Tick-borne diseases have strong links to the environment, particularly in forested and agricultural landscapes, where ticks and their hosts are found and where human risk activities are carried out. These elements are however not homogeneously distributed in space. A spatially explicit understanding of the underlying processes in the transmission system is crucially needed to explain the spatial distribution of risk of tick-borne zoonoses. This particularly involves environmental change issues, such as climate change, land management and risk activities. One aspect of the spatial heterogeneity observed in tick-borne diseases distribution is the spatial distribution of hosts. Ticks can parasitize a wide range of host species, some of which are reservoir-competent for pathogens while others are not but nevertheless important for tick reproduction. Typically, most existing models are not spatially explicit and therefore assume homogenous spatial distributions for all involved species. Besides, many are instances of empirical modelling, rather than based on mechanistic or biological processes. This is because in many cases the underlying relationships between the pathogen transmission and environment or/and human activities are poorly understood. These factors are influencing the biological behaviours of and habitat suitability for both ticks and their hosts, and thereby shifting the pathogen transmission patterns.

The tick-borne disease transmission model presented here incorporates spatial dynamics and multi-host structure using a Cellular Automata framework. Spatial dynamics are included by abstracting the impacts of moving hosts on tick population and pathogen transmission. Two types of hosts are considered: reservoir hosts and reproduction hosts. Populations of the two host types and ticks are involved in the model. These populations are classified into three categories following the classical SIR model, and for each cell in the Cellular Automata, the densities of these classes are stated at every discrete step of modelling time, and can be related to varying habitat suitability throughout the landscape.

We illustrate how this model can be theoretically built and adapted to investigate spatio-temporal dynamics of tick-borne transmission systems. We also present an application of the prototype model which simulates pathogen dispersal in dynamic tick and host populations. The model will be used as a basis for our study to understand the effect of host distributions on Lyme borreliosis transmission in Belgium. We believe that, for the same case study, the model can be further developed to assess the influences of environmental factors including climate and human activities in a spatially explicit way. Understanding the heterogeneity and dynamics of the tick-hosts-pathogen system can contribute to the development of disease management programmes.

Distribution of tick species on the vegetation of different climatic areas in Spain

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Climate and vegetation in Spain varies from North to South affecting tick distribution and consequently presence of tick-borne diseases. The aim of this study was to investigate the distribution of the different exophilic tick species present on the vegetation in 18 areas located in Central (CAM-CACLM) and Northern Spain (CAPV) along a two-year study. Sampling sites were sampled on a monthly basis by blanket dragging during different time intervals (20-30 min). To compare tick counts from different samplings, a “tick abundance index” (TAI) was calculated by dividing the number of ticks collected by dragging time in minutes. Tick species and stages were counted and morphologically identified. A total of 12 species

belonging to the genera *Ixodes*, *Haemaphysalis*, *Rhipicephalus*, *Dermacentor* and *Hyalomma* were identified. Immature ticks were recovered from all genera but *Dermacentor*. The most frequent and abundant species in Northern Spain were *Ixodes ricinus* and *Haemaphysalis punctata*, whereas *Hyalomma lusitanicum* and *Dermacentor marginatus* were the most abundant tick species in Central Spain. Other species like *Haemaphysalis hispanica*, *Ixodes ventralis* and *Ixodes frontalis* were only scarcely found. *I. ricinus*, *H. punctata*, *D. marginatus* and *Rhipicephalus bursa* were captured in both regions, but with significantly different TAI indexes. Hence, *I. ricinus* accounted for 66.7% of adult ticks identified in Northern Spain, whereas in Central Spain this species only represented 0.3%. Similarly, prevalence of adult *H. punctata* was higher in Northern Spain (8.1%) than in Central Spain (0.04%). Conversely, *H. lusitanicum* represented 85.5% of the adult ticks captured in Central Spain, being absent in the Northern part. There were important differences in the monthly seasonal patterns according to the tick species. Thus, nymphs and adults of *I. ricinus* showed similar seasonality, being more abundant between March and May, whereas larvae were active between May and September. The adults of the three main *Haemaphysalis* species showed different activity patterns, and whereas *H. inermis* and *H. punctata* were more abundant in colder months, *H. concinna* was active from May to September. *Dermacentor* species predominated in autumn and winter months, with longer activity periods for *D. marginatus*, also active in spring months (March-June). Finally, adults of *H. lusitanicum* and *R. bursa* were only collected at the end of spring and beginning of summer, with peaks in June and July. All sampling areas were frequented by people, livestock and/or wildlife. In general, the presence of ungulates (roe deer, red deer, wild boar) was not associated with the abundance of any particular tick species. However, *D. marginatus* and *H. lusitanicum*, which were found mainly in dry areas with continental climate (Central Spain), were associated to the presence of rabbits. *Rhipicephalus pusillus* was also associated to sites with abundance of rabbits. *D. reticulatus* and *H. punctata* were associated to areas with mixed grazing livestock, especially free-ranging horses, and presence of wild carnivores. These results highlight important differences in tick distribution in neighbouring areas and the need to continue surveillance schemes to monitor tick population dynamics and the prevalence of tick-borne pathogens.

Tick-borne zoonotic bacteria in ticks in Northern Spain

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PCR and reverse line blot hybridization were used to study the distribution of the tick-borne zoonotic bacteria *Borrelia* spp., *Anaplasma phagocytophilum* and *Rickettsia* spp. in *Ixodes ricinus* ticks from Northern Spain. A total of 620 adults and 1,800 nymphs (in 360 pools of 5 nymphs each) collected from 6 areas in the Basque Country and La Rioja were analysed. DNA was extracted from all the specimens, including a negative extraction control every 20 samples, using the QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany). A probe targeting the *msp2* gene was designed to detect *Anaplasma phagocytophilum*. Two approaches were used for the detection of *Borrelia* spp. A generic one that targeted the 16S rRNA gene, and another based on the nested PCR amplification of the 5S-23S rRNA intergenic spacer followed by RLB hybridization with genospecific probes (Gern L, unpublished). *Rickettsia* spp. was detected using primers that amplified the 23S-5S rRNA intergenic spacer and hybridization with specific probes for a variety of species, a generic probe for all *Rickettsia* species and two additional probes, specific for the Spotted Fever and Typhus Groups (Jado, et al 2006). An internal amplification control was added to evaluate the presence of PCR inhibitors. The sensitivity of the assays was determined using 102, 10 and 1 genomic equivalents (GE) of *B. burgdorferi sensu stricto*, *B. garinii*, *B. afzelii*, *B. valaisiana*, and *B. lusitaniae*, and *A. phagocytophilum*, respectively in each PCR.

Overall, between 2.4 and 8.0%, and between 6.4 and 10.6 % of *I. ricinus* were positive for *Borrelia* spp. and *A. phagocytophilum*, respectively. One of the sites in the Basque Country (Site 3) had the highest percentage of infection. Nine ticks were found positive for both pathogens. *Borrelia* spp. was detected in 7 samples by 16S rRNA but not by 5S-23S rRNA amplification, and they were confirmed by sequencing. *Rickettsia* was detected in 18 specimens (10 pools of nymphs and 8 adults), identifying 5 *R. monacensis* and 5 *R. helvetica* among them. The detection limit for the combined PCRs and subsequent hybridizations was good in all cases, ranging between 10 and 1 GE.

Ixodes ricinus as vector of human tick borne zoonoses in Tuscany

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Ixodes ricinus is the European tick vector of *Borrelia burgdorferi* sensu lato and other zoonotic agents. In Tuscany the Regional public health department is actively involved in arthropod born diseases control and the analysis of the current regional report of Lyme disease (1995-2007) suggests an increasing risk of the disease.

In this work we describe a 5-year-long study (2005–2009) conducted by the tick borne zoonoses surveillance system implemented in Lucca Local health unit (ASL 2). A total of 331 patients with diagnoses of tick bite were interviewed. The ticks were classified and stored until DNA extraction. In the same area, in 2005-2006, host-seeking ticks were monthly collected together with ecological data, in 12 different sites in homogeneous deciduous woodland.

A total of 331 patients were interviewed and the mean age was 47 years. The majority of patients were male (ratio male/female was 1.54) and they also related an higher previous infestations frequency rate ($P < 0.05$).

I. ricinus accounted for 85.2% of the total collected ticks. *Dermacentor marginatus*, *Rhipicephalus sanguineus*, *Hyalomma marginatum* and *Haemaphysalis punctata* were also collected.

I. ricinus bite events were recorded mainly in wood (42.4%) and related to occupational activities (70.1%).

A total of 3509 host seeking ticks were collected. *I. ricinus* nymphs peaked in April-May and larvae in August. Density of nymphs resulted correlated to density of Roe deer (Spearman test, P

Dermacentor marginatus and Spotted Fever Group rickettsiae in Tuscany

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In Italy, rickettsial agents have been detected in different tick species, but human rickettsioses were traditionally considered to be caused by *Rickettsia conorii*, the agent of Mediterranean Spotted Fever (MSF) transmitted by *Rhipicephalus sanguineus*. Recently, the tick-borne zoonoses surveillance system established at the Lucca local health unit (ASL 2) indicated the emerging role of *Rickettsia slovaca*, the causative agent of Tick Borne Lymphadenopathy (TIBOLA), as a human pathogen. This evidence highlights the role of *Dermacentor marginatus* as primary vector of SFG rickettsiae in the area.

Patients admitted to emergency units in order to remove a tick from the skin were included in the study. A total of 331 patients were followed for 40 days; epidemiological and clinical data were collected using a standardized questionnaire. Ticks were classified and stored until DNA extraction. At the same time the ecology of TIBOLA was investigated: 494 *D. marginatus* ticks were collected from 109 hunted wild boars, during the 2007-2008 hunting season in Lucca province. All ticks were tested for the presence of *Rickettsia* spp. by molecular biology methods.

D. marginatus is involved in 26 out of 331 of the tick bite events recorded at the surveillance system, second in importance only to *Ixodes ricinus*. *D. marginatus* was recovered on the scalp, trunk, arm and leg. Twelve (46%) patients showed TIBOLA symptoms, the ratio male/female was 0.33 and the mean age in TIBOLA

cases was 33 years. All TIBOLA patients reported a history of tick bite and tick collection during free time. TIBOLA cases occurred from October to June peaking in April. TIBOLA patients main symptoms were: enlarged cervical lymph node (83%), eschar and fever (28%), alopecia (25%). Field survey of *D. marginatus* was conducted in sixteen hunting districts located in the province of Lucca, during hunting season 2007-2008. The 98.2% of all the ticks specimens collected on hunter-killed wild boars were *D. marginatus*. Hundred nine out of 163 (66.9%) wild boars were found infested with *D. marginatus* ticks. Overall, 494 *D. marginatus* were collected from the animals, 112 of which were tested for the presence of *Rickettsia* spp. Sequence analysis identified *R. slovaca* in 36 samples and *R. raoultii* was detected in 2 ticks. The result of the study field confirm the primary role of *D. marginatus* as vector of SFG rickettsiae in the area. *D. marginatus* adult is strictly connected to wild boar that, as known, support the *D. marginatus* life cycle by feeding them. Wild boars are widespread in woods, but in hilly area of the province this ungulate is seen in proximity to the dwellings. As a consequence of the abundance of *D. marginatus* and the high prevalence of infectious in ticks, we lately recorded the highest incidence of SFG rickettsiosis of the last 20-30 years and a peculiar distribution of the risk among the residents. Our results highlight the importance of ecological and epidemiological survey, both on field and by surveillance systems, in collaboration with public health emergency units., in order to support the diagnosis and the management of SFG patients.

The occurrence of tick borne encephalitis virus (TBEV) in *Ixodes ricinus* ticks in north-eastern Poland. Preliminary results.

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Ixodes ricinus, the most commonly observed tick species in Poland, is a known vector of such pathogenic organisms as *Borelia burgdorferi* s.l., *Anaplasma phagocytophilum*, *Rickettsia helvetica* and Tick-Borne Encephalitis virus (TBEV) in our country. TBEV (Flaviviridae, Flavivirus) is an etiologic agent of tick-borne encephalitis (TBE), the most important arboviral human infection involving the central nervous system. The disease is endemic in a zone extending from central and eastern Europe to Siberia and Japan, and corresponds to the distribution of the ixodid ticks, which act both as the vectors and reservoir of TBEV. Humans acquire infection by the bite of an infected tick. A continuous increase of TBE cases throughout Europe has been observed over a period of 30 years. First clinical cases of the disease in Poland were reported from the Bialowieza Primeval Forest over 50 years ago. Although between 2003 and 2007, ca. 260 cases annually were reported, little is known about the occurrence of TBEV in native *I. ricinus* populations. The objective of this study was a preliminary determination of the infection level of ticks collected in North-eastern Poland, the endemic area of TBE. Questing *I. ricinus* ticks (adults, nymphs and larvae) were collected by flagging the lower vegetation in 46 locations in north-eastern Poland in 2006-2008. A total of 1906 ticks were collected and sorted by the collecting site, stage and sex, and placed in pools of not in more than 20 adults and 50 nymphs in mixture of phenol and chaotropic salts (Fenozol A&A Biotechnology) and frozen in -20oC. Total RNA were extracted using the phenol-chloroform method and frozen in –65oC for further investigation. The reverse transcription reactions were performed with random primers (Invitrogen) and M-MLV reverse transcriptase (Invitrogen). Two pairs of primers for nested PCR were used from the 5’-NCR and the 5’-terminus of the C protein coding region, which are highly conserved among the TBEV isolates. A total of 237 tick pools were examined, in this number: 646 females, 511 males, 704 nymphs and 45 larvae. The average number of ticks in one pool was 8.04. Using RT-PCR it was possible to detect RNA of TBE virus in 16 pools (6.75%). A total number of ticks in infected pools was 153 (68 females, 46 males, 26 nymphs and 13 larvae), the average number of ticks in one infected pool was 9.56. The minimum infection rate of ticks with TBEV was estimated in total area as 0.84%. The prevalence of viral infection in ticks is a useful indicator of TBE virus circulation and may be used for risk assessment of the degree of natural focus activity and of the risk to contact TBE in a particular natural habitat.

Bird-associated cryptic cycling of common and novel strains of *Borrelia burgdorferi* in the Midwestern United States

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Wildlife sentinels and genetic investigations have elucidated an active invasion of blacklegged ticks, *Ixodes scapularis*, and the Lyme disease pathogen, *Borrelia burgdorferi*, in Michigan in the Midwestern United States (Hamer et al. in review). Previous laboratory and field studies have implicated *I. dentatus* – the eastern bird-rabbit tick – as a competent vector that maintains *B. burgdorferi* sensu lato in ‘cryptic cycles’ in areas with sympatric *I. scapularis* (Telford and Spielman 1989a,b). We hypothesized that *I. dentatus*-mediated cryptic cycling occurs in areas of Michigan prior to the invasion of *I. scapularis*. Furthermore, we predicted that the diversity of *B. burgdorferi* strains found in cryptic cycles would differ from those in nearby *I. scapularis*-inhabited zones.

From May–November of 2004–2008, we used mist nets to capture 24,828 wild birds of 119 species at a focal site in Lower Michigan that is 90 km to the east of the zone of blacklegged tick invasion. Eleven percent of birds were infested with a total of 11,555 ticks, of which *I. dentatus* comprised over 94%. The only three individual birds found to harbor *I. scapularis* were fall migrants that likely picked up blacklegged ticks in endemic foci to the north. We trapped white-footed mice (*Peromyscus leucopus*) and eastern chipmunks (*Tamias striatus*)- the most sensitive wildlife sentinels for low-density *I. scapularis* in the region- and found that none of 91 mammals were infested at a time when over 85% harbored *I. scapularis* in the nearby invasion zone.

We used a nested PCR and sequencing of an internally transcribed spacer of *Borrelia* species to determine that 3.8% of 1879 nymphs and larval pools of bird-derived ticks were infected with *B. burgdorferi*, suggesting cryptic transmission in the absence of *I. scapularis*. Additionally, *B. andersonii* (a Lyme borreliosis group spirochete not associated with human disease) and *B. miyamotoi* (a relapsing fever group spirochete of unknown disease risk) were detected in less than 2% of bird-derived ticks. In the *I. scapularis* invasion zone, 12 strains of *B. burgdorferi* were found among 169 infected *I. scapularis*, of which six of these strains were also found among 55 infected *I. dentatus* at the cryptic transmission site. An additional twelve novel sequence variants were identified in *I. dentatus* of which a majority were single, double, or triple-nucleotide variants of more common types found in *I. scapularis*. We hypothesize that the presence of *B. burgdorferi* in avian hosts and *I. dentatus*, which precedes the arrival of *I. scapularis*, may reduce the time lag between subsequent *I. scapularis* invasion and build-up of infection prevalence. Thus, mobile, infected birds and cryptic cycles may have the potential to accelerate the increase in human disease risk and contribute new strains within an invasion zone.

Monitoring Tick activity in Recreational Areas: Temporal and Spatial Dynamics and Dependence on Environmental Factors

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Methods: Season and weather dependent *Ixodes ricinus* tick activity was monitored during 2009 in Southern Germany by standardized methods compatible to the EDEN procedures (flagging defined, 100 m² sized areas). With respect to Public Health, 6 different types of recreational areas were selected: Parks in a large city (Munich, central park "English Garden") and a smaller town (Herrsching, town park), along a popular hiking route in the Alps (Garmisch, mountain "Wank", 800 m altitude difference), inside a National Park ("Bayerischer Wald"), along a well-frequented river valley (river "Altmuehl"), and a forest area near a university (Freising). In each location, four, six or eight 100 m² sites were marked, GPS coordinates taken,

described in respect to vegetation and surroundings, and photographed. Temperatures and relative humidities were continuously monitored within each sampling site using miniature data loggers ("iButtons"). Each site was probed for ticks once to four times per month.

Conclusions: As data collection is just finishing at this point of abstract preparation (November 2009), data analyses, interpretation and modelling are still ongoing. However, we expect up-to-date results to be available at the time of the conference.

Crimean-Congo hemorrhagic fever virus circulation in the environment of French soldiers

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by the CCHF fever virus, a member of the genus *Nairovirus* in the family *Bunyaviridae*. The virus is transmitted to humans through infected tick bites, crushed ticks or from direct contact with viremic animals or humans. Numerous domestic and wild vertebrates have been reported to present antibody response and/or viremia, including cattle, goats, sheep, horses, pigs, hares, ostriches, camels, donkeys, mice, and domestic dogs. The virus causes severe hemorrhagic diseases in humans with a 30-40 % mortality rate. On the opposite, infection is asymptomatic in animals. Outbreaks of CCHF have been documented in Africa, Middle East, Eastern Europe and Western Asia where ticks of *Hyalomma* spp. are distributed. CCHF represents a real risk for Armed forces. Large military troops are deployed in areas where CCHF is endemic or epidemic: Balkans, Africa and Afghanistan. Soldiers are particularly exposed due to their occupations (overseas operations in unsafe situations with a deployment in natural environments, drills and commando training on the field, in rural areas, in contact with herds and vector).

Since 2003, we have been conducting epidemiological surveys in the surrounding areas of the French troops, using domestic animals as sentinels for the human infection.

In total, 1034 blood samples and 1089 ticks were collected from animals (495 cattle, 30 sheep, 71 goats, 381 dogs, 57 horses) in areas of deployment of French military forces (Congo DR, Gabon, Ivory Coast, Chad, Kosovo, Benin...).

Analyses were performed in the French Forces Institute of Biomedical Research. Serum samples were assayed for antibodies against a recombinant virus nucleoprotein using an enzyme-linked immunosorbent assay. All the ticks were identified using morphologic criteria, but only *Hyalomma* spp. were tested for CCHF virus, with a novel real-time detection RT-PCR using TaqMan technology.

We have identified different prevalence of CCHF in domestic animals sera from 0 to 37% depending on the region and animal species. It was nil in dogs and goats in Gabon, in dogs in Ivory Coast, in goats in Kosovo, in sheep and zebus in Chad, but positive in cattle in Kosovo (2.2 %; 2/91), in goats in Benin (5 %; 2/34), in horses in Congo (15 %; 3/20), and in horses in Chad (37 %; 14/37). In addition, CCHFV was detected in 5.8 % of *Hyalomma* spp. (28/482) in Chad.

Our studies showed that in general CCHF is prevalent in cattle, goats and horses in all studied regions except Gabon and Ivory Coast. CCHF virus was for the first time detected in Chad.

The risk for contracting CCHF should be considered for all the troops deployed in these regions. In this regard, military personnel in endemic regions should use personal protective measures that include the avoidance of areas where ticks are abundant and predominantly when the tick vector population is particularly active, the regular examination of clothing and skin for ticks and their removal, and the use of repellents.

Incidence and trend of disseminated stages of Lyme Borreliosis in the province of Brabant Wallon (Belgium): retrospective data of a reference center

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Background: Lyme Borreliosis (LB) is the most common vector-borne disease in Europe (Bacon 2008). Epidemiological serological data in Belgium show an increasing incidence since 1993 (Ducoffre 2008). However, this serological data does not allow measuring the rate of disseminated forms, which constitute the burden of the disease. Using the annual incidence of disseminated stages of LB from a reference hospital (Clinique Saint-Pierre, Ottignies-Louvain-la-Neuve), we aimed at describing the clinical and epidemiological features of cases over an 11-years period (1997-2007) **Methods:** Hospital cases records of all LB diagnosed from January 1st, 1997 to December 31th, 2007 were collected. To estimate the incidence of disseminated LB in Brabant Wallon, we reported annual cases to the number of people living in the catchment area of the hospital. **Results:** Ninety-eight (98) cases of LB were registered, representing a mean of 8.9 cases per year. All cases were diagnosed at second or later stages of the disease. No linear trend in incident cases was observed. Most of the cases (75%) were recorded between June and November. Neuroborreliosis was the dominant clinical form every year, with a mean of 76.5%, similarly to a previous report in 1989 (Bigaignon 1989). Considering the catchment population during the 11 years of our study, the cumulative estimated incidence of disseminated LB was 19.5/100,000 person-years with an annual mean rate of 1.8/100,000 persons. The minimum estimated incidence was observed in 2002 (n=2, 0.48/100,000 person-years) and the maximum in 2004 (n=14, 3.37/100,000 person-years). In 2008, the Belgian Scientific Institute of Public Health reported a LB seropositivity rate of 12.8/100,000 person-years for Belgium and 6.4/100,000 person-years for the province of Brabant Wallon (Ducoffre 2009). Our results probably underestimated the actual incidence because patients may choose to be treated elsewhere. Nevertheless, compared with this seropositivity rate, our clinical LB disseminated rate indicated 1 LB clinical case requiring hospitalization for 3 positive LB sera. **Conclusion** Our study does not show an increase over time in clinical LB cases hospitalized in the province of Brabant Wallon between 1997 and 2007. Clinical cases of LB that required hospitalization represented one-third of seropositivity to LB, keeping in mind that many cases of Lyme arthritis do not require hospitalization. To estimate more accurately the trend of LB infection and the rate of complications, we need a sentinel surveillance of clinically defined LB cases and a follow-up of individuals exposed to tick bites such as servicemen, forest workers or scouts.

Detection and Characterization of Babesia in Estonia

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Babesia, the causal agent of babesiosis, is tick-borne protozoa, which causes human babesiosis as well as disease in domestic animals. The presence of *Babesia* spp. has never been investigated in Estonia. Therefore, a survey of the occurrence of *Babesia* species and their tick vectors in Estonia was conducted.

The general objectives of our study were 1) to investigate the presence and distribution of *Babesia* spp. in Estonian ticks 2) to characterize *Babesia* by genetical and phylogenetical methods.

Materials and methods

For the current study, ticks were collected by flagging during 2006-2008, at the period of tick activity (April - October) in different parts of Estonia (Andineme, Oonurme, Laeva, Järvselja, Kilingi-Nõmme, Are, Puhtu). Ticks were pooled (five ticks/pool), tick suspensions were made, followed by DNA extraction. Screening of

ticks for *Babesia* spp. was done by Reverse Line Blot (RLB). Sixteen samples positive in RLB were chosen for amplification and sequencing of partial 18rRNA gene. Phylogenetic analysis was performed by PHYLIP program (Felsenstein, 1993).

Results

A total of 2160 ticks (390 adults and 970 nymphs - *I. ricinus*, 520 adults and 280 nymphs – *I. persulcatus*) were analyzed. The presence of *Babesia* spp. was detected in 36 (1.6%) of the tick pools. In 18 (0.83%) *B. microti* was found, in 3 (0.13%) *B. divergens*, while in 15 (0.69%) the species could not be determined due to hybridization with an universal *Babesia* spp "catch all" probe only. *B. microti* was detected in 4 adult *I. persulcatus* ticks in eastern part of Estonia and in 14 *I. ricinus* nymphs which were collected from the western part of Estonia. *B. divergens* was determined only in 3 *I. persulcatus* adult ticks in eastern Estonia. Fifteen tick pools (13 nymphs of *I. ricinus* and 2 of *I. persulcatus*) with undetermined *Babesia* species were found and were distributed through all collecting sites.

Sequence analysis of 8 *B. microti* strains revealed that seven of them were identical to each other in 379 bp amplicon of 18S rRNA and had a homology of 100% with a *B. microti* strain amplified from a patient (GenBank EF413181). One *B. microti* differed from all other strains by two amino acid substitutions at positions 206 and 291. Two *B. divergens* samples were identical to each other and had a homology of 99% with *B. divergens* (GenBank AY144688) amplified from rabbit blood and with a nucleotide substitution at position 227. Six samples positive only with a universal *Babesia* spp probe were defined after sequencing as European *Babesia* species (EU1), with a homology of 100% with *Babesia* spp EU1 (GenBank EF185819) amplified from *I. ricinus*.

The present study revealed for the first time the presence of *Babesia* species in Estonia. Phylogenetic analysis showed that *B. divergens*, *B. microti* and *Babesia* spp EU1 strains from Estonia are close related with other European strains.

Detection of Ehrlichia and Anaplasma in ticks infesting dogs in Tunisia

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Ehrlichiosis and anaplasmosis are considered as important emerging diseases of humans and animals. They are caused by several microorganisms of the genus *Ehrlichia* and *Anaplasma*, respectively. These obligate intracellular bacteria infect dogs and are characterized by their association with Ixodid ticks.

A total of 225 unfed adult tick samples removed from dogs, between June and September, in five bioclimatic zones in Tunisia, were used. Additionally, blood samples were taken from 227 healthy and sick (having signs of any disease) dogs. *E. canis*, *A. platys* and *A. phagocytophilum* DNA were detected, in ticks, by polymerase chain reaction amplification (PCR) using primers targeting 16S rRNA gene. The seroprevalence of *E. canis* and *A. phagocytophilum* was investigated using species-specific immunofluorescence antibody (IFA) test. For the molecular detection of *Ehrlichia* and *Anaplasma* species infecting dogs, the reverse line blotting (RLB) technique was performed.

Among 225 ticks, 18.2% were positive for *E. canis* and 2.2% for *A. platys*, while no ticks was infected by *A. phagocytophilum*. Among 227 collected sera, 56.8% and 37.4% were seropositive for *E. canis* and *A. phagocytophilum*, respectively. A RLB hybridization was then used to identify isolated *Ehrlichia* and *Anaplasma* species infecting dogs (n=227). Among them, only two dogs were infected by *A. phagocytophilum*; ten sample dogs were demonstrated infected by *E. canis* and ten infected by *Ehrlichia* sp., from which one dog showed a mixed infection with *A. phagocytophilum* and *E. canis* and one with *A. phagocytophilum* and *Ehrlichia* sp. PCR and RLB findings were confirmed by sequencing.

Our molecular results report for the first time, in North Africa, that canine ehrlichiosis and anaplasmosis are caused by *E. canis*, *A. platys*, and *A. phagocytophilum*. These would help veterinary clinicians who would be aware of the different *Ehrlichia* and *Anaplasma* species in naturally infected dogs in Tunisia and understand the epidemiology of these important emerging tick-borne diseases in both humans and animals.

Remotely sensed climatic factors associated with tick phenology and infestation of hosts

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Many continents, including Europe, are facing an increasing risk of introduction or spread of tropical vector-borne diseases transmitted by insects, ticks and rodents threatening human and animal health (Randolph 2006; Senior 2008; Rizzoli et al. 2009). Since arthropod vectors such as ticks and insects are ectotherms, their activity depends directly on environmental conditions (IPCC 2008). Especially relevant is temperature, which affects many aspects of tick biology.

Up to now, temperature data has been commonly interpolated from meteorological stations; however, satellite based indicators are sometimes more suitable for epidemiological applications than traditional methods (Tatem et al. 2004; Scharlemann et al. 2008). In fact, satellite-based Land Surface Temperature (LST) data appeared to be a valid proxy for analysing the temperature profile in relation to tick activity (Randolph 2004). The optimal match of temporal and spatial resolution is currently provided by the MODIS sensor, flown on the Terra and Aqua satellites, from which daily temperature time series can be reconstructed (Neteler *subm.*).

In this study, longitudinal field data (2006-2008) of questing tick larvae and nymphs were obtained by dragging in three EDEN project sites, Candaten and Losego (IT4 and 5), both located in the Province of Belluno, Italy and Lamar (IT2), Province of Trento, Italy. A series of phenological indices were extracted from this dataset: for example, date of beginning/end of questing activity, date and values of seasonal tick density peaks. These indices were coupled with information on ticks feeding on hosts measured from an empirical survey on rodents, aimed at comparing contrasting geographic sub-regions. A series of variables was extracted for the tick sampling sites and sampling dates from aggregated reconstructed daily MODIS LST data (Neteler *subm.*). The variables include: autumnal cooling of the previous year, spring warming of the current year, 7°C and 10°C temperature threshold for the observed onset of tick activity, decadal mean LST (16days), and Growing Degree Days. We performed a series of statistical analyses to explore the influence of climatic factors on seasonal dynamics of questing ticks, and on its effects on host tick burden. Since a strong regional effect on tick seasonality and host tick infestation has already been noted for these sites, but only using data from terrestrial weather stations or an appropriate proxy (Cagnacci et al. *submitted*; Tagliapietra et al. *submitted*), this effect was explored in more detail using the LST data.

[Analyses are ongoing and this abstract will be updated before publication.]

Tick-borne encephalitis caused by unpasteurized milk in Hungary

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Tick-borne encephalitis cases caused by infectious milk products are not unknown in Hungary. The latest large outbreak involving 25 patients of 154 exposed persons occurred in August 2007. None of the patients had a history of tick-bite, however, all of them drank unpasteurized raw goat milk from the same farm. The National Center for Epidemiology together with veterinarian researchers aimed to identify the goats on the farm, which could have spread the infection through their milk. Blood samples were taken from 75 goats on the farm and were examined by various serological methods, namely indirect immunofluorescent assay, hemagglutination inhibition, microneutralization and an ELISA adapted to testing material from goats, to determine antibody levels in the serum. It has been concluded that serological results for goats by the different methods should be compared before final diagnosis because the specificity of methods in use can differ significantly.

To extend our knowledge on the risks of milk-borne infections and to characterize the TBE infection of goats, an *in vivo* experiment was conducted, in which goats were infected with TBE virus and their blood

and milk samples were examined for the presence of the virus by molecular and classic virological methods. TBEV was detectable in the blood and milk of the infected goats by both methodologies, although with different sensitivity. Regarding the interval while virus is shed by the milk of the goats, results are in accordance with earlier reports. Further studies are planned to investigate the possibilities of immunization of goats against TBEV.

Detection and characterization of *Anaplasma phagocytophilum* in ticks in Estonia, and Baltic regions of Russia and Belarus

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Anaplasma phagocytophilum is a tick-borne gram-negative obligate intracellular bacterium, which causes human granulocytic anaplasmosis (HGA). In Estonia the presence of *A. phagocytophilum* in tick populations as well as their significance for human morbidity remains unclear. Aims of the study were: to investigate the distribution and prevalence of *A. phagocytophilum* in the tick populations in Estonia as well as the Baltic regions of Russia and Belarus, and to genetically characterize the detected *A. phagocytophilum* strains.

Materials and methods

Ticks were collected in Estonia, Russia and Belarus. The total numbers of analyzed ticks were 2160 from mainland Estonia (390 adults and 970 nymphs - *I. ricinus*, 520 adults and 280 nymphs – *I. persulcatus*) and 342 of adult *I. ricinus* from Saaremaa island; 394 adult *I. persulcatus* from the Baltic region of Russia and 83 adult *I. ricinus* from the Kuronian Split and 344 adult *I. ricinus* from Belarus (Minsk region). Screening of ticks for *A. phagocytophilum* was performed by real-time PCR (Courtney et al. 2004). 21 positive samples were chosen for PCR amplification and sequencing of the partial groESL heat shock operon. In addition, 12 and 5 positive samples were sequenced in the 16S rRNA and ankA regions, respectively.

Results

Prevalence: *A. phagocytophilum* were detected in 60 *I. ricinus* ticks by real-time PCR, all *I. persulcatus* were negative. The overall prevalence of *A. phagocytophilum* in ticks from different sites in Estonia varied from 0 to 4%; in Russia *A. phagocytophilum* were detected in 7,2% of *I. ricinus* ticks collected only in the Kuronian Split; in Belarus 3,7% ticks were infected.

groESL: A total of 1256 bp of the groESL heat shock operon were analyzed for the 21 real-time PCR positive samples. The sequences were 98.0 to 100% identical to each other. The first 402 predicted amino acids of the groEL sequence were analyzed. We found unique amino acid substitutions at positions 139, (Glu→Arg in two Estonian strains) and at position 398 (Glu→Gly in one Belarussian strain). According to the substitutions at position 242, all analyzed sequences (21 from this study and retrieved 37 from GenBank) two groups were distinguished, one with Serine and another with alanine at that position. The splitting of *A. phagocytophilum* into two genetic lineages was confirmed by phylogenetic analysis, and did not correspond to the place or origin of *A. phagocytophilum* isolation or detection. Strains detected in ticks from Estonia, Russia and Belarus belonged to both genetic lineages.

16S rRNA gene: Sequence analysis of 1157bp of partial 16S rRNA gene was performed for 15 samples and revealed high level of identity (99,6-100%) between samples. Three genetic variants of *A. phagocytophilum* (according to von Stedingk et al., 1997) were revealed in Estonian, Russian and Belarussian strains. However, separation of *A. phagocytophilum* lineages was not supported by significant bootstrapping values due to the high similarity of the 16S rRNA gene sequences.

ankA: Sequence analysis of 5 samples was performed for partial ankA gene (1862bp). Sequences showed a high diversity on both nucleotide and amino acids levels, 69,1%-99% and 69,5%-99,3%, respectively, and revealed three distinct sequence types. On a phylogenetic tree, the Estonian, Russian and Belarussian strains of *A. phagocytophilum* clustered within different lineages. The clustering was not supported by geographical origin of the strains.

Conclusions: Co-circulation of different *A. phagocytophilum* lineages in *I. ricinus* ticks were detected in Estonia, Russia and Belarus by sequence and phylogenetic analyses.
Work granted by EDEN and VISBI projects.

Identification and quantification of *Borrelia* species in *Ixodes ricinus* ticks in Tri-City agglomeration and surrounding areas (northern Poland).

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Lyme disease is one of the most common emerging infectious disease occurring in the Northern Hemisphere. The number of cases of borreliosis in Poland has increased over the last few years and currently about 8,000 cases are reported in our country annually. *Ixodes ricinus* tick is the main vector of spirochetes *Borrelia burgdorferi* sensu lato, an etiological agent of Lyme disease, in Europe. Current researches in our country reveal that percentage of infected ticks depends on localization and can vary from 0 to ca. 60%. Moreover, recent studies proved that the effectiveness of transmission of spirochetes from tick to vertebrate host depends on the number of bacterial cells in arthropod vector. Taking this into consideration, better estimation of the risk of acquiring disease requires not only detection, but also evaluation the amount of pathogens in tick organism. The aim of this study was detection and quantification of *Borrelia burgdorferi* s.l. in *Ixodes ricinus* ticks depending on: developmental stage of the tick, seasonal activity and type of area (environment with high and low anthropopression). Ticks were collected by flagging lower vegetation in four localizations in Tri-City forests and surrounding areas. The species, stage of development and gender were identified and tick specimens were preserved in 70% ethanol. DNA was extracted by lysis of crushed ticks in ammonium hydroxide. The Real-Time TaqMan PCR assay targeting 23S rRNA gene of *Borrelia* sp. was used to detection and quantification of spirochetes in individual ticks. Genospecies of *Borrelia* were identified in positive samples by PCR-RFLP analysis of the flagellin gene. A total of 1392 *I. ricinus*, including 526 females, 552 males and 314 nymphs were examined individually. 34,5% of all samples were positive for *Borrelia* sp. No significant differences between percentage of positive samples in males and females were detected (38.9% vs. 40.3%) while the percentage of infected nymphs was two times lower (16.9%). Five different *Borrelia* s.l. genospecies were identified: *B. afzelii* (32.6% of infected ticks), *B. garinii* (31.0%), *B. valaisiana* (10.6%), *B. lusitaniae* (9.1%) and *B. burgdorferi* sensu stricto (2.9%). In 2.7% of infected ticks other species of *Borrelia*, closely related to relapsing fever spirochetes, *B. miyamotoi* was detected. The number of bacterial cells in individual ticks varied from 12 to 372,760 with the mean of 18,914 spirochetes per tick. The mean number of spirochetes per tick was different depending on developmental stage (25,574 for females, 14,488 for males and 7,046 for nymphs) and species of *Borrelia* (the highest level of infection was detected in ticks infected with *B. miyamotoi* and *B. garinii*: mean numbers of bacterial cells were 59,976 and 32,066 per tick, respectively).

The highest percentage of infected ticks was detected in August (44.8%), which was correlated with the highest level of infection in ticks (mean number of spirochetes reached 50,668 per tick in this month). Obtained results confirm that ticks from forests in Tri-City agglomeration and surrounding areas are potential source of infection with *Borrelia burgdorferi* s.l. and can serve as a vector of other spirochete species.

Molecular survey of tick-borne pathogens in the Czech Republic

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Introduction: Ixodid ticks (mainly *Ixodes ricinus* in Central Europe) represent significant health risk for humans as well as for many other vertebrate species being vectors of multiple pathogens of which the most important are flavivirus of tick-borne encephalitis complex (TBE virus), *Borrelia burgdorferi* sensu lato (s.l.),

Francisella tularensis, Anaplasma phagocytophilum, Rickettsia helvetica, Babesia microti, B. divergens and Babesia sp. EU1.

Aims: Within the project EDEN (Tick-borne pathogens group) we have conducted molecular based survey on prevalence of selected tick-borne pathogens, namely TBE virus, A. phagocytophilum, B. burgdorferi s. l. and Babesia spp. in nymphal and adult host-seeking ticks on four study sites (Brno, Cvilín, Znojmo, Valtice), three of which (Brno, Cvilín and Znojmo) are recognized as natural foci for TBE virus.

Methods: A total of 2400 host-seeking I. ricinus ticks (300 nymphs and 300 adults per site) were collected by flagging low vegetation and examined for the presence of studied microorganisms using TaqMan Real-time PCR procedure (for TBE virus and A. phagocytophilum) or conventional PCR followed by Reverse line blotting (for B. burgdorferi s.l., Babesia spp.), respectively.

Results:

TBE virus: Total prevalence of TBE virus was found to be 0.04. % (0.16 % in Brno; 0.0 0% in Cvilín; 0.0 % in Znojmo; 0.0 % in Valtice). A. phagocytophilum: Total prevalence of bacterium A. phagocytophilum was found to be 3.08 % (2.67 % in Brno; 3.17 % in Cvilín; 3.83 % in Znojmo; 2.67 % in Valtice). B. burgdorferi s.l.: Total prevalence of B. burgdorferi was found to be 16.0% (14.0 % in Brno; 17.5 0% in Cvilín; 15.5 % in Znojmo; 17.0 % in Valtice). Babesia spp. Total prevalence for protozoan Babesia spp. was established only in Brno (1.2 %). Accurate identification of B. burgdorferi s.l. into genomic species as well as differentiation between Babesia spp. are still ongoing.

Conclusion: Molecular survey of tick-borne pathogens represents scientific background for the comparison between data of other european countries involved in EDEN project and significantly complements missing information concerning prevalence of Babesia spp. in the tick I. ricinus in the Czech Republic.

This study was funded by EU grant GOCE-2003-010284 EDEN.

The effect of temperature on the viability of Rhipicephalus sanguineus infected with Rickettsia conorii

Cristina SOCOLOVSCHI, Didier RAOULT and Philippe PAROLA

Mediterranean spotted fever, caused by Rickettsia conorii conorii, is one of the oldest tick borne diseases endemic in Southern Europe and Northern Africa. Rhipicephalus sanguineus, the brown dog tick, is the vector and potential reservoir of disease. Transovarial and filial infection rate of R. conorii conorii are very high, but the prevalence of infected ticks in nature is very low. In this study we show that engorged infected nymphs not survive at change temperature. Rh. sanguineus overwinter as engorged nymphs and adults. The hypothesis that R. conorii conorii infected Rh. sanguineus ticks would not pass the winter is discussed.

Emergence of Human Granulocytic Anaplasmosis in France.

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Human Granulocytic Anaplasmosis (HGA) is a tick-borne zoonotic infection caused by Anaplasma phagocytophilum, an intracellular bacterium that belong to the order Rickettsiales, family Anaplasmataceae. It is transmitted by ticks, particularly Ixodes, and describe mainly in United States and Europe. First case in Europe was described in 1997 in Slovenia, first and only case in France was described in 2003. To date, only 70 human cases are described in Europe. Epidemiology in France is still little known and little reported. This emerging disease appears most commonly as an undifferentiated febrile illness after tick exposure occurring in the spring or summer, when ticks are most active.

In the National Reference Center for rickettsial diseases, in Marseille, France, we recently described 5 cases of human infection with Anaplasma phagocytophilum between the end of June and September 2009. All patients presented similar clinical symptoms: fever, headache, myalgia and 2 patients presented abdominal

pain. Three patients reported recently ticks bites but all are frequently exposed to ticks in forest or in contact with animals. Five patients live in north eastern of France. One patient reported recently travel in Slovenia and an other patient in Sologne, a forested region of central France. Results of laboratory investigation showed leucopenia and thrombocytopenia, biologic inflammatory syndrome and moderate elevation of liver enzymes. Giemsa stain of a buffy coat specimen revealed the presence of morula inside polymorphonuclear leucocytes for 2 patients. Serology with indirect microimmunofluorescence technique was positive for 5 patients and a seroconversion was observed for 3 patients. Real time PCR using primers and probes targeting the *yqaB* specific gene of *A. phagocytophylum* was positive in blood or serum samples for 3 patients. This result was confirmed using primers and probes targeting a second specific gene and PCR of 16 S rRNA gene and sequencing. The sequence of *A. phagocytophylum* was identified with 98.68% (301/305) of similarity with Genbank sequence (AY776165). RT-PCR analysis has high sensitivity during 0 to 7 days after infection, so allow an earlier diagnosis than serology. No complications were observed. All patients cured; with treatment with doxycycline for 2 patients and 3 patients resolve their illness spontaneously.

In fact, these tick borne related illness was probably underestimated because lack of knowledge to this disease and lack of specific clinical symptoms. Human Granulocytic Anaplasmosis is considered as an emerging disease because of their relatively recent description and their steady increase incidence in several parts of the world. Various factors have contributed to the emergence of these tick-borne illnesses, including better awareness by physicians, better diagnostic tools, changes in the environment with the expansion of the animal reservoirs and the growth of susceptible human population. That's why, Human Granulocytic Anaplasmosis must be suggested during summer flu, associated with bicytopenia, after ticks exposure.

First molecular evidence of potentially zoonotic *Babesia microti* and *Babesia* sp.EU1 in *Ixodes ricinus* ticks in Belgium.

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We report the first molecular evidence of the presence of *Babesia* sp.EU1 and *B. microti* in *Ixodes ricinus* ticks in Belgium. During a one-year national survey, 1005 ticks were collected from cats and dogs, randomly submitted to veterinary practices. A PCR technique amplifying a part of the 18s rDNA was used and in order to detect false negative results due to PCR inhibition and to validate the efficiency of the DNA extraction, an additional PCR targeting a 325 bp DNA fragment corresponding to the tick 16s rDNA gene was included. Eleven ticks out of 841 selected and validated ticks were found to be positive for *Babesia* spp. All of them belonged to the species *Ixodes ricinus*. A subsequent sequencing allowed the identification of *B. microti* (n=3) and *B. sp.EU1*. (n=6). This study shows a low prevalence (1.31% with 95% CI: 0.65-2.33) of *Babesia* spp. carriage in *I. ricinus* ticks in Belgium and reports for the first time 2 potentially zoonotic species belonging to this genus. Co-infection with *B. microti* and *Borrelia* spp. was also demonstrated. Phylogenetic analysis was performed from consensus and reference sequences by using the neighbor-joining method with Mega 4 program (Tamura et al., 2007). In addition, to assess potential inhibition due to engorgement of the ticks, the effect of DNA dilution on results was assessed on three hundred thirty five DNA samples. This study clearly demonstrates that inhibitors of PCR amplification are present in engorged ticks. A 1/10 DNA dilution is indicated in order to determine accurately *Babesia* spp. infection rates.

Influence of biotic factors on the incidence of tick-borne encephalitis in Slovenia

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Tick-borne encephalitis virus is the causative agent of the most prevalent arboviral human infection in Europe. Three subtypes have been identified: European (TBEV-Eu), Far Eastern (TBEV-FE) and Siberian (TBEV-Sib). The vector for transmission of TBEV-Eu, is the tick *Ixodes ricinus* and *I. persulcatus* is the vector of the other two subtypes. Many different vertebrates have been implicated in the maintenance and circulation of the TBEV. Major role in Central Europe seems to belong to *Apodemus flavicollis* and *Myodes glareolus*, not only because they are abundant in the regions where TBE incidence is high and they are excellent hosts for both nymphal and larval stages of the tick, but also since they promote TBEV transmission by co-feeding.

With long-term monthly tick samplings (2005-2008) we confirmed the presence and dynamics that facilitates the presence of TBE of *Ixodes ricinus* ticks in most parts of Slovenia. The sampled ticks were tested for the presence of TBEV using real time RT-PCR. We determined the overall 0.4% prevalence of the TBEV infection in Slovenian ticks: 0.54% in 2005, 0.43% in 2006 and 0.24% in 2007. However, significant variation in the prevalence of infection depending on the location of sampling and year of sampling was noted. Also the detected infection rate in ticks significantly correlated with the TBEV incidence rates in selected areas. The prevalence of infection in rodents sampled from 1990 to 2008 all over Slovenia was assessed using an immunofluorescence assay (IFA). It varied greatly according to rodent species, with the highest rate in *Myodes glareolus* (14 %) and lower in the *Apodemus* species (2- 4 %). The prevalence of infection differed in various regions, with the highest rate confirmed in areas with the highest incidence of TBE in humans in Slovenia. Additionally, 753 rodents were screened by real-time RT-PCR. In 5 cases TBE virus was detected without the presence of antibodies, 4 *Apodemus* species and 1 *M. glareolus* and in 16 cases the virus was detected in serologically positive animals, in 8 of those cases the only positive organ was the brain of the rodent (5 *Apodemus* rodents and 3 *M. glareolus*). These findings indicate that rodents develop long-term viremia.

It has also been implied that roe deer are one of the key factors necessary for TBE circulation. Data on deer abundance in Slovenia has been collected for the period from 1970 to 2008. We can see a gradual increase in the number of animals in the past decades. More detailed analysis is still being performed.

Even though the influence of biotic factors on the incidence of TBE seems to be substantial, it is not enough to explain the big changes viewed in the past decades. In order to fully comprehend the causes for these changes one has to include the human factor into account and perhaps some, as of yet, unknown components.

TBE virus in Switzerland: Microclimate and co-feeding transmission

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Tick-borne encephalitis (TBE) virus is known to be restricted to specific areas in Europe. Its focal distribution appears to depend mainly on co-feeding transmission between *Ixodes ricinus* infected nymphs and uninfected larvae. In order to better understand the role played by the co-feeding transmission, we followed the tick infestation of small mammals and the influence of microclimate on the seasonal evolution of questing tick population. We carried out a 3-year study in 4 sites, 2 of them were TBE foci. Free-living ticks and rodents were monthly collected in each area since spring 2006, and climatic data [relative humidity (RH) and temperature at 60 cm above soil] were recorded. Questing ticks and ticks feeding on rodents were analyzed for TBE virus after their molt by real-time PCR (modified from Schwaiger and Cassinotti, 2003). A decrease in questing tick density and in tick infestation of rodents was observed in 2007. In spring 2007, a very low RH associated with an increase in temperature, observed during the period when questing tick activity usually peaks, might explain the low tick activity. The same year the proportion of hosts carrying both larvae and nymphs was also lower in all sites. However, the prevalence of hosts infested by both larvae

and nymphs did not differ statistically between 2006 and 2008, except in one site. More than 6000 questing ticks and 3000 ticks from 435 rodents were analyzed for TBE virus infection. Overall, 7 pools of questing ticks from 3/4 sites were found infected and two rodents from one endemic site carried TBE infected larvae but no infected nymphs. During this study, we identified a new TBE focus amongst the 2 sites not recognized as such at the beginning of this work. This site showed a stable proportion of hosts carrying co-feeding ticks between 2006 and 2008 as observed in the 2 TBE foci.

Ixodes ricinus ticks seasonal dynamics in correlation with some climatic parameters in North Eastern Poland.

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Ixodes ricinus ticks are reservoirs of many infectious agents pathogenic for humans. For many years north-eastern part of Poland has been regarded as endemic for tick-borne diseases based on recorded epidemiological data. Aim of the study The analysis of *Ixodes ricinus* ticks number (larvae, nymphs, adults) in six places of Podlaskie region in vegetation period (from IV to X) and correlation with meteorological parameters from that area. Material and Method The numeration of ticks (adults- females and males, nymphs, larvae) was conducted for three years (2006-2008) every month (April to October) in six localizations of podlaskie voivodship. The number of collected ticks was estimated per 100/m². To collect ticks flagging method was performed (1x1m flannel blankets pulled 100 times on 2,5 m sections were used). Due to ticks susceptibility to changing from day-to-day climatic factors (temperature, moisture, rainfalls, wind) monthly mean number of larvae, nymphs, females and males was calculated. The meteorological data (minimal and maximal temperatures [T_{max}, T_{min}], mean rainfalls[R_f], number of days with snow cover[S_c]) collected in three stations (Białystok, Suwalki, Białowieża) were used for correlations - Spearman's rank correlation coefficient. Results *Ixodes ricinus* females – there was a difference in mean number per 100m² in the following months in all six localizations (from 5,42 to 1,1). The maximal number of collected females in all six places was stated in May (up to 22/100m² in Białystok district) and the minimal in the autumn (many places 0/100m²). *Ixodes ricinus* males – there was also a difference in mean number per 100m² in the following months in all six localizations (from 8,7 to 1,0). The maximal number of collected males was 18/100m² in Grajewo district in August). *Ixodes ricinus* nymphs – mean number per 100m² in the following months in all six localizations varied from 25,1 to 3,3). May and June were the time when the maximal number of collected nymphs was stated. Achieved results were correlated with meteorological parameters from current and previous(-1) months of collection and winter. The following correlations were stated a) between three years females number and T_{min}-1(p 0,042, r=-0,448), b) between three years males number and T_{max} (p 0,043 r=,0,445), c) between 2008 nymphs number and T_{min} (p 0,012, r=-0,0893). The comparison of winter parameters did not allow to state correlation but there is visible difference in ticks numeration pattern after mild (2007, 2008) and strong(2006) winters. Conclusions 1. The highest number of *Ixodes ricinus* ticks was reported in late-spring – May, June 2. No evident correlation between number of collected ticks and meteorological parameters was stated

European subtype tick-borne encephalitis virus in *Ixodes persulcatus* and *Myodes glareolus* in Simo, Finnish Lapland

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Finland is in the northernmost edge of tick-borne encephalitis (TBE) endemic area in Europe. TBE is focally endemic in a few areas in Finland: Åland and Turku archipelago, archipelagos of Helsinki in the south and Kokkola in the west, and in south-eastern Finland near Lappeenranta. In addition to these established endemic foci, human cases have occurred at the northernmost coastal area of the Baltic Sea (at the Gulf of Bothnia in Simo, Finnish Lapland), approximately 100 km south from the Arctic Circle, in 2008 and 2009. Both tick species *Ixodes ricinus* and *I. persulcatus* are found in Finland but their distribution areas seem not to overlap. Instead, *I. ricinus* has been found in southern and central parts of the country while, notably, we have found *I. persulcatus* in scattered foci along the western coast, including Kokkola archipelago, where *I. persulcatus* carries Siberian subtype of the TBE virus (TBEV). In the archipelagos of Helsinki, Turku, and Åland, and in Lappeenranta region, *I. ricinus* and European TBEV have been found. In June 2009 we collected 97 *I. persulcatus* ticks and 17 *Myodes glareolus* bank voles from Simo at places where TBE patients had likely contracted a tick bite. We pooled the ticks to 47 pools of 1-3 ticks; the rodents were handled individually. We isolated RNA by TriPure Isolation Reagent (Roche) from the tick pools and from brain and lung samples of the bank voles. Two out of 47 tick pools were positive for TBEV-RNA by a conventional RT-nPCR method targeting a 172-nt stretch from the 5'NCR (Shrader and Süss, 1999). In 15 out of 17 bank voles at least lung or brain was positive for TBEV RNA by a real-time RT-PCR (Schwaiger and Cassinotti, 2003). For virus isolation experiments we chose the two TBEV-RNA positive tick pools of two ticks as well as four rodents which had detectable levels of antibodies to TBEV by IFA and TBEV RNA seen by real-time RT-PCR in both lung and brains and by 5'NCR-RT-nPCR in brains. All the 6/6 virus isolation trials were successful. Based on an about 1000-nt amplicon from the E gene, all the TBEV isolates from Simo, that is, 2 from ticks and 4 from bank voles, were very closely related to each other and 94-98% identical to previously published European subtype TBEV strains. We conclude that Simo in Finnish Lapland is a new and by far the northernmost TBE endemic focus known in the world. The tick species in Simo is *I. persulcatus* (the taiga tick), similarly to Kokkola archipelago approximately 200 km to the south. However, the isolates represented the Eur-TBEV subtype, unlike other strains reported from *I. persulcatus* e.g. in Kokkola, Russia, or the Baltic countries, suggesting that the virus and the tick vectors have had different dispersal routes and histories.

Molecular Investigations on Romanian Ticks during the EDEN Project

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During the EDEN sampling years the Romanian TBD team collected, identified and preserved in RNA later solution over than 3500 ticks from 6 sites located in 3 different counties (Comana-Giurgiu; Macin and Ciucurova-Tulcea; Brateiu, Cristian and Sadu-Sibiu).

Starting with 2009 we investigated by molecular techniques the content of those samples. The used techniques were the ones agreed by EDEN-TBD but with minor modifications in parameters, according to our equipment without affecting the sensibility & specificity of those methods (each method was set up with positive controls provided by LMU-EDEN-TBD Partner).

Pools of 5-20 ticks were analyzed with real time PCR for detection of TBEV presence.

Borrelia burgdorferi genospecies were determined first by PCR and then by RLB starting from each individual tick. The same samples were investigated for blood-meal analysis and for detection of *Babesia* sp. Few samples from all the sampling sites were sent to LMU (Dr. Philippe de Mendonça) for detection of *Anaplasma* sp.

The results of molecular analysis for pathogens and blood-meal detection for each sampling site will be discussed in correlation with the seasonal abundance of ticks and the availability of vertebrate hosts in those sites.

Tick-borne encephalitis virus persists in wild rodents during winter

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RNA of European and Siberian subtypes of tick-borne encephalitis virus (TBEV) was detected in brain and internal organs of *Microtus agrestis* and *Myodes glareolus* rodents, respectively, during two subsequent winters several months after the tick-feeding season in Finland. This may indicate, that small mammals act besides as tick co-feeding host and when viremic as source for infection of ticks, as long-term reservoir for the virus.

The three known subtypes of TBEV are designated as European, Siberian and Far eastern. Vector for European subtype is *Ixodes ricinus* – tick whereas the two other subtypes are spread by *Ixodes persulcatus* – ticks. Both European and Siberian subtype of TBEV has been shown to exist in ticks in Finland. The rodents were caught at areas representing both subtypes, in Kokkola archipelago (Siberian subtype) and on Isosaari island, outside of Helsinki (European subtype). 201 rodents and insectivores were trapped in February and early March 2008 and 2009 by snap trapping. Viral RNA was detected at both areas in brain and organ pools of rodents using real-time reverse transcription (RT). Further to real-time RT-PCR, viral RNA was detected by nested RT-PCR and sequenced. Sequence analysis showed, that strains were similar to those detected in ticks at the areas in question.

The characterization of gene Bc37/41, an orthologue of the merozoite surface antigen Bd37 allows the molecular differentiation of *B. capreoli* from *B. divergens*

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Babesia divergens and *Babesia capreoli* are closely related species with distinct host ranges. Although *B. divergens* is known to be zoonotic, the development of *B. capreoli* in human erythrocytes has never been demonstrated. Roe deer, the natural host for *B. capreoli*, has often been suspected but never proven to be a reservoir for the zoonotic *B. divergens*. The two species are 99.8% similar in the 18S rDNA gene sequence and indistinguishable by morphological or serological means, leading to confusion as to their species status. It is thus essential to develop new molecular tools, based on variable genes, to differentiate the two species, especially within their common vector, the tick *Ixodes ricinus*. We were able to amplify a single PCR product from *B. capreoli* genomic DNA using primers specific for the *B. divergens* merozoite surface protein coding gene *bd37*, and sequencing confirmed the presence of a *bd37* orthologue in *B. capreoli*, named *bc37/41*. Restriction fragment length polymorphism (RFLP) analysis of the amplicons revealed an extreme polymorphism and yielded distinct profiles between *B. capreoli* and *B. divergens*, allowing their differentiation without sequencing. The full sequences of the *bc37/41* genes and their intron-exon structures were obtained for two cloned lines of *B. capreoli*. They suggest functional homologies between *Bd37* and *Bc37/41* such as their surface localisation, their role in immune escape mechanism and in the initial non specific attachment to the erythrocyte. IFAT and western blot analysis of *B. capreoli* merozoite protein extracts suggest a role of *Bc37/41* in the serological cross-reactions already described between these two species.

The best way to survive a Dutch winter for *Borrelia burgdorferi*

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In a large part of Europe, winter can be severe enough to prevent ticks from questing for hosts, which cuts off the only transmission route between individual vertebrates for *Borrelia burgdorferi* (the causative agent of Lyme disease). To understand how *B. burgdorferi* spreads over the tick- and host population after the winter, it is vital to know what fractions of hosts and of ticks are still infected. Ticks that acquire the bacterium with their larval blood meal are known to be well able to retain the bacterium over winter and to appear as infected nymphs in spring. But, the infectivity of the hosts after the winter is largely unknown. In the Netherlands small rodents are the primary hosts for immature ticks. A fundamental difference between ticks and rodents is that ticks do not reproduce over winter (i.e. the number of infected ticks does not get diluted by recruitment), whereas rodents reproduce in late autumn when large quantities of food are available (mast), which increases the fraction of individuals that never encountered ticks (or *B. burgdorferi*). To determine this demographic turnover of the rodent population over the winter period when ticks are inactive, we monitored sheep tick (*Ixodes ricinus*) activity and measured the population demography of both wood mouse (*Apodemus sylvaticus*) and bank vole (*Clethrionomys glareolus*) from August 2007 till May 2008 in an oak-dominated deciduous forest in the Netherlands. Measurements were done every ten weeks on six different plots each approximately 1 hectare sized. Ticks were monitored using a blanket-dragging method. The blanket was dragged for 25 meter after which ticks were counted. For adults (n=18), nymphs (n=12) and larvae (n=6). In total 15826 larvae, 3340 nymphs and 169 adult ticks were caught. Rodents were trapped on four consecutive days (twice a day; at dusk and at dawn), using 12 Longworth life traps per plot. By a mark-recapture method, 986 individuals rodents were identified. Ticks were considered inactive when the medians of the tick counts were zero and the percentage of ticks compared to the July count was

Consequences of Climate Change in Northern Europe

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Climate change will affect the transmission and geographical distribution of several vector-borne diseases in Europe. Impacts of changes in the length of the different seasons will be most noticeable in areas close to the latitude and altitude distribution limits of the vectors/diseases. In this paper the most recent data on the relationships between key climate indicators and the northernmost distribution of *Ixodes ricinus* in Europe will be presented, and projected changes in risk areas of Lyme borreliosis in Scandinavia during this century discussed.

Influence of host migration between woodland and pasture on the population dynamics of the tick *Ixodes ricinus*: a modelling approach

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Ticks act as vectors of pathogens that can be harmful to animals and/or humans. Epidemiological models can be useful tools to investigate the potential effects of control strategies on diseases such as tick-borne diseases. The modelling of tick population dynamics is a prerequisite to simulating tick-borne diseases and the corresponding spread of the pathogen. At the landscape level, the relationships between woodland and pasture play a pivotal role in tick population dynamics and in disease spread when cattle represent a reservoir

for pathogens. The tick population dynamics that we have developed is thus applied at the landscape level to investigate the effect of host movement between woodland and pasture on tick densities.

We have first developed a dynamic model to simulate changes in tick density at different stages (egg, larva, nymph adult) under the influence of temperature. We have focused on the tick *Ixodes ricinus*, which is widespread in Europe. The principal processes governing the biological cycles of ticks were taken into account: egg laying, hatching, development, host (small, mainly rodents, or large, like deer and cattle, mammals) questing, feeding and mortality. This model was first applied to a homogeneous habitat, where simulations revealed realistic changes to tick densities. We then considered a multi-habitat model, where three different habitats (woodland, ecotone and meadow) were connected through host migration. Based on this second application, it appears that migration from woodland, via the ecotone, is necessary to sustain the presence of ticks in the meadow. Woodland can therefore be considered as a source of ticks for the meadow, which in turn can be regarded as a sink. The influence of woodland on surrounding tick densities increases in line with the area of this habitat before reaching a plateau. This type of modelling approach provides insight into the influence of spatial heterogeneity on tick population dynamics.

Species Diversity, Relative Abundance and Crimean-Congo Haemorrhagic Fever Virus (CCHFV) Prevalence of Ixodid Ticks of Humans

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Ixodid ticks (Acari: Ixodidae) transmit various pathogens, such as Crimean-Congo Haemorrhagic Virus (CCHFV) to humans. Numerous CCHF cases reported from various provinces of Turkey, including Amasya. Identification of ixodid tick species infesting humans is crucial for the control of ticks and tick-borne diseases. In the present work, a tick survey performed on human ticks to determine the species diversity, relative abundance and CCHFV prevalence of ixodid ticks infesting humans in Amasya province of Turkey. A total of 2528 ixodid ticks (1925 adult, 593 nymph and 10 larvae) were collected from humans from April to November in 2008. Adult ticks were identified according to their distinct morphological characteristics. In addition, 23 *Hyalomma* tick pools were tested for the presence of CCHFV by using RT-PCR. Results showed that 25 ixodid tick species from six genera; *Hyalomma*, *Rhipicephalus*, *Boophilus*, *Dermacentor*, *Haemaphysalis* and *Ixodes* were found on humans. The *Hyalomma* species ranked first in relative abundance (60%) and 17% of the *Hyalomma* tick pools were CCHFV positive. Results indicated that variety of ixodid tick species infest humans and *Hyalomma* species have high potential to transmit CCHFV to humans in the province.

On the Estimation of the Basic Reproductive Number for Tick-Borne Diseases from Field Data

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The basic reproduction number (R_0) is a useful parameter to study the transmission and maintenance of infectious agents. Several mathematical models focusing on R_0 have been generated to study tick-borne pathogens in the past few years, some referring to LB in particular. Many of these models capture all factors influencing the pathogen transmission dynamics, but consist in complex formulations that are hardly applicable to fieldwork data ([1],[3],[5]).

We have recently ([4]) proposed a model to qualitatively estimate R_0 for two different genospecies of *Borrelia burgdorferi* s.l. from field data. This model is designed to capture their interaction with *Ixodes ricinus* immature stages and two vertebrate host species, each competent for one genospecies. This model mainly resides on the assumption that only systemic infection transmission is considered. For those ecological systems where co-feeding transmission plays an important role in the epidemiological dynamics ([2]), the proposed model should be seen as a lower bound estimation of basic reproductive number.

Here we propose a generalization of this model that allows to estimate R_0 of those tick-borne disease systems where both systemic and co-feeding transmissions of the pathogen are present. Our biological source of inspiration is the transmission of the etiological agent responsible for tick-borne encephalitis (TBE). TBE is an endemic disease in Europe and Asia, existing in discontinuous, woodland foci that harbor tick vectors and small mammalian hosts. TBE system is particularly suited for a discrete mathematical model mainly because tick's life cycle is divided in three stages and during each stage a tick can take a single blood meal on a host. The epidemiological cycle of TBE virus raises an intriguing question. In fact, it has been seen that the main route of transmission for TBE virus is the co-feeding, where the virus can be transmitted from an infected to a susceptible tick feeding on the same host without involving the host in the transmission process. This peculiar route of transmission will introduce in the model a higher level of complexity. The formulation of the equation is derived in order to still be applicable to fieldwork data in order to give a first approximation of the basic reproductive number of the epidemiological dynamics in a particular scenario. Some of the ingredients of the model can be found in the literature: the duration of the host infectious period of competent host species, the duration of the larvae feeding period on competent host species, the systemic transmission probabilities from an infected host to a susceptible larva and from an infected nymph to a susceptible host, the survival probability from feeding larva to feeding nymph, the transmission probability from an infected larva to its nymph stage, and the non-systemic transmission probability from an infected nymph to a susceptible larva feeding on the same host. In order to apply the model, fieldwork should be planned to be able to estimate the different competent and non-competent host species densities, together with the expected number of larvae and nymphs infesting and coinfesting them.

Molecular detection of *Rickettsia*, *Anaplasma*, *Ehrlichia*, *Coxiella*, and *Borrelia* in common ixodid ticks infesting camels and cows in Egypt

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The aim of this study is to determine pathogenic bacteria of the genera *Rickettsia*, *Anaplasma*, *Ehrlichia*, *Coxiella*, *Borrelia*, *Bartonella* and *Wolbachia* in common ixodid ticks infesting camels and cows in Egypt. Ticks were collected from 6 different localities in Egypt, Giza, Qalet El Nakhl, El Arish, Minufia, Qina and New Valley during the period from August to October, 2008. Pathogenic bacteria were identified by using PCR and sequencing. The *gltA* (citrate synthase) gene and outer surface protein gene (*ompA*) were used for the initial detection of *Rickettsia* spp. Further characterization of positive samples involved the use of primers targeting outer surface protein gene (*ompB*), Cell surface antigen, (*sca4*) and intergenic spacers (*mppA*, *dksA* & *rpmE*). We used the primers designed to amplify the portion of *groEL* and *rpoB* genes to detect both *Anaplasma* spp. and *Ehrlichia* spp., intergenic spacer and *rpoB* for *Coxiella burnetii*, *groEL* and *ftsZ* for *Wolbachia* spp., intergenic spacer for *Bartonella* spp. and *flaB* for *Borrelia* spp. Results showed that: 1) *Hyalomma dromedarii*; 57.1 % of ticks collected from camels in Qalet El Nakhl were infected with *Rickettsia africae* (99.1-100% similarities), 80.8% of ticks collected from camel in Giza were infected with *Anaplasma phagocytophilum* (99.8% similarity) and 20 - 71.4 % of ticks collected from Giza and Qalet El Nakhl were infected with *Coxiella burnetii* (99.5-100% similarities). 2) *Hyalomma anatolicum excavatum*; 41.2% of ticks collected from cows in New Valley were infected with genotype close to *A. centrale* (98.9% similarity), 76.5 of ticks collected from the same locality and host were infected with two genotypes close to *Ehrlichia canis* (96.0% similarity), *Ehrlichia* sp. (93.5% similarity) and 100% of ticks collected from cows in Qina were infected with genotype close to *E. canis* (96.0% similarity). 3) *Hyalomma impeltatum*, 26.7% and 73.3% of ticks collected from camels in El Arish were infected with *R. africae* (98.3-100% similarities) and *R. aeschlimannii* (97.9-100% similarities), respectively and 80% of ticks collected from camels in Qalet El Nakhl were infected with *C. burnetii* (100% similarity). 4) *Hyalomma marginatum marginatum*; 33.3% of ticks collected from camels in Qalet El Nakhl were infected with *R. africae* (99.1-100% similarity) and *R. aeschlimannii* (99.3-100% similarity). 5) *Boophilus annulatus*; 71% of ticks collected from cows in Qina were infected with *C. burnetii* (100% similarity), 41.9 % of ticks collected from the same locality and host were infected with two genotypes close to *E. canis* (96.0% similarity) and *Ehrlichia* sp. (93.5% similarity) and 20% of ticks collected from cows in Minufia were infected with *Borrelia* sp (100% similarity) which was

identified in *B. microplus* in Brazil. All examined ticks were found negative for *Wolbachia* spp. and *Bartonella* spp. Moreover, *R. africae*, *A. phagocytophylum* and *Borrelia* sp. are identified for first record in Egypt as well as the three new genotypes; close to *A. centrale*, *E. canis*, and *Ehrlichia* sp might be indicated to new species. This data shows the importance of tick borne diseases in Egypt.

Two years of seasonal questing activity of *Ixodes ricinus* at two locations in Germany determined by direct observation

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The hard tick *Ixodes ricinus* is the main European vector of the causative agents of Lyme borreliosis and tick-borne encephalitis virus. Its pattern of seasonal questing is rather flexible in time and space and may also vary from year to year at a given location. As a consequence, only a regular monitoring of the level of questing tick activity in a given area allows a realistic assessment of the tick bite risk in exposed people.

The main aim of the present study was to investigate the power of the used observation method to monitor *I. ricinus* questing (mainly nymphs and adults) all the year round at two locations in parallel in 2008 and 2009. This study was carried out in two forest areas, one in Berlin and one other approximately 500 km away, close to Giessen, a town north of Frankfurt/Main (Germany). We fed field-collected ticks at three different times a year and released them a few days after engorgement onto fenced field plots (each 1 m² in size), where they moulted to the next life stage in the following summer. Later on, they climbed the offered vertical wooden rods when questing. On each occasion, the numbers of questing ticks were counted by direct observation.

Expecting short-term changes in tick questing, we made observations two times a week from March to mid-November and every week or every two weeks from mid-November to February. For comparative reasons, we also collected ticks by flagging every 2 weeks in both locations.

Released *I. ricinus* survived for up to almost 2.5 years in the field plots. It is evident from the results that weather, especially rainfall and temperature, largely affected the course of seasonal questing but apparently with seasonal differences. This will be substantiated exemplarily.

Direct observation of questing *I. ricinus* ticks on field plots proved to be an objective and very reliable methodological approach for determining seasonal activity. In contrast to flagging, it allowed tick counts independently from the existing weather conditions in any short intervals. This qualifies this method for being widely used for monitoring of questing *I. ricinus* and probably also for other ixodid ticks with an ambushing host-seeking strategy.

Exploring diversity of tick-borne pathogens and tick-associated bacteria from different Italian *Ixodes ricinus* populations by pyrosequencing.

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Ixodes ricinus ticks play a major role in the transmission of a wide range of bacterial pathogens of medical importance (i.e., *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia*) [1, 2] Nevertheless, ticks may also harbor bacterial endosymbionts and non-pathogenic microflora whose biological significance in the host-pathogens interaction is still poorly understood [3]. The aim of this study

was to explore the bacteria communities, including tick-borne pathogens and tick-associated bacteria, in *I. ricinus* tick populations from two distinct geographic areas of Northern Italy (Trento and Belluno provinces). For this purpose, we established a massively parallel 454 pyrosequencing based on a targeted approach combined with a DNA barcoding system. By means of this method, we ultra-deep sequenced amplicons generated from the V6 hypervariable region of the 16S rRNA gene for the simultaneous identification of tick-borne pathogens and tick-associated bacteria, and the partial flagellin gene for *Borrelia* genospecies discrimination. An overall of 485.880 amplicon sequences were generated from the V6 region of the bacterial 16S rRNA of the DNA extracted from 100 nymph and 20 adult host-seeking ticks collected from each geographic area. The taxonomical composition of bacteria, based on the V6 regions, showed γ -Proteobacteria as dominant bacteria class, followed by α -Proteobacteria, Actinobacteria and Bacteroidetes. Several bacteria of medical importance were simultaneously identified in both geographic areas, namely *Borrelia* and *Rickettsia*, whilst *Anaplasma* was found only in tick population from Belluno province. Importantly, the analysis of the amplicon sequences of the *Borrelia* partial flagellin gene showed geographical differences in the circulation of *Borrelia* genospecies. In particular *B. garinii*, *B. valaisiana* were identified in the Trento province, whilst *B. garinii* and *B. burgdorferi sensu stricto* in the Belluno Province. Notably, we identified the arthropod endosymbiont *Wolbachia* in both geographic areas and sequences belonging to the genus *Rickettsiella*, whose significance require further investigation considering its close phylogenetic relation to vertebrate pathogenic bacteria of the genera *Coxiella* and *Legionella*. Among the tick-associated bacteria, we confirmed the presence of *Staphylococcus*, *Rhodococcus*, *Pseudomonas*, *Moraxella* and *Williamsia* [4, 5, 6]. Nevertheless, we found several bacteria not previously reported by traditional molecular methods, which instead require cultivation and cloning approaches, providing a complete view of tick microbial composition. Our results showed how the recent advances in sequencing technologies offer new perspectives for a rapid surveillance of pathogens implicated in tick-borne diseases in *I. ricinus* population, and for the simultaneous microbial characterization which may provide information about the tick microbiota and habitat distribution.

Climate, microclimate and tick activity (*Ixodes ricinus*) in Germany

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Various scientific results from recent years indicate an association between climate data, an increasing tick activity and the incidence of tick borne diseases. Other investigations support the opposite view. The factors influencing tick activity are highly diverse and range from climate and microclimate factors to socio-economic and political changes. However, even for simple questions like the correlation of tick activity and climate data, no validated results are available.

We therefore started studying climate and weather data, tick activity and tick borne diseases at seven locations from North- to South-Germany in a long time period project. Two different tick biotopes, a meadow and a deciduous forest, were investigated at every of the seven locations.

At all locations, 4 plots of 50 x 50 metre with a homogenous plant community were selected and connected to the exact GPS data. In 2009, ticks were collected in these plots several times per year both in the morning and in the afternoon by “flagging”. The plots were flagged alternately according to a very sophisticated and standardized method. The collected ticks were cooled immediately, transported to the lab, sorted, counted and stored at -80°C until extraction for determination of tick borne pathogens, especially TBE virus. Additionally, weather data (e.g. air and soil temperature, humidity, wind speed) directly at the plots and climate data (e.g. temperature, precipitation, humidity, air pressure, duration of sunshine, global radiation, wind speed) at the closest weather stations in Germany were also collected.

In 2009, several tick collection series at the different plots could be finished: 16 in Thuringia, 6 in Saarland, 4 in North Rhine-Westphalia, 6 in Lower Saxony, 6 in Mecklenburg Western Pomerania, 9 in Baden-Wuerttemberg and 8 in Bavaria. All collected ticks, in total 9320, could be classified as *Ixodes ricinus* 589 female, 566 male, 6740 nymphs and 1425 larvae. The relationship between tick activity, climate and local

weather data for several locations, in different biotops and at different times of the day and year will be presented. First results on tick borne pathogens (especially TBE) will also be shown. The described investigations are the initial step of a long-term study which will be carried out over the next years.

In a further step 30-year-old climate data and indirect data of the tick activity will be compared with data from 2009 and 2010 in order to find a possible direct influence of climate and microclimate change on tick activity and spread of tick borne diseases. To our opinion, these results will provide us with a solid prognosis for tick activity and tick borne diseases over the next decades.

Simulation of vector-borne dynamics on network-based models

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Network structure is clearly emerging as a key factor for modeling biological systems. In the last decade, we can observe a growing interest in the study of the influence that the interconnecting topology of a population of agents may have on the dynamical processes on a complex system, in particular on the effects of the small-world and scale-free properties of the graph (Newman 2002, Caldarelli 2007, Barrat et al. 2008).

Our source of inspiration is the transmission of the *Borrelia burgdorferi sensu lato* between ticks and rodents or lizards. Epidemiological spreading of pathogenic agents that can only pass between vectors and hosts can be naturally represented using bipartite graphs. In these structures nodes are divided in two classes and edges can only exist between nodes belonging to different classes. This is true in particular for *B. burgdorferi s.l.* epidemiological system, thanks to its very low probability of transovarial and cofeeding transmission (Gern and Rais 1996, Matuschka et al. 1998, Randolph et al 1995). We thus focus on the simulation of epidemic dynamics on these graphs.

An interesting feature of *B. burgdorferi*'s system is that ticks are observed to have an aggregate behavior on hosts. The resulting bipartite graph seems to show a binomial distribution of the ticks' nodes degrees (typical of random graph), and a scale-free distribution of the hosts' nodes degrees. This characteristic is currently investigated by statistical analysis of field data collected in a natural reserve in Tuscany, central Italy. Preliminary fitting results confirm that frequency distributions of ticks on mice and lizards are best described by power law equations, showing exponents between 1 and 2.

Susceptible-Infected-Susceptible (SIS) and Susceptible-Infected-Recovered (SIR) models can be used to investigate the dynamics of this pathogen, in particular their epidemic threshold (ET), the transmission probability above which the system is in an endemic state. SIR and SIS models were shown to have, as the population grows, a constant epidemic threshold greater than zero on random graph. On the other end, the ET for the same models on scale-free networks decreases when increasing the network size (Pastor-Satorras and Vespignani 2001).

The main question we address is whether the scale-free or the random graph character influences the behavior of the dynamical processes taking part on a bipartite graph. We test the epidemic spreading on three different kind of bipartite networks, according to the degree distributions of the two families of nodes: random – random (R-R), scale-free – random (SF-R), and scale-free – scale-free (SF-SF). Simulations show that, in those graphs where at least one family of nodes have SF propriety, the ET decreases as the network size grows. Similar results were found by Gomez-Gardenes et al. (2008) for SF-SF graphs representing heterosexual contacts.

This result shows that it's easier for *B. burgdorferi* to become endemic in a territory when factors like climatic variations or decreasing of incompetent hosts populations increase the probability for ticks to feed on reservoir hosts. Moreover, the topological characteristic of the system implies that a targeted removal of the more connected hosts would result in an efficient measure in the control of the pathogen spread.

Detection and identification of tick-borne bacteria from ticks collected on sheep and vegetation in Majorca Island (Balearic Islands, Spain).

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The aim of the study was to survey the Ixodidae fauna on sheep and vegetation in Majorca (Balearic Islands, Spain), as well as the pathogens which are capable of being transmitted by them. Sampling on sheep was conducted from May to July 2007 in six farms located in the northernmost mountain range of Majorca. Furthermore, four farms located in the plain area of Majorca were surveyed from May to September 2009. Adult ticks were collected from ears, face, eyes, perianal zone, udders and legs. The ticks were preserved at -70°C for molecular analysis. Sampling of ticks from vegetation was carried out by using dipping technique in mountain, plain, and coastal areas of Majorca during October 2008 as well as from May to August 2009.

The most abundant species found on sheep were *R. sanguineus* and *R. turanicus*, whereas only one specimen of *Haemaphysalis punctata* was recorded. Sampling of vegetation recorded the following species: *Dermacentor marginatus*, *Haemaphysalis punctata*, *Hyalomma lusitanicum*, *Ixodes frontalis*, *I. ricinus*, *I. ventralloi*, *Rhipicephalus pusillus*, *Rhipicephalus sanguineus* and *R. turanicus*.

From the total of ticks collected on sheep, 100 specimens (36 *R. sanguineus* and 64 *R. turanicus*) were molecularly analyzed for bacteria and virus detection. A duplex PCR for DNA amplification of *Borrelia* and *Rickettsia* species, as well as for *Francisella* and *Bartonella* species was performed. For the identification of *Anaplasma* only one single PCR was used. The bacteria species were identified by RLB hybridation and sequencing.

From the total of ticks analyzed 45 specimens were positive to bacteria. Three genera of bacteria of medical and veterinary importance were detected: *Anaplasma*, *Rickettsia* and *Borrelia*. No tick borne viruses were detected in any of the specimens. A total of 37 specimens were positive for *Anaplasma* and *Ehrlichia*, from which 10 were for *Anaplasma ovis*, 5 for *Ehrlichia canis*, 2 for *E. spp. China* and one for *A. ovis*, *E. canis* and *E. spp. China*. In regards to *Borrelia*, from 10 positives to the genera, only the species *B. burgdorferi* was identified. Finally, we found 10 positive specimens to *Rickettsia*, from which only two species, *R. conorii*, and *R. massiliae* were identified.

Prevalence of Anaplasma, Babesia, Borrelia burgdorferi and Rickettsia spec. in different hard ticks species in the area of Berlin, Germany

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The risk to be infected by a tick-borne disease depends on the density of ticks, its species and the pathogens' prevalence. Every four weeks from September 2008 until December 2009 we collected ticks in seven representative districts of Berlin with the different methods "flagging" and "dragging". The collected ticks - in total 1038 - were further investigated for different pathogens by PCR. Therefore we extracted the DNA, analysed it by specific PCR and revealed the product on a Lateral Flow- Stripe. This was performed for the four pathogens *Anaplasma*, *Babesia*, *Borrelia burgdorferi* and *Rickettsia spec.*

The two most common tick species in the districts were *Ixodes ricinus* and *Dermacentor reticulatus*. Two districts which represented the parkland within the city of Berlin were found completely free of ticks. In all other districts we regularly found ticks. *Ixodes* ticks of all stages were found in five out of seven districts.

Adult ticks of *Dermacentor reticulatus* were found in three of seven districts. *Dermacentor reticulatus* was found mainly in the southern districts but we could reveal this species even in the north of Berlin. In one district more than 50% of the caught ticks were *Dermacentor reticulatus*.

Thirty ticks of every district were analysed for the pathogens *Borrelia burgdorferi sensu lato*, *Babesia spec.*, *Anaplasma spec.*, and *Rickettsia spec.* in more detail. Additionally we analysed the ticks' density and the pathogens' prevalence with a geographic information system (GIS) in order to gain an overview of the risk situation for every pathogen in the different areas.

It was noticeable that the tick species *Dermacentor reticulatus* is wide-spread in the area of Berlin. In most cases these ticks were infected by *Rickettsia spec.* (66%). In total 47% of all ticks were infected by *Rickettsia spec.* and 9% of all ticks were positive for *Borrelia burgdorferi sensu lato*.

Further investigation showed that the *Rickettsia* species we found in *Dermacentor reticulatus* was *Rickettsia raoultii*. Sometimes the *Rickettsia*-positive *Dermacentor* ticks were even co-infected by *Borrelia burgdorferi sensu lato* (1%).

Ixodes ricinus was found to be infected by *Rickettsia spec.* (30%), too. 16% of these *Rickettsia*-positive ticks were co-infected by *Borrelia burgdorferi sensu lato*. *Babesia spec.* was found only in few *Ixodes* ticks (5%) but not in *Dermacentor reticulatus*. *Anaplasma spec.* was found in 3% of the *Ixodes* ticks. The DNA extracted of *Dermacentor reticulatus* was never found positive for *Anaplasma spec.* No co-infection with *Anaplasma* or *Babesia spec.* was observed.

Surveillance of tick borne zoonotic pathogens in vegetation and in wild and domestic animals in Madrid Region (Spain)

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Ticks are obligate blood-sucking arthropods that parasitize vertebrates (Merino et al., 2005). They are important vectors and reservoirs of various pathogenic bacteria, protozoa, and viruses that can cause disease in humans and animals (Parola et al., 2001). Some of these agents are important emerging vector-borne pathogens for humans and are receiving growing attention.

The Environmental Health Service from the Autonomous Government of Madrid launched a Wildlife Surveillance System in 2008. The monitoring of the ticks present in this Spanish Region is included among the activities of this System.

The present work was designed to identify the causative agents of emerging infectious diseases infecting ticks in the Comunidad of Madrid, as a part of the surveillance activities carried out during the period 2008-2009.

Tick collection was carried out by Forest Guards from the Cuerpo de Agentes Forestales, and staff of the Animal Protection Centres of Alcalá de Henares, Fuenlabrada, Henares-Jarama, Leganés and Móstoles as well as Patrimonio Nacional del Real Sitio de El Pardo. Specimens were collected from vegetation, domestic (dog) and wild animals, placed in a sample tube containing alcohol 70% and sent to the VISAVET Surveillance Centre for identification and later molecular analysis. Briefly, ticks were subjected to DNA extraction techniques and nucleic acids obtained were then analysed using PCR methods (Anda et al., 2005) for detection and identification of DNA from *Rickettsia* and other species.

More than 500 ticks, mainly from *Hyalomma*, *Rhipicephalus* and *Dermacentor* genera, were recovered from wild animals (n=379), vegetation (n=45) and domestic animals (n=119). Among the pathogenic species under study (mainly from genus *Rickettsia*, *Borrelia*, *Francisella* and *Anaplasma*) molecular analysis

revealed the presence of DNA from pathogenic bacteria such as *R. massilae* and *R. raoultii*, therefore highlighting the importance of these parasites as potential reservoirs of zoonotic diseases.

The diversity and complex cycle of these vectors, as well as the possible changes that can happen due to dynamic factors such as climate change, make necessary a continuous surveillance (Barandika et al., 2007) in order to evaluate the situation and prevent risks for transmission to humans.

The monitoring of the wild and peridomestic cycles of tick-borne pathogens will improve our understanding of the epidemiology of these zoonotic organisms and provide tools to design public health interventions for prevention and control.

Multilevel study of the environmental factors of the spatial distribution of tick-borne diseases

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The spatial distribution of vector-borne diseases is tied to environmental conditions in two primary ways. Vectors will only thrive under certain habitat conditions. These can be defined in terms of appropriate microhabitats, best documented at the local scale. This scale is also appropriate to describe hosts habitat, and, if relevant, human use of the land and hence risk activities. Vector habitat can also be described in terms of climatic (e.g. thermal) ranges, which can also be relevant to pathogen persistence. Climatic ranges are best described at the regional scale. In the current context of climate change, large uncertainties remain on the role played by climatic factors on the incidence of tick borne diseases in Europe, and particularly so when compared to the role of other environmental factors such as those operating at the landscape, local scale. Indeed, important environmental factors have been identified at both scales, but most studies have only examined one scale at a time, including studies using remotely sensed data. Our study aims at investigating how much variability in disease incidence can be attributed to environmental factors operating at a regional scale (such as climate), and to landscape-level environmental factors. Both low- and high-resolution remotely sensed data have been successful at mapping either vectors or disease cases, however, we think that combining factors acting at regional and local scales would improve the use of remotely sensed data in spatial epidemiology.

Among diseases for which a role of the environment at various scales has been identified, tick-borne diseases are an important group, as well as the major group of vector-borne diseases in Europe. Our study focuses on tick-borne encephalitis (TBE) in the Baltic States and Lyme borreliosis (LB) in Belgium. In our study, eco-climatic regions (as defined by thermal range, humidity, etc) favourable to the establishment of a disease transmission cycle will be characterised by using MODIS images (low-resolution remote sensing data). The amount and quality of vector's microhabitat will be characterized using classified LANDSAT images (high-resolution land cover maps) that will be analysed for landscape composition and structure. Multilevel statistical methods, a promising statistical approach to such hierarchical datasets, will allow to study this combination of scales applied to spatial epidemiology. This information can eventually be combined with the spatial distribution of human risk activities, as reflected by land use, through the help of a GIS.

Vertical distribution of *Ixodes ricinus* tick and pathogens transmitted by them in the Martinské hole mountains (Central Slovakia).

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At present, tick-borne pathogens causing many important diseases primarily in tropic and subtropic areas represent an enhanced risk in temperate climate. Climatic changes induce changes in the geographic

distribution and density of ticks as well as in occurrence and prevalence of tick-borne pathogens. In Europe, *Ixodes ricinus* is the most important vector of tick-borne pathogens (tick-borne encephalitis virus, spirochaetes of the *Borrelia burgdorferi* s.l. complex, anaplasmae, rickettsiae and babesiae). Data on ticks and tick-borne diseases in the Central Slovakia, namely in the northern areas such as Turčianska kotlina basin, have been sporadic. Moreover the data on the occurrence of ticks in higher altitudes are very brief. The aim of our work was to determinate the distribution and density of ticks in higher altitudes in Central Slovakia. The presence and genetic variability of tick-borne pathogens in *Ixodes ricinus* ticks was also tested. Questing ticks have been collected by dragging the vegetation in three different altitudes (600 – 650 m above sea level, 800 m a.s.l. and 1000 m a.s.l.) in the Turčianska kotlina basin since 2006. Genomic DNA was isolated from ticks using alkaline hydrolysis. *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. were detected by PCR of 16S rRNA and PCR-RFLP of 5S-23S rRNA, respectively, *Babesia* sp. was detected by TouchDown PCR and *Rickettsia* sp. was detected by PCR of *gltA* and *ompA* genes.

In total, 23% prevalence of *Borrelia*-infected ticks was detected. Among the detected genospecies, *Borrelia lusitaniae* was dominant at 600–650 m above sea level (a.s.l.), *B. afzelii* at 800 m a.s.l. and *B. valaisiana* at 1000 m a.s.l. Partion of collected ticks was examined also for the presence of *Anaplasma phagocytophilum*, *Babesia* sp. and *Rickettsia* sp. infection. The prevalence of *Rickettsia*, *Babesia* and *Anaplasma* – infected ticks was 18%, 10% and 1%, respectively. Positive samples will be further analyzed by sequencing.

Acknowledgements: This study was supported by the Slovak Research and Development Agency under contract No. APVV-51-009205, grant VEGA No. 2/0161/09 and EU grant GOCE-2003-010284 EDEN.

Genetic variability of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in Slovakia and Czech Republic

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The most common tick in Central Europe, the sheep tick - *Ixodes ricinus* transmits viral (e.g. tick-borne encephalitis virus), bacterial (e.g. *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*) as well as protozoan pathogens (e.g. *Babesia microtii*, *Babesia divergens*) to the humans and animals. In the recent years due to the global warming, as well as human impact on the environment, several tick-borne pathogenic organisms have (re)-emerged in Europe. The main aim of our study was to obtain new information on the prevalence and genetic variability of the *B. burgdorferi* s.l. and *Anaplasma* spp. in Slovakia and Czech Republic. Furthermore the DNA-chip for the detection of all relevant tick-transmitted bacterial species in one step have been developed and tested.

A total of 3499 ticks from five different geographical areas in Slovakia and three in the Czech Republic were tested by PCR-RFLP, RLB, or SSCP analysis for the presence and genetic variability of *Borrelia burgdorferi* s.l. The DNA of 978 ticks from five different areas in Slovakia and two in the Czech Republic was also tested for the presence of *A. phagocytophilum* and the other members of the family Anaplasmataceae.

The overall borrelial prevalence varied between 9-31% with the mean average 20.6%. *B. afzelii*, *B. garinii*, *B. valaisiana*, were presented in all sites. *B. lusitaniae* was presented at the locality in north-central Slovakia where 80% of all positive ticks belonged to this species. *B. garinii* predominated in Eastern Slovakia. The most common species at the localities in Czech Republic was represented by *B. afzelii* (52%). *B. spielmanii* was detected only in Southern Czech Republic. The sequencing of the 5S-23S rDNA intergenic spacer and *flagellin* gene revealed high intraspecific variability within the *B. garinii* and *B. afzelii* genospecies. The prevalence of *A. phagocytophilum* varied from 0% in South-eastern Slovakia to 7.8% in North-central Slovakia. Phylogenetic analysis of the *msp4* gene showed that most of the our samples clustered within a monophyletic clade together with *A. phagocytophilum* isolates from various ruminant species. Other members of the family Anaplasmataceae in ticks were represented by *Neoehrlichia*

mikurensis, *Ehrlichia muris* and *Anaplasma*-like. Based on the analysis of the obtained sequences, oligonucleotide probes for the development of the DNA-chip were designed. This method has a potential of use in the large scale epidemiological studies as well as fast diagnostic tool.

Surveillance on the *Borrelia* and *Anaplasma* prevalence as well as accurate identification and typing of these microorganisms is the key factor for understanding the ecology and epidemiology of the tick borne diseases.

Acknowledgement. This work was supported by the Slovak Research and Development Agency under contract NO. APVV-51-009205, grant VEGA NO. 2/0161/09 and EU grant GOCE-2003-01 0284 EDEN.

Rodent-borne diseases

Separating history from natural selection during the co-evolution of hantaviruses and their rodent hosts in Europe

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Hantaviruses are RNA-viruses of the Bunyaviridae family that usually occur in rodents/insectivores, but can also be transmitted to humans, often with more or less severe pathogenic symptoms as a consequence (1). As new hantaviruses, including pathogenic ones, are still being discovered, they are classified as important emerging diseases (2).

Hantaviruses and their rodent/insectivore hosts are often presented as textbook examples of parasite-host co-evolution. But after closer scrutiny, this relationship is not that straightforward: the genetic variation within hantavirus lineages shows topological patterns that are considerably different from those of their hosts. In Europe this could possibly be related to the recolonization pattern since the last ice age(s), but the reasons and mechanisms behind this pattern remain to be elucidated. Furthermore, hantaviruses have an as yet unexplained heterogenous occurrence and pathogenicity (3,4).

Phylogenetic studies of Puumala virus have revealed a complex genetic pattern that does not match the Quaternary history of its host (5,6), even though this would be expected on the basis of strong host specificity. Possible reasons for this could be that besides co-evolution, other processes like local adaptation, local extinction or host-switching have had a hand in shaping the current distribution. Although the other European Hantaviruses have been studied in less detail, similar processes can be expected (7,8).

In this context, and following up on EDEN results and insights, we introduce the start of a study that aims to unravel evolutionary and historical processes (local adaptation and extinction, co-speciation, host-switching, genetic drift) and their relative roles in shaping the current geographic and allelic distribution of 4 enzootic European hantaviruses and their hosts (5) (Puumala virus in *Myodes glareolus*, Dobrava virus in *Apodemus flavicollis*, Saaremaa virus in *Apodemus agrarius* and Tula virus in *Microtus* sp., the first three of which cause haemorrhagic fever with renal syndrome in humans).

These problems will be approached by constructing and comparing detailed phylogenies and phylogeographies of both the viruses and their hosts, based on neutral markers and functional genes, and by genetic analyses using state-of-the-art methods in computational molecular genetics, thus untangling historical and selective processes.

Enterohepatic *Helicobacter* species in Swedish wild rodents

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Current knowledge concerning ecological niches occupied by enteric *Helicobacter* species in wild rodents is scarce. We therefore investigated the status of *Helicobacter* species in wild rodents from different parts of Sweden. Specimens of bank vole (*Myodes glareolus*), wood mouse (*Apodemus sylvaticus*) and field vole (*Microtus agrestis*) were obtained from the northern, mid and south of Sweden. Genomic DNA was extracted from colon tissue samples (n=84) and analyzed by *Helicobacter* species-specific PCR assays (*H. hepaticus*, *H. bilis*, and *H. rodentium*) as well as a *Helicobacter* genus-specific PCR assay. None of the samples were positive using any of the species-specific PCR assays whereas all animals tested positive in the *Helicobacter* genus-specific PCR assay. PCR-products were therefore characterized by DNA-sequencing. 16S rDNA sequences demonstrated similarity to *H. aurati*, *H. ganmani*, *H. winghamensis*, and *H. sp MIT*

01-6451. Enterohepatic *Helicobacter* species were presumptively identified by DNA sequence analysis. Our observations suggest that rodents serve as a significant reservoir for enterohepatic *Helicobacter* species in the wild.

Mapping the Risk of Hemorrhagic Fever with Renal Syndrome in Northern Europe by Use of Low-Resolution Remote-Sensing

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Hemorrhagic fever with renal syndrome (HFRS) in Europe is mainly caused by Puumala virus (PUUV), a hantavirus within the family Bunyaviridae, that is naturally carried and shed by bank voles (*Myodes glareolus*). In northern Sweden, HFRS is the most prevailing serious febrile viral infection, second only to influenza. Sweden reported an all time high of 2.195 HFRS in 2007.

Environmental conditions at 862 geo-referenced sites of human PUUV exposure (in 1991 to 1998) in northern Sweden were extracted from satellite data derived from the MODIS sensor on the NASA Terra satellite and temporal Fourier processed to extract environmental signatures or finger-prints of seasonality, using middle infra-red, day- and night-time Land Surface Temperatures, and the Normalised Difference and Enhanced Vegetation Indices data. The HFRS and satellite data were used within a bootstrap, non-linear discriminant analytical framework to produce risk maps of PUUV exposure.

Overall, kappa values were highly significant ranging between 0.763 (± 0.057) for the best ten models and 0.658 (± 0.108) for the worst ten models out of the 100 bootstrap samples. The equivalent figures for Sensitivity were 93.2% ($\pm 1.76\%$) and 89.93 ($\pm 3.48\%$) and for Specificity were 80.02 (± 2.94) and 70.77 ($\pm 5.86\%$) respectively. Elevation of the sample sites was the best discriminator of HFRS presence and was selected in 99 of the 100 models, followed by the mean day-time Land Surface Temperatures selected in 96 of the models. These models show that in Sweden the disease is present in areas of lower temperature and at lower altitudes compared with areas of disease absence.

How this combination of low temperature and altitude determines successful PUUV circulation and/or bank vole numbers requires further investigation. PUUV viability outside the bank vole is known to benefit from lower temperature. Low altitudes in northern Sweden imply less snow-cover, as compared to higher altitudes. Lack of snow may drive bank voles to dispersal, which can result in the invasion of human dwellings.

Epidemiology of hantaviruses in Slovenia

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Hemorrhagic fever with renal syndrome and hantavirus cardiopulmonary syndrome are complex human diseases characterized by different set of symptoms. Despite the differences in the course of both disease syndromes, the causative agents are hantaviruses. Hantaviruses are worldwide distributed zoonotic agents maintained in nature by evolving tight relationship with their specific rodent carriers. It is well known that each hantavirus is associated with a specific rodent host or few closely related host species. Additionally molecular evidence of hantavirus outside its primary host reservoir or outside closely related species exists, which poses a question whether the spillover events are far more common and important as previously thought.

In Slovenia the biannual rodent capturing has been conducted since 1990 and 4 different hantaviruses (Dobrava, Puumala, Saaremaa and Tula) were proven to coexist in a single region of endemicity. The

virological testing of patients with clinical signs of hemorrhagic fever with renal syndrome has begun in 1985 and since then 287 patients with HFRS have been identified. Despite 4 different hantaviruses circulating in local rodent population only Puumala and Dobrava virus have so far been confirmed to cause disease in humans in Slovenia. Although PUUV-associated HFRS is generally less severe than DOBV-associated HFRS, we observed that both viruses cause a wide spectrum of disease. On average there are about 10 HFRS cases per year, with slight variations depending on the rodent cycle and the masting year. In the year 2008 there was an increase in number of HFRS patients, with 46 cases, which was preceded by high abundance of rodents in autumn 2007. In addition to an increasing trend of HFRS cases we have also noticed, that there are more PUUV-associated cases every year. Biogeographical factors that could submerge to these findings are currently under investigation, but we believe that the changes in environment led to the invasion of bank voles into naive areas.

Genetic characterization of arenaviruses in small mammals in Europe

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Arenaviruses (family Arenaviridae) are enveloped negative-stranded RNA viruses that are associated with specific rodent reservoirs. In rodents, arenaviruses cause chronic infections, while in humans, they may cause hemorrhagic fevers. In Europe, the only arenavirus reported is lymphocytic choriomeningitis virus (LCMV), traditionally associated primarily with the common house mouse, *Mus musculus*. Humans infected with LCMV are usually asymptomatic or they show mild flu-like symptoms, but the infection may lead to aseptic meningitis, meningoencephalitis, and congenital abnormalities. LCMV or LCMV type viruses are circulating also among wild rodents, and several rodent species around Europe have been found seropositive in EDEN studies. Attempts to amplify arenavirus genome sequences from the wild rodents have been mainly unsuccessful, and only recently an independent lineage of LCMV was described from wood mice (*Apodemus sylvaticus*) captured in Spain (1). We have screened wild rodents for arenavirus genomes, and perform unbiased whole genome sequencing to recover full length genomes of arenaviruses from rodent tissue samples collected in EDEN.

As an example of our approach, wild rodents were trapped in Latvia and tested for arenavirus antibodies by using immunofluorescence assays (IFAs). From the study sites in which positive animals were found, 66 samples were chosen for screening by nested-RT-PCR targeting conserved motifs of the N gene. The collection of samples included both seropositive (32%) and seronegative rodents of the species *Myodes glareolus*, *Microtus arvalis* and *Apodemus* spp. LCMV-like genome sequences were recovered from seronegative bank voles. This is the first time ever when arenaviruses are reported from voles (Arvicolinae). Amplification of full genomes was attempted by high-throughput pyrosequencing. RNA extracts from rodent tissues were depleted of ribosomal-RNA, and transcribed to cDNA with a combination of random primers and primers targeting to the conserved viral termini. After target amplification, the libraries for the pyrosequencing were created, and subjected to unbiased sequencing.

The new sequencing technologies have proven to be a powerful tool for the discovery of pathogens. The genetic characterization of novel arenavirus genomes in wild rodents will now be facilitated by the use of these methods.

Phylogenetic analysis of Puumala hantavirus and its rodent host in northern Europe suggests no co-divergence on intra-species level

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Genus Hantavirus (Bunyaviridae family) includes negative strand RNA viruses carried by persistently infected species of rodents and insectivores. Puumala virus (PUUV), carried by bank voles (*Myodes glareolus*), is a major hantavirus pathogen that causes a mild form of HFRS in Europe. Here we present new sequences of PUUV recovered from the northern and central regions of Scandinavia, and comparative phylogenetic analysis of PUUV and *M. glareolus* genetic lineages from northern Europe. Our analysis shows that in that region representatives of the three bank vole phylogroups carry four phylogenetically distinct lineages of PUUV. Topological contradictions between phylogenetic trees based on the host and virus sequences suggest that genetic lineages of PUUV did not co-diverge with lineages of *M. glareolus* that carry them. Furthermore, co-phylogeny analysis and comparison of the divergence time estimates suggest that PUUV did not co-evolve with the bank vole, but was acquired via a host switching from the grey-sided vole (*M. rufocanus*) approximately 15 000 years ago. After the host switching, PUUV was most probably transmitted between the established genetic lineages of the new host during post-glacial recolonization of Europe.

Evidence for co-circulation of two Puumala hantavirus genetic lineages in a bank vole population in northern Finland

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The natural host of Puumala virus (PUUV) is the bank vole, *Myodes glareolus*. The bank vole is widely distributed throughout Europe, from the British Isles to the Urals, excluding some northernmost regions and the Mediterranean coast. Its range continues eastward into central Siberia (IUCN 2007). All along its distribution, the genetic variants of PUUV show phylo-geographical clustering. Bank voles have recolonized Fennoscandia after the last glaciation, 12 000 - 9 000 BP, from different directions. The Western European bank vole lineage, defined by mitochondrial DNA, was confined in Central European glacial refugium (Deffontaine et al., 2005). This lineage migrated to Fennoscandia through a land-bridge, at that time connecting Denmark and Sweden, and colonized southern Norway and Sweden (Björck 1995a,b; Jaarola & Tegelström 1996). The Eastern European bank vole lineage expanded from the environs of the Carpathian mountains towards northern Europe up to central Finland (Deffontaine et al. 2005; Kotlík et al. 2006; Deffontaine-Deurbroeck 2008). The Ural bank vole lineage moved from southern Urals over northern Russia towards north of Fennoscandia (Deffontaine-Deurbroeck 2008). The Ural lineage, migrating from the north-east, met the Western European lineage in North-Central Sweden-Norway and the Eastern European lineage in Central Finland establishing two contact zones (Jaarola & Tegelström 1999). It seems that each of the above mentioned bank vole lineages carried its own distinct genetic lineage of PUUV. As a result, three PUUV lineages are currently found in Fennoscandia: (1) the South-Scandinavian lineage presented by strains from south Norway and central Sweden up to the contact zone in North-Central Sweden, (2) the Finnish lineage that comprises strains from south-central Finland and Russian Karelia and (3) the North-Scandinavian lineage from northern Sweden. For the first time, two distinct main genetic lineages of PUUV, the Finnish and North-Scandinavian ones, have been found within a small sampling area, and within a single host genetic lineage, the Ural clade, at Pallasjärvi, in northern Finland.

Lung tissue samples of 171 bank voles were screened for the presence of PUUV N-antigen and 25 were found positive. Partial sequences of PUUV S, M, and L genome segments were recovered from these samples using RT-PCR. Phylo-genetic analyses revealed two main genetic groups of PUUV sequences that belonged to the Finnish and the North-Scandinavian lineages.

This presented a unique opportunity to study inter-lineage reassortment in PUUV; indeed, the 32% of the studied bank voles appeared to carry reassortant virus genomes. Thus the frequency of inter-lineage reassortment is comparable to that of intra-lineage reassortment in PUUV observed earlier (Razzauti et al. 2008). Of six possible reassortant S/M/L combinations, only two were found at Pallasjärvi and, notably, in all reassortants both S and L segments originated from the same genetic lineage suggesting a non-random pattern for the reassortment. These findings raise an interesting question: how and when PUUV genetic variants of FIN lineage were transferred to the Ural bank vole clade? Presently the PUUV FIN lineage extends 500 km into the range of Ural clade of *M. glareolus*, and the contact zone of main PUUV lineages is in the middle of the range of Ural clade of the bank vole.

RoBo pathogens recorded in northern Italy: a summary review

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The yellow necked mouse, *Apodemus flavicollis*, and the bank vole, *Myodes glareolus*, are widespread rodent species inhabiting forest biomes of EU. These species play an important role as reservoirs of a series of emerging zoonotic pathogens such as Hantaviruses, Lymphocytic Choriomeningitis Virus, Murine Herpes Virus, Cowpox virus and Ljungan virus. We performed cross-sectional studies on eleven sites across northern Italy, and a longitudinal study on a long term study site in the Province of Trento from 2000 to 2008. Rodents were trapped either using snap and live trapping. For each rodent standard biometric parameters were recorded and a blood sample and/or organs were taken for serological screening. The prevalence of all the pathogens screened was recorded and a summary of the results is reported. In some cases the prevalence of infection was lower with respect to other areas of central and northern Europe (Bennet et al. 1997; Chantrey et al. 1999; Vapalahti et al. 2003; Kallio-Kokko et al. 2005; Laakkonen et al. 2006). More in detail, for the intensive longitudinal study site, we report the results of the temporal dynamic of the Lymphocytic Choriomeningitis Virus within a population of yellow necked mouse. A significant correlation of the infection prevalence with rodent abundance was observed. At individual level, the transmission of this virus was horizontal, also showing an important role played by maturation and behavioural changes as well as social and spacing behaviour of this species (see Tagliapietra et al. 2009).

Transmission dynamics of PUUV hantavirus in its rodent host in boreal zone

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Nephropathia epidemica (NE), caused by Puumala hantavirus (PUUV), is most common in the northern boreal zone, where the host populations, i.e. bank voles (*Myodes glareolus*) show cyclic fluctuations of high amplitude. Here we present the results of our intensive, 7-year longitudinal study on PUUV transmission dynamics in a boreal bank vole population. In this study the animals were individually marked, their survival was monitored and infection status determined on each trapping session around the year, allowing the

determination the onset of PUUV infection. Monitoring was done once a month except in mid-winter when two month intervals prevailed.

The prevalence of PUUV showed seasonal variation, being highest in spring and lowest in fall. No density dependence was observed in PUUV transmission, as the prevalence was usually high in spring also in low densities. There was a steady increase in prevalence from fall to spring, during which period voles are non-breeding subadults and do not behave aggressively. The highest prevalences were observed after maturation in late spring, when up to 100% of voles can be seropositive. During the breeding season the prevalence decreased notably due to the influx of young voles into the population, reinforced by the presence of maternal antibodies in young animals (see also the abstract of Kallio et al.). Understanding the seasonal breeding and variation in the population structure is essential in Puumala dynamics. The cyclic phase can, to some extent, modify these basic patterns.

In several studies hantavirus infections have been associated more often with males than females, especially in Sigmondontinae and Neotominae. Our longitudinal intensive monitoring of marked individuals allowed an analysis of individual factors that promote the risk of acquiring PUUV infection by the following capture. Our results showed that in strongly seasonal boreal environment host sex is not a significant factor of infection risk, whereas the sexual maturity of the bank voles plays an important role. The individual infection risk appears to decrease markedly during the breeding season, which is probably a result of a high proportion of young individuals that are protected by maternal antibodies.

Detection and Characterization of a Novel Hepatitis E-like Virus in Wild Norway Rats (*Rattus norvegicus*) out of the sewer system in Hamburg, Germany

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Hepatitis E is a rare human disease in developed countries. It is caused by hepatitis E virus (HEV), which is most likely zoonotically transmitted to humans from domestic pigs and wild boars. Multiple reports on the detection of HEV-specific antibodies in rats suggested the presence of an HEV-related agent; however, infectious virus or a viral genome could not be demonstrated in these rodents so far. Here, a nested broad-spectrum RT-PCR protocol was developed capable of detecting different HEV types including those derived from wild boar and chicken. Initially, a screening of 30 faecal samples of wild Norway rats (*Rattus norvegicus*) from Hamburg (Germany) resulted in the detection of two sequences with similarities to human, mammalian and avian HEV. Virus particles with morphology reminiscent of HEV could be demonstrated by immune electron microscopy in one of these samples and the virus was tentatively designated as rat HEV. Inoculation of the samples onto three different permanent rat liver cell lines did not result in detectable virus replication as assayed by RT-PCR with cells of the fifth virus passage. Investigation of tissue from trapped sewer-rat individuals resulted in the assessment of the complete nucleotide sequence showing a typical genome organization of HEV. Phylogenetic analyses suggest this virus as separate HEV genotype, and real-time RT-PCR and immunohistochemistry investigations indicate a hepatotropism. The zoonotic potential of the virus and its possible future application in an animal model for human hepatitis E is discussed.

Long-term trends in geography of rodent cycles and hantaviral disease in Finland

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Nephropathia epidemica (NE), a mild form of hemorrhagic fever with renal syndrome (HFRS), is a rodent-borne disease caused by Puumala hantavirus (PUUV). PUUV is the most common hantavirus in Europe, and most of the HFRS in Europe are caused by PUUV. The host species, bank vole, *Myodes glareolus* is found in the whole of Europe, except in the very North and in Mediterranean regions. The bank vole is characterized by strong population cycles in Northern Europe, and by more stable seasonal dynamics, with occasional mast driven (good seed years of deciduous trees) outbreaks in temperate Europe, resulting in geographically different patterns of human epidemiology. We report here the long-term human patterns of NE in relation to the geographic cyclic patterns in the bank voles.

Finnish Forest Research Institute runs a country-wide rodent monitoring program, with trappings around Finland in spring and autumn. We know the general geographic patterns of rodent cycles in Finland.

From 1970s to late 1990s, the cycles were geographically clearly more asynchronous than since the late 1990s, and since then the 3-year vole cycles have been geographically synchronous over the whole southern half of Finland, within 150 000 km², resulting in regular NE epidemic years in the area where most of the human population live.

There are two clear trends in the Finnish NE data. The long-term increasing trend is apparently partly based on better awareness and diagnostics of the disease. The increasing trend is also clear in local data sets and in between-peak years. However, the most important feature is that at the national scale the human epidemiology has changed from a multi-annually rather stable pattern to a strongly cyclic one.

The change in NE dynamics is obviously due to the change in the geographic synchrony of vole fluctuations in Finland. In the southern half of Finland, where most human NE data come from, a vole cycle usually lasts for 3 years, though 4-year cycles are also known. From the 1970s until the late 1990s, the geographic coverage of a vole peak was not as extensive as it is presently. Earlier, a vole peak occurred simultaneously in western and south-central Finland, and a year later in southeastern Finland, etc. In the late 1990s, a change in geographic synchrony took place, and since then, the vole peak has occurred in the same year through most of the southern half of Finland from the western coast to the eastern border. As a result, the number of human cases that earlier were dispersed over 2 or 3 years in geographically different areas now occur in the same year in the whole area. The reason for this geographic change in the synchrony of vole cycles is not well known, but changes like this have taken place earlier and may therefore not be related to any present sudden environmental change.

The Finnish rodent monitoring serves as an excellent early warning system in a country with highest incidence of hantaviral disease in Europe.

Host specificity and genetic diversity of Arenaviruses in Morogoro, Tanzania

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Arenaviruses are primarily rodent-borne RNA viruses that include the etiologic agents of lymphocytic choriomeningitis and hemorrhagic fevers in humans. Specific Muridae species are the principal hosts of arenaviruses. In Africa, the multimammate mouse, *Mastomys natalensis*, is the reservoir host of the highly pathogenic Lassa virus in West Africa and Mopeia virus, for which human pathogenicity has not been reported, in Eastern Africa. In a previous study, a hot spot of arenavirus circulation has been described in Morogoro, Tanzania. This virus, Morogoro, is considered as a subspecies of Mopeia virus and it has been isolated from *M. natalensis* tissues. This virus seems a promising model to study virus-host dynamics and test rational rodent control measures for arenaviruses sharing *M. natalensis* as a host. However, before being used as a model, the degree of specificity of Morogoro virus for its host reservoir has to be assessed since secondary reservoir species may play a role in the transmission and maintenance of the virus in natural habitats. The purpose of this study was thus to determine the limit of specificity of the Morogoro virus in *M. natalensis*.

Between October 13-December 3 2008, 558 small mammals were trapped in Morogoro, Tanzania. Blood samples were tested for presence of antibodies against Morogoro virus by indirect immunofluorescent antibody (IFA) assay and for the presence of the virus by one step RT-PCR and sequencing targeting a portion of the L gene. A total of 61 blood samples from *M. natalensis*, 1 from *Lemniscomys rosalia* and 1 from *Mus minutoides*, showed positive results with the IFA test. With RT-PCR, we obtained positive results for 52 individuals, 50 *M. natalensis*, 1 *L. rosalia* and 1 *M. minutoides*. The sequences derived from *M. natalensis* individuals showed between 97.1% and 100% amino acid homology with the Morogoro prototype L sequence. In contrast, the 2 sequences derived from the blood samples of *L. rosalia* and *M. minutoides* showed only 69.3% and 65.2% amino acid homology with the Morogoro prototype L sequence. These 2 sequences were compared with sequences of other Old World arenaviruses. The viral amino acid sequence of the virus isolated from *M. minutoides* shows 93.7% homology with that of the Kodoko virus, which has been previously isolated from *M. minutoides* in Guinea. The amino acid sequence of the virus isolated from *L. rosalia* clusters with the sequence of the Ippy virus, which has been previously isolated from *Arvicanthis niloticus* in the Central African Republic.

In conclusion, in high-density habitats of *M. natalensis* where Morogoro arenavirus transmission occurs, sympatric murine species do not seem to be secondary reservoirs for the virus. In contrast, 2 murine species, *L. rosalia* and *M. minutoides* appear to be reservoirs of 2 other Old World arenaviruses, 1 of which may be a new species. Our study illustrates that African arenaviruses may be highly diverse and demonstrates the efficiency of the recently developed pan-OW arenavirus RT-PCR for identifying new Old World arenaviruses.

Genetic determinants of rodent species susceptibility to hantaviruses: Insights into integrins.

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Recent investigations in rodent systematics and epidemiology have led to the discovery of numerous Hantavirus species in Asia. As seroprevalence are very low, the screening of thousands of rodents is required to genetically identify these hantaviruses and to determine their human pathogenesis and their rodent reservoirs. Pathogenic and non-pathogenic hantaviruses use different integrin receptors to enter cells. In particular, human integrins $\alpha IIa\beta 3$ and $\alpha v\beta 3$ can mediate cellular entry of hemorrhagic fever with renal syndrome (HFRS)- and hemorrhagic pulmonary syndrome (HPS)-causing hantaviruses. In contrast, non-pathogenic or low pathogenic hantaviruses were indicated to enter the cell via integrin $\alpha v\beta 1$. We tested the hypothesis that variation in $\beta 3$ chain integrin sequences could provide keys to determine a priori i) the possibility for a rodent species to carry a hantavirus or ii) the susceptibility to hantavirus of an individual rodent. We sequenced 330 bp of the Plexin-Semaphorin-Integrin domain of $\beta 3$ chain integrin, for 62 individuals of 15 Asian rodent species. We found 60 variable sites and a high level of divergence between species (until 10%). Although the genetic variation reflected the neutral phylogeny of these species, we found one site differing between rodent species carrying pathogenic hantaviruses and non-reservoir rodent species. We also detected one variable site that was under positive selection, but only in rodent species that carry pathogenic hantaviruses. This site did not exhibit any genetic variation in rodent species that do not carry any hantavirus. In consequence, it is likely that knowing the amino-acid present at this position for a given rodent species could help us determining a priori whether this species is a reservoir of pathogenic hantaviruses. Further investigations are required to examine whether amino-acid changes at this position could mediate conformational changes that would prevent the fixation of pathogenic hantaviruses.

The role of migration, drift and selection at immune genes in the epidemiology of the Puumala hantavirus in a bank vole metapopulation in Finland

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The increasing number of (re)-emergent zoonoses emphasizes the importance of studying immunogenetics in wild animal reservoir populations. This approach may provide key information on the immune gene factors influencing the geographic distribution of zoonotic agents and the risk of emerging infectious diseases in humans. Our project focuses on the mild form of hemorrhagic fever with renal syndrome (HFRS): nephropathia epidemica (NE), in Europe. This infectious disease is caused by Puumala virus (PUUV), a hantavirus carried by bank voles (*Myodes glareolus*). The timing of disease outbreaks seems to be associated with peak phases of fluctuations of rodent population densities. Besides, neutral and selective pressures acting on immune genes involved in PUUV / *M. glareolus* interactions are likely to influence virus transmission, but also PUUV virulence through local adaptation phenomenon. We studied the role of evolutionary forces acting on bank voles and affecting PUUV epidemiology within a metapopulation of Central Finland (Konnevesi) exhibiting three year density cycles. We analysed the population genetics of *M. glareolus* between two outbreaks: 2005 and 2009. We genotyped bank voles at 19 neutral microsatellite markers and at one immune gene previously shown to influence the susceptibility to PUUV infection (Drb, Mhc class II gene). The neutral genetic pattern observed at microsatellites will allow to determine the gene flow and drift occurring within the bank vole metapopulation, both in space and time. The comparison of kinship and gene flow estimated for PUUV infected and non-infected voles will provide information on the modes of PUUV transmission. We will compare these spatial and temporal genetic patterns observed at neutral microsatellites with those obtained at the Drb immune gene. Contrasted patterns will reveal selection acting on the Drb gene. Associations between PUUV infection and Drb polymorphism, as well as previous results obtained on the genetics of PUUV in this area will be used to infer the influence of Drb polymorphism on PUUV epidemiology and local adaptation.

Shedding dynamics of Puumala hantavirus by the bank vole

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Puumala hantavirus (PUUV) is carried by bank voles (*Myodes glareolus*) and is spread via contaminated excretions. Although the rodent host is chronically infected, little is known about the shedding of the virus to the environment. Understanding the shedding patterns is crucial for modelling the transmission dynamics and human infection risk.

In the first part of the study, we subcutaneously inoculated colonized bank voles with PUUV (strain Kazanwt) and sampled excretions until day 133 post infection. Levels of shed viral RNA peaked within 11-28, 14-21 and 11-28 days post infection for saliva, urine and faeces, respectively, and the virus was found in excreta 80 days post infection. In contrast, blood still contained viral RNA in five out of six animals at day 133 post infection, indicating that bank voles secrete virus only during a limited time of the infection. Intranasal inoculations with bank vole saliva, urine, or faeces, were all infectious for naïve bank voles, indicating that all these three transmission routes may occur in nature and that rodent saliva might play a larger role in transmission to humans than previously believed. The relative importance of direct (via saliva through biting) and indirect (via aerosolised excretions) transmission routes may depend on the season and breeding status of the voles, as the behaviour of bank voles depends essentially on maturation status, and in breeding voles on sex. Aggressive behaviour and biting is primarily found among breeding males in summer.

To compare our laboratory results with PUUV shedding dynamics in nature, we studied the shedding patterns in naturally infected, wild bank voles in field. Based on local (Konnevesi) PUUV gene sequences, the RT-PCR was redesigned and optimized. Individually marked voles were captured in monthly intervals, and saliva, urine, faeces, and blood samples were drawn on each capture. The onset time of PUUV infection was approximated by detection of PUUV-specific antibodies in blood samples, and consequent shedding

patterns in excretions were analysed. This procedure allowed us to study the shedding patterns in different seasons and in different functional (maturity) groups.

Epidemiological role of *Apodemus agrarius* (Rodentia) in Slovakia

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Striped field mouse (*Apodemus agrarius* Pall.) occurs especially in the Eastern and Northern Slovakia. Long-term study confirmed that this species is presently abundant in a broad habitat spectrum. The distribution history of *A. agrarius* in Slovakia is characterised by distinctive changes, and the species has evidently spread into its present range during last forty-fifty years.

About 1400 specimens of *A. agrarius* in lowlands of Slovakia were caught during 1996-2006 to evaluate its epidemiological role. We present serological and microbiological analysis of blood serum and other tissues on presence of many pathogens (*Chlamydia* sp., *Leptospira* sp., *Borrelia* sp., *Toxocara* sp.). Antibodies against *Chlamydomphila* (*Chlamydia*) *psittaci* were confirmed in 15.3 % of rodents, the chlamydial antibody titres ranged from 1:16 up to 1:512.

Smear slides from approximately four hundred animals were examined for the presence of blood parasites. They were detected in 18,9 % of *A. agrarius* – *Trypanosoma* sp. (prevalence 12.7 %), *Bartonella* sp. (7.3%), *Babesia* sp. (0.8 %).

More than 800 *A. agrarius* animals were examined microscopically and about 900 serologically for *Leptospira*. Microscopic agglutination test showed seroprevalence of 16.4 % (151/800 animals), Antibodies were found against five *Leptospira* serovars, most frequently *pomona* (74.8 %), next *grippothyphosa* (11.9 %), *sejroe* and *australis* (both 4.0 %) and *bataviae* (0.7). Seven samples had mixed infections. Through cultivation of kidney sections, 116 strains of *Leptospira* were isolated (13.6 of host prevalence), the serovar *pomona* in 82 animals (70.7 %), the serovars *grippothyphosa* in 23 (19.8 %), *sejroe* (6; 5.2 %), *australis* (4; 3.4 %) and *bataviae* (1; 0.9 %). Our study has confirmed earlier knowledge that *A. agrarius* is the main reservoir of serovar *Leptospira pomona* in natural foci of diseases.

Serological and molecular analysis confirmed the circulation of a hantavirus, borreliae and *Anaplasma phagocytophilum* in Slovakia.

Additionally, 102 individuals of *A. agrarius* were analysed for gastro-intestinal helminths in 2007. The helminth prevalence was 78.4 %. A total of 67.6 % of mice were infected with nematodes, while the prevalences of cestodes and trematodes were 32.4 % and 3.9 % respectively. No Acanthocephala were detected. Nine helminth species were found: *Brachylaemus recurvus*, *Plagiorchis elegans* (Trematoda), *Hymenolepis diminuta*, *Mesocestoides literratus* larvae, *Passerilepis crenata*, *Rodentolepis fraterna* (Cestoda) and *Ganguleterakis spumosa*, *Heligmosomoides polygurus*, *Syphacia stroma* (Nematoda). The most frequent parasites were *Heligmosomoides polygurus* and *Syphacia stroma*, the prevalences were 45.1% and 31.4 % respectively.

During 2001 – 2008, a total of 1320 individuals were serologically examined for *Toxocara* sp. Seroprevalence was 11,8 %. Long-term study confirmed the higher *Toxocara* antibody level as well as seroprevalence than other rodent species. Seroprevalence was highest in spring, decreased in summer and increased in autumn and winter.

Long-term studies of rodents in Slovakia confirmed that *Apodemus agrarius* plays an important role as reservoir of many pathogens in natural foci of zoonotic infections.

Acknowledgements. The study was partially supported by EU grant GOCE-2003-010284 EDEN, project VEGA No. 2/0043/09 and 2/0137/10.

Leishmaniasis

Leishmaniasis in southern Spain: changes over the past two decades and risk factors

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Southern Spain is a good area for studying changes in the prevalence of canine leishmaniasis (CanL) in a changing environment, because long-term quality data on leishmaniasis caused by *Leishmania infantum* and its sandfly vector are available.

We conducted a cross-sectional seroprevalence study and a survey from April to June 2006, in order to examine prevalence changes and risk factors for CanL. We then compared current CanL seroprevalence data with data from two cross-sectional surveys conducted in 1984 and 1991 (J. Martín-Sánchez et al., 2009. *Emerging Infectious Diseases* 15, 795-8). The villages sampled for all 3 studies were similar and had been selected at random from within each of three bioclimatic zones: thermo-, meso- and supra-Mediterranean.

Sandfly sampling was undertaken at 7 sites on a monthly basis (from April to October) in 2005, in order to investigate changes in vector density. Sandflies were captured using sticky traps which were left at the capture sites for one week. The sandfly densities were compared with data obtained in 1978 at the same locations. The density variations were then compared with the temperature variations recorded over these years.

We constructed a risk map for exposure to *P. perniciosus* that could be used in leishmaniasis control programmes. For this, sandflies were captured in the month of July with sticky-paper traps which were placed in 170 sandfly resting sites for a period of 4 days. From each sampling site, geographical, environmental and ecological data were assembled using a GIS and notes taken in situ using a PDA (independent variables). We carried out uni- and multivariate logistic regression studies with all the independent variables to model the presence/absence of *P. perniciosus* as our dependent variable.

Findings: Over the past 22 years, CanL seroprevalence has progressively increased at the meso-Mediterranean zone. CanL risk was highest for dogs that were older (>4 years), larger (>25 Kg), worked as guard dogs or sheepdogs, slept outside, and lived in a village where the *P. perniciosus* density was > 4 sandflies/m². Over the past 25 years, the densities of *P. perniciosus* and *P. ariasi* have remained constant in the thermo-Mediterranean zone and have fallen in the meso-Mediterranean zone. If the monthly results were examined, however, *P. perniciosus*-*P. ariasi* density was found to increase in the month of May in the meso-Mediterranean zone and in September in the thermo-Mediterranean zone. The average monthly temperature variation was negatively associated with the variation in density, with the bioclimatic zone having an influence ($p < 0.05$). The main vector in the area, *P. perniciosus*, had increased its activity period at some trap sites, while the season of activity of *P. ariasi* ended earlier at some trap sites.

This work was partly funded by EU grant GOCE-2003-010284 EDEN (www.eden-fp6project.net).

Potential role of *Phlebotomus halepensis* in the transmission of cutaneous leishmaniasis: faunistic studies in two endemic sites in Turkey

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Both human cutaneous leishmaniasis (CL) and human visceral leishmaniasis (VL) are prevalent in Turkey. The studies on the sandfly vectors have been carried out in different endemic provinces in the Aegean, Mediterranean, Marmara, East, Middle East and Southeast Anatolia regions, revealing a total of 21 different *Phlebotomus* species and 4 *Sergentomyia* species. *Leishmania tropica* was found to be the causative agent of CL, and *P. sergenti* its vector, in the hyper endemic areas located in the Southeast and East Mediterranean

regions. However, in preliminary studies, *P. halepensis* was found to be the dominant sandfly in two villages of Afyon and Nigde cities, where CL is endemic. In the present study, we aimed to determine the sandfly fauna in all endemic areas in Afyon and Nigde provinces and to investigate the vectorial role of *P. halepensis*.

The field work was carried out in August 2008 and July 2009 in three villages of Nigde and in 5 villages of Afyon provinces with CL cases caused by *L. tropica*. The predominant species was found to be *P. halepensis* (40-96%), indicating a possible vectorial role in these endemic areas. Other *Phlebotomus* species included: *P. papatasi*, *P. neglectus*, *P. balcanicus*, *P. perfiliewi* and *P. simici* in Afyon province and *P. papatasi*, *P. sergenti*, *P. simici*, *P. mascittii*, *P. neglectus* and *P. alexandri* in Nigde province.

This work is a part of an ongoing PhD thesis aimed at investigating the vectorial role of *P. halepensis*. The study will further focus on isolating *Leishmania* parasites from sandflies using microscopy and real-time PCR.

This work was funded by EU grant GOCE-2003-010284 EDEN (www.eden-fp6project.net).

Molecular Taxonomy of *Phlebotomus neglectus* and *P. syriacus*, vectors of *Leishmania infantum*

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Within the subgenus *Larroussius*, the taxonomic status and distribution of two species of the *Phlebotomus major* complex were studied. While *P. neglectus* is a proven vector of *Leishmania infantum* as well as of Phleboviruses, *P. syriacus* is assumed to be a probable vector on circumstantial evidence, namely, its distribution, biting habits and taxonomic position.

Previously described species-specific morphological characters of various populations of *P. neglectus* and *P. syriacus* were compared for specimens from different localities in Italy, Croatia, Montenegro, Albania, Hungary, mainland Greece, Crete, Turkey, Syria, Lebanon and Israel. Furthermore, the populations were studied deploying several molecular biology techniques, namely RAPD analysis and comparative sequencing analysis of nuclear (internal transcribed spacer 2) and mitochondrial (cytochrome b, cytochrome oxidase I) markers.

Classical morphological characters proved to be useless for species determination. However, all the molecular methods confirmed the general conclusion that *P. neglectus* is present in the north-central part of the Mediterranean (Italy, Balkan countries) while *P. syriacus* is confined to the eastern part. Turkey is presumably the country that harbours the border between the two species. However, the taxonomic status of populations from different parts of Turkey remains to be resolved. Moreover, a detailed survey revealed a sympatric occurrence of both species in Crete as well as a possible existence of a new cryptic species. As neither of the studied species had been recorded from Cyprus - an island in a geographical position that would favour their presence - an entomological survey was carried out in both southern and northern Cyprus. Since proper morphological identification of these two closely related species from certain areas of distribution is impossible, it is desirable to develop a set of molecular markers which would readily distinguish them. Such diagnostic tools would be highly useful for rapid and routine species identification and distribution assessment. In order to develop such tools, two approaches were deployed: a multiplex PCR assay and a PCR-restriction fragment length polymorphism (RFLP) assay.

Susceptibility of sandfly vectors for *Leishmania donovani* strains emerging in the Mediterranean

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Leishmania strains belonging to the *L. infantum/donovani* complex have a wide spectrum of clinical forms, and manifestation of the disease is often dependent on the parasite genotype. Recently, we described a focus of cutaneous leishmaniasis in Çukurova region (South Anatolia, Turkey). Small, non-ulcerating lesions prevailed and patients were negative in rK39 tests for antibody detection for human visceral leishmaniasis. The most abundant sandfly species, *Phlebotomus tobbi*, was found to be positive for *leishmania* promastigotes with a prevalence of 1.4% (13 out of 898 dissected females). The high number of CL patients (up to 10% in some villages), relative scarcity of dogs and frequent feeding of *P. tobbi* on humans suggests that the transmission cycle could be anthroponotic (*Int J Parasitol* 2009, 39, 251-6). MLST and MLEE analysis of isolates from patients (CUK1) and sandflies (CUK2 and 3) revealed the new *L. donovani* zymodeme MON-309. We tested development of this emerging *L. donovani* strain in sandflies. CUK3 isolate was compared with a viscerotropic strain of *L. infantum* from Turkey. Females of two major *L. infantum* vectors, *Lutzomyia longipalpis* and *Phlebotomus perniciosus* were infected by membrane feeding. Localisation and intensity of the infection were evaluated under a microscope two and eight days postinfection, and parasite numbers were estimated by qPCR (SYBR Green). Both *Leishmania* strains were able to produce heavy late-stage infection in *P. perniciosus* and *L. longipalpis* and colonize their stomodeal valve. The q-PCR showed that in both sandfly species the infection intensity was significantly higher.

Mapping risk areas for visceral and cutaneous leishmaniasis related to the distribution of vector species in the western part of Turkey using Geographical Information Systems

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Human leishmaniasis is present in two clinical forms, visceral and cutaneous, in Turkey. While the annual number of recorded cases is only around 50-60 for visceral leishmaniasis (VL) and the disease is seen in every region of Turkey, it is > 2000 for cutaneous leishmaniasis (CL) and the disease is spreading throughout the country. The aim of the present study was to carry out an entomological survey and produce leishmaniasis risk maps for the probable sandfly vectors in western Turkey.

For determining the vector species, an entomological survey was carried out in Kusadasi town and rural areas of Aydin province, where VL, CL and canine leishmaniasis (CanL) are endemic. The study area was 48x88 km² (divided in 66 squares of 16 km²) and at least one location in each square was sampled during the field work. Detailed ecological information was also collected for each location.

The results of entomological studies indicated that the probable vector species are *Phlebotomus tobbi* and *P. neglectus* for VL and CanL, and *P. similis* for CL. Maps were produced to show the distribution of vector species in the study area using a geographical information system (GIS) and then risk maps were developed based on the known distribution of these three species in the western part of Turkey and using multivariate and linear regression analyses. Altitude, aspect, Normalized Difference Vegetation Index (NDVI) and Land Surface Temperature (LST) values were used as explanatory parameters. Altitude and aspect were derived from the SRTM data set, while NDVI and LST values were calculated from Landsat TM and MODIS data of the study area. The methodology provided useful information for guiding control program interventions.

This work was funded by EU grant GOCE-2003-010284 EDEN (www.eden-fp6project.net).

First epidemiological and entomological studies of canine leishmaniasis in Kırklareli province, northwestern part of Turkey

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Human visceral leishmaniasis (HVL) and canine leishmaniasis (CanL) caused by *Leishmania infantum* are mainly observed in the Aegean and Mediterranean Regions of Turkey. The overall seroprevalence rate of CanL is 15.76% in Turkey. Kırklareli province is located in the northwestern part of Turkey and borders Bulgaria. The aim of this study was to carry out entomological and serological dog surveys in this province where CanL had not been investigated.

This study was carried out between 23 and 28 July 2007. The province is divided into four geographical areas and three or four villages were selected from each one. In these villages, physical examinations and blood sampling were carried out in a total of 131 dogs. Anti-*Leishmania* antibodies were detected by IFAT: two dogs (1.51%) were found to be seropositive (1/80 titre) in two villages, while 32 (24.42%) were considered as borderline (1/60 titre).

In order to determine the vector species, 15 CDC miniature light traps and 290 A4-size sticky papers were set up in seven villages. Only 48 sandfly specimens were collected: *Phlebotomus tobbi* (10.41%), *P. similis* (2.08%) and *Sergentomyia* species (87.7%).

These findings indicated that CanL could be being transmitted by known vectors of *L. infantum* in northwestern Turkey.

This work was funded by EU grant GOCE-2003-010284 EDEN (www.eden-fp6project.net).

QuantiFERON® (QFN) as an epidemiological tool for evaluating the exposure to *Leishmania* infection

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The diagnoses of visceral (VL) and cutaneous leishmaniasis (CL) are often made by the demonstration of amastigote forms of the parasite in aspirated materials, mainly from the bone marrow for VL and lesions for CL. The parasite demonstration and isolation rates are rather poor from cutaneous and mucocutaneous lesions due to low parasite load and high rate of culture contamination. There is a need for a simple and rapid diagnostic tool for the diagnosis of leishmaniasis especially for epidemiological studies examining large number of subjects. The Leishmanin Skin Test (LST) is still used for epidemiological surveys on the prevalence of leishmanial infection in some endemic regions of the World. But injection of antigen might cause some side effects and also certain social problems. A new screening tool is needed for epidemiological studies on leishmaniasis especially in Europe.

The aim of the present preliminary study was to investigate the potential of measurement of IFN- γ secretion by T cells into blood plasma using QuantiFERON® (QFN) assay with leishmanial antigens to determine exposure to *Leishmania* infection. Stored blood samples from 18 visceral leishmaniasis cases, 20 cutaneous leishmaniasis cases and 20 healthy controls were tested. Responses to *L. major* H2B and *L. infantum* H2B antigens were detected from the majority of treated VL cases but not from controls.

Establishment of a new assay for epidemiological screening might provide an alternative application of this tool for disease monitoring and even for evaluating the efficacy of vaccine trials.

Preliminary data on a seroepidemiological study of canine leishmaniasis in Tirana district, Albania, 2007-2008

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Objective: This study aimed to determine the seroprevalence of canine leishmaniasis among dogs with known owners in the district of Tirana.

Methods: During 2007-2008, a sero-epidemiological study for visceral leishmaniasis was carried out using the indirect immunofluorescence test (IFAT). 98 dogs were sampled from 17 places in the district of Tirana. Bio-statistical and geospatial methods were used for statistical analysis.

Results: Of the 98 samples tested by IFAT, 17.3% were positive at 1:160 cut-off. 61.1% of positive cases were asymptomatic and only 38.8% were symptomatic. Specific anti-*Leishmania* antibodies were detected in both sexes and no statistically significant difference was found between them. The highest specific percentages (10-30%) were observed among dogs aged 2-6 years.

Conclusions: Tirana district is an endemic zone for canine leishmaniasis, and the seropositivity of 61.1% means that there is a risk of infection to humans. Follow-up studies will extend the sampling and include isolation and identification of the parasite.

Current data about the geographical spread and the increase of the incidence rate of visceral leishmaniasis in Tunisia

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Visceral leishmaniasis (VL) rages in Tunisia in its Mediterranean infantile form. Until the late 1970s, the disease was restricted to the Northern districts of the country with less than 25 cases yearly. The aim of this study is to update the incidence rate and the geographical distribution of VL in Tunisia and to discuss the factors associated with its recent emergence.

In order to register the maximum of VL cases diagnosed between 1996 and 2006 in the country, most university and regional hospitals, particularly pediatric departments, were visited. For each confirmed case, epidemiological data, mainly the age and the home address, were noted. Data related to the Tunisian population were also collected, to calculate VL mean annual incidence rates (MAIR).

One thousand and ninety-six VL cases were registered. The mean annual incidence was 99.6 cases/year. Children under 5 years were obviously the most affected (82.6% of all cases). The mean annual incidence rate in this age group was 9.6 cases/100000, revealing an important and statistically significant increase compared to former studies.

Targeting the trypanothione pathway of *Leishmania*: metal compounds inhibiting trypanothione reductase

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Leishmaniasis is a disease characterized by high morbidity, which is deeply linked to malnutrition, humanitarian emergencies and environmental changes that affect vector biology. It causes an estimated 70,000 deaths annually, a rate surpassed among parasitic diseases only by malaria. Trypanosomatids have a unique thiol-based metabolism, in which trypanothione (N1-N8-bis(glutathionyl)spermidine) and trypanothione reductase (TR) replace many of the antioxidant and metabolic functions of the

glutathione/glutathione reductase and thioredoxin/thioredoxin reductase systems present in the host. TR is necessary for the survival of these protozoans and is considered among the best targets for drug development. Our group is focusing on the study of TR inhibition by metal complexes, with the aim of identifying lead compounds for developing new drugs against leishmaniasis.

Methods: We solved the crystal structures of TR complexes using Ag(I) and Auranofin, an anti-rheumatic agent. Native crystals of TR were soaked in solutions of AgNO₃ and Auranofin, respectively. The structures have been solved using datasets collected at the Synchrotron Radiation source BESSY (Berlin) at a resolution of 3.3 Å and 3.5 Å, respectively. We also carried out enzymatic activity experiments in the presence of metal-containing compounds, in order to identify the mechanism of action and the inhibiting properties of the compounds.

Major conclusions: Recently, we solved the structure of TR in a complex with Sb(III), disclosing the molecular basis of its inhibition and of its interaction with antimonials, the first choice drugs against leishmaniasis. Sb(III) binds to the catalytic pocket of the enzymes engaging the residues involved in the catalytic mechanism in a complex formation, thereby inhibiting TR activity. Sb(III) is bound to Cys52 and Cys 53, to His461' of the two-fold symmetry related subunit, and to Thr 335. The structure of TR in complex with Ag(I) shows that this metal also binds to the catalytic cysteines, with higher affinity with respect to Sb(III). A further bimetallic metal-binding site has been found at the interface between the two TR subunits, where one Ag(I) binds to Cys444 of both subunits and the second metal ion to Met447. The whole Auranofin molecule has been found in the pocket where trypanothione binds. Therefore, Auranofin inhibits TR with a dual mechanism, i.e. both by binding the residues involved in trypanothione reduction, namely Cys52, Cys57, and His461' of the 2-fold symmetry related subunit, and by competing with the binding of trypanothione to the pocket lined by Leu399, Ser464, Glu467 and Phe496. The identification of new TR inhibitors represents the starting point for drug development against leishmaniasis.

Responses of the sandfly *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae) to an oviposition pheromone associated with conspecific eggs

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The chemical ecology of *Lutzomyia longipalpis*, the South American vector of *Leishmania chagasi infantum*, has been extensively studied and this species complex is known to produce both sex and oviposition pheromones. By contrast, very few studies on the pheromones of Old World sandflies have been performed. Our preliminary studies have shown that female *P. papatasi* produce an oviposition pheromone.

Lipophosphoglycan-independent attachment of *Leishmania* in permissive sandfly species

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Depending on their ability to support development of one or a few strains vs. a broad spectrum of *Leishmania* parasites, sandfly species can be classified into two groups, termed specific or permissive vectors. We explored the molecular mechanisms underlying the permissiveness of certain sand -fly species. We followed the development of two *Leishmania major* glycoalyx mutants within different types of vectors. The lpg1-mutant, which specifically lacks lipophosphoglycan (LPG), was able to survive normally in two permissive species, *Phlebotomus argentipes* and *P. perniciosus*, but was only able to survive within the specific species *P. duboscqi* for a limited time prior to defecation of blood remnants. The lpg2- *L. major* mutant, which is a broader mutant and lacks all phosphoglycans including LPG and proteophosphoglycans, was unable to survive in all three vector species tested. These results gave supporting evidence for the existence of an LPG-independent mechanism for survival in sandflies. Using electrophoretic and

microscopical techniques we identified glycoconjugates displaying GalNAc residues localized on the microvillar border of the sandfly midgut as potential *Leishmania* ligands, as they bind to the promastigotes *in vitro*. We detected the presence of such glycoconjugates in the midgut of all the permissive species tested, while they were absent from the specific vectors *P. papatasi*, *P. sergenti* and *P. duboscqi*. In *Lutzomyia longipalpis* midgut, the candidate molecule is a 50kDa glycoprotein GPI-anchored in the membranes of the epithelial cells. This new binding modality has important implications for the co-evolution of the parasite with the vector, as it enables successful transmission of *Leishmania* in new permissive vectors.

Differences in the salivary effects of wild-caught versus colonized *Phlebotomus papatasi* (Diptera: Psychodidae) on the development of zoonotic cutaneous leishmaniasis in BALB/c mice.

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Several studies have shown that immunization of mice with saliva of *Phlebotomus papatasi* or their pre-exposition to uninfected bites provided a significant protection against *Leishmania major*, etiologic agent of zoonotic cutaneous leishmaniasis (ZCL). The main question is why people succumb to leishmaniasis particularly when non-infective bites are more common than infective ones? It is of major importance to point out that all these studies were performed with long-term laboratory colonized sandflies. Laboratory colonies of insects are often accepted as being representative of field populations from which they have been derived, but this assumption may not be valid, as colonies frequently incorporate only a fraction of the original populations' genetic variability. To date there are few published studies on the effects of saliva from wild-caught *P. papatasi* on ZCL development.

Pre-immunization of mice with salivary gland homogenates (SGH) of long-term colonized (F29) female *P. papatasi* induced protection against *L. major* co-inoculated with the same type of SGH. In contrast, pre-immunization of mice with SGH of wild-caught female *P. papatasi* did not confer protection against *L. major* co-inoculated with the same type of SGH. Similarly, pre-immunization of mice with SGH from recently colonized (F1) female *P. papatasi* did not protect mice against *L. major*.

The striking difference observed between the effects of SGH from long-term colonized and those of wild-caught or recently colonized sandflies is most probably due to the impact of long-term colonization. These results emphasize the inaccuracy of using long-term colonized material when making inferences concerning natural populations. It is better to examine directly wild populations of *P. papatasi*, and to avoid interpretation based solely on long-term colony material. These results suggest that a sandfly saliva-based vaccine should take into consideration the natural population variation of *P. papatasi*.

Rabbit-rearing as a zooprophylactic measure to control zoonotic cutaneous leishmaniasis

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Control of *Phlebotomus papatasi* is largely based on residual spraying of insecticides and impregnated-treated curtains or bed nets. The effectiveness of these methods is variable in addition to their potential for negative environmental effects. Habitat modification such as the destruction of rodent burrows is not cost-effective in rural areas where houses are scattered. Therefore, alternative methods for controlling sandfly populations are much needed. Zooprophylaxis is the use of animals to deviate vectors from humans, and we

hypothesized that increasing biodiversity in the peridomestic areas may reduce the contact between humans and sandflies and subsequently reduce the risk of acquiring zoonotic cutaneous leishmaniasis (ZCL).

The indoor abundance of *P. papatasi* in houses with rabbit holes in the peridomestic areas is significantly lower than the indoor abundance in houses without rabbit holes in their peridomestic areas. Introduction of rabbits in artificial underground holes in peridomestic areas reduced significantly the indoor abundance of *P. papatasi*. The ecological niche made around houses in endemic areas by creating active rabbit holes is attractive to *P. papatasi* and therefore it may deviate the vector from humans to rabbits. While rabbit holes are breeding sites for *P. papatasi*, rabbits are not competent reservoirs for *Leishmania major*.

This study showed the effect of biodiversity on a vector-borne disease like leishmaniasis. Our results suggest that zooprophyllaxis based on breeding rabbits in man-made underground holes located in the peridomestic areas is potentially a practical option for long-term control of ZCL in endemic areas. Such a method is welcomed by villagers, because it provides them with food, a source of income, and protects them from sandfly bites. A large community-based study is needed to evaluate the impact of breeding rabbits on the incidence of ZCL in rural endemic areas.

Punique virus: a novel phlebovirus related to Sandfly fever Naples virus, isolated from sand flies collected in Tunisia

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Sandflies are widely distributed around the Mediterranean and human populations in this area are exposed to sandfly-transmitted diseases, including those caused by phleboviruses. While there is substantial data in countries located in southern Europe, there is very little data available from North Africa. A total of 1,489 sandflies was trapped in 2008 in Tunisia from two sites, bioclimatically distinct, located 235 km apart, and identified via morphological criteria. Sandfly species consisted of *Phlebotomus perniciosus*, *P. longicuspis*, *P. papatasi*, *P. perfiliewi*, *P. langeroni*, and *Sergentomyia minuta*. PCR screening resulted in two pools positive for a novel virus, closely related to Sandfly fever Naples virus. Virus isolation was obtained from one pool. Genetic and phylogenetic characterization based on sequences in three genomic segments showed that it was a novel virus distinct from other recognized members of the species. This new virus was provisionally named Punique virus.

Leishmaniasis in Morocco: the current situation

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In recent years, several vector-borne parasitic or zoonotic diseases have (re)-emerged and spread all over the world, which has had major health, ecological, socio-economical and political consequences. Most of these outbreaks are linked to global and local changes caused by either climate change, human-induced landscape changes or the direct impact of human activities. Among these emergent health problems in Morocco, leishmaniasis is a zoonotic disease transmitted by sandflies belonging to the genus *Phlebotomus*. Dogs, rodents and humans are the main reservoirs of infection. There are several different forms of leishmaniasis. Common are cutaneous leishmaniasis (CL), muco-Cutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL). All these forms exist in Morocco. Most leishmaniasis patients are low income and live in poor socio-economic environments, which are often in remote rural locations.

Sporadic cases have been seen in different regions of Morocco, e.g. in 1993 about 40 cases were recorded in different parts of Morocco. Nowadays, disease incidence has dramatically increased, with an epidemic score of 2000 cases. The aim of this study was to investigate the extent of the problem caused by leishmaniasis,

and to map the geographic distribution of the three forms in Morocco. Also, intervention strategies will be outlined.

Vector studies of *Phlebotomus* species (Diptera; Psychodidae) in the Balearic Islands, Spain

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The Balearic Islands have been described as an endemic location for *Leishmania infantum* (Kinetoplastida; Trypanosomatidae), a parasitic protozoan transmitted by different species of the genus *Phlebotomus* (Diptera; Psychodidae). The prevalence of canine leishmaniasis is very high in Majorca and Ibiza (more than 50%), thus contributing to the spread of the disease in periurban environments. Despite the medical and veterinary importance of this disease, few studies on the species composition and seasonality of the vectors have been conducted. Hence, the main aim of these studies, conducted in 2003 and 2005, was to improve our knowledge about the populations of Phlebotominae in the Balearics.

In 2003, the collections of Phlebotominae were conducted by using two suction light traps. The traps were located in the municipality of Santa María (Majorca), where several cases of canine leishmaniasis had been detected in previous years. Traps were operated nightly and were placed near the resting places of dogs. All the collected Phlebotominae were counted and identified to species level. Sampling was conducted three times per week from September to December 2003. Furthermore, Phlebotominae were also identified from weekly collections made with 22 CDC traps (Black light U.V. John Hook Co.) placed in farms distributed in Majorca, Minorca and Ibiza during 2005. These farms and traps are included in the Entomological Surveillance Program for Bluetongue in the Balearics.

In 2003, 72 samples were collected from which 153 females and 146 males of Phlebotominae were recorded. Three species were detected in the area: *Sergentomyia minuta* (Rondani, 1843) never described as a vector of *Leishmania*, *Phlebotomus sergenti* (Parrot, 1917), a candidate vector for *Leishmania tropica*, and *Phlebotomus perniciosus* Newstead, 1911, which is known to be a major vector of *L. infantum* in the western Mediterranean Basin. The latter species was the most abundant, followed by *S. minuta* and *P. sergenti* (only one female). The peak of abundance of *S. minuta* and *P. perniciosus* occurred in September, and none were found in November-December.

In 2005, 1,086 samples were obtained from January to December. A total of 10,037 Phlebotominae were obtained. The species *P. perniciosus* was again the most abundant species (81.5%), followed by *S. minuta* (18.5%). The first detection of both species in the three islands was in March, whereas the last captures were obtained in late October. In Majorca, the peak abundance of *P. perniciosus* occurred between June and July, meanwhile in Minorca and Ibiza it occurred between August and September.

Impact of climatic changes and habitat degradation on Phlebotominae (Diptera: Psychodidae) distribution and leishmaniasis dispersion in Brazil

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We examined changes in the phlebotomine fauna and the spread of cutaneous and visceral leishmaniasis resulting from human intervention and climatic changes in the biomes of 5 regions of Brazil. Areas of natural undisturbed forest or highly degraded forest were compared for the overall abundance and richness of sandfly species. Overall abundances were higher in undisturbed forests (Amazonian and Atlantic Forest) with species richness greater than in degraded habitats. This seems to maintain a natural balance for

Leishmania infection in sylvatic animals. Moreover, several studies point to important changes, such as drought in the Amazon and extreme rainfall in the south of Brazil, as contributing to the spread of leishmaniasis foci. In disturbed areas a few sandfly species, mainly *Leishmania* vectors, are dominant but highly adaptable to this environment. In areas of visceral leishmaniasis, the sandfly fauna was dominated by *Lutzomyia longipalpis* (Lutz & Neiva), vector of *Leishmania infantum chagasi* (Cunha & Chagas), independent of its natural biome. The same is observed for *Nyssomyia intermedia*/*N. neivai* (vectors of *Leishmania braziliensis* (Vianna)), both with a tendency to predominate in degraded environments in South and Southeast Brazil. Species such as *N. whitmani*, another common vector of cutaneous leishmaniasis in less degraded areas, are now transmitting the disease in degraded environments in several regions of Brazil. The zoophilic species *Bichromomyia flaviscutellata* (Mangabeira), a natural forest vector of *Leishmania amazonensis*, may be spreading to new biomes and may represent a new threat to man.

Malaria

The decline of malaria in Western Provence (France) in the light of historical data

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Malaria was a common endemic disease in Provence by the 18th century and prevalent principally near the Rhône river and along the coasts around salt marshes and swamps. Moreover, during the second half of this century, moderate to major epidemics occurred relatively frequently but at irregular intervals. During these epidemics, the mortality rates shot up dramatically. Whereas analyses of historical sources suggest that this period was probably the worst affected by malaria, the decline of this disease began in the latter half of the 18th century and continued during the 19th century without any truly deliberate counter-measures.

The long term declining trend of malaria in Western Provence was compared to all the factors considered as possible explanations for disappearance of malaria in Europe. For this aim, the recent analyze of incidence of malaria in Provence and corresponding responses to this disease from the 16th to the 19th centuries^{1,2} and additional data collected from different sources were used. Moreover, although long series of climatological data are rare for this period, databases of homogeneous daily series of temperature (minimum and maximum) and rainfall over Marseille since the middle of the 18th century, and over Avignon since the beginning of the following century are available^{3,4}. The correlation between temperature and rainfall alone or taken together, and the number of outbreaks has been tested on annual, seasonal and monthly levels. Moreover, analyses of the number of consecutive days during which the average temperature was less than or equal to 0°C or greater than or equal to 24°C could also play a deleterious role, as well as civil engineering works after Rhone floods. A correlation between famines and malarial outbreaks has also been observed and the inter-relationship of malnutrition and malaria is generally accepted as being synergistic, the one promoting the other. The severe winter of 1708-1709, which was a time of cold record, has occasioned famine and malignant fevers in all the French Kingdom. To date, the various factors known to be able to play a role in malaria extinction (use of quinine, changes in environmental sanitation, economic and demographic changes, etc…) give inconclusive results. In addition, the mosquito populations in this case can be ruled out as an explanatory factor in the extinction process of malaria. Indeed, whereas the Anopheles species which transmitted malaria will probably remain unknown owing to absence of biogeographical data, studies of ancient DNA from human bones could help to determine the Plasmodium species and strains implied in the past malarial outbreaks and endemicity in Provence.

In Provence, the indigenous epidemic outbreak of malaria seriously faded out during a short period at the end of the 18th century, with limited or no counter measures or medication. This represents one of the very few opportunities for detailed studies of natural malaria dynamics, which aim is to understand the factors involved in the extinction of malaria outbreaks in a temperate country. The knowledge of these factors might help modern strategies against Plasmodium.

Potential malaria anopheline vectors of North Lazio (Central Italy)

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During 2007-2008 a longitudinal entomological survey was carried out in Central Italy, in the northern part of Lazio region belonging to Viterbo province and bordering on Tuscany and Umbria regions. This study area includes a narrow coastal plain (the natural southern continuation of Tuscan Maremma) extending from Tyrrhenian Sea to the hill slopes, and an internal hilly area. Data on distribution and bionomics of Anopheline populations, and also environmental parameters that could influence their dynamics were

collected and analysed. Furthermore, in 2008 a sporadic survey was carried out in Umbria region, in Terni province, to define the anopheline composition in an internal area.

Although many environmental changes have occurred in this region, the main potential malaria vector *Anopheles labranchiae* is still present.

Monthly entomological surveys were carried out from April to October in selected sites (eight farms with neighbouring potential breeding sites), placed in Viterbo, Monteromano, Tarquinia, Montalto di Castro and Tuscania municipalities. Indoor resting mosquitoes were collected in both animal shelters and human dwellings and subsequently identified in laboratory by morphological and molecular analyses.

A total of 867 specimens were collected belonging to 15 species. Out of total sample, 82.3% (N=714) were anophelines, 96.3% (N=688) of which belonging to the *An. maculipennis* complex and the remaining portion was identified as *Anopheles claviger*, *Anopheles petragani* and *Anopheles plumbeus*. To identify all specimens of *An. claviger* complex were used ITS-2 sequences, whereas 96% (N=661) of the *An. maculipennis* sample were recognized by egg-morphology and multiplex specific PCR. Only three species of this complex has been recorded: *An. labranchiae*, *Anopheles maculipennis* ss and *Anopheles melanoon*. The dynamics of anopheline species show seasonal trends differing in each site. In particular our results highlight that *An. labranchiae* is the most abundant species, representing 65.8% in 2007 and 74.4% in 2008, with respect to *An. maculipennis* ss (32.2% and 22.3%, respectively) and *An. melanoon* (2% and 3.3%, respectively). This so high difference in abundance of *An. labranchiae* in the eight collecting sites seems clearly related to the kind of local environment, climatic parameters and anthropic activities.

As expected, along the four coastal sites this species results predominant, while *An. maculipennis* ss is the most abundant species in the internal areas. However our findings confirm that *An. labranchiae* has a range wider than supposed in the past, being this species also detected in hilly areas, as previously showed in Tuscany (Di Luca et al, 2009). In 2007 *An. labranchiae* adults (11/92) were recorded in Viterbo (the inner site of the study area) and in 2008 (3/61) in Umbria (Borgheria site).

This study aims to firstly characterize the mosquito fauna in North Lazio in order to bridge a gap due to a lack of faunistic and ecological data in several areas of Central Italy, where malaria was endemic up to the 1950's. The assessments of further biological (blood meal analysis, parity rates etc.) and ecological factors involved in determining of mosquito biodiversity, along with more detailed data on environmental, climatic and social condition changes, are in progress.

This research was partially founded by EU grant GOCE-2003-010284 EDEN.

Entomological studies on anopheline mosquito (Diptera: Culicidae) fauna belonging to two former endemic malaria areas in order to assess the possibility of malaria re-emergence in Romania

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Malaria was officially declared eradicated in Europe in 1975 but its former vectors, mainly members of the *Anopheles maculipennis* complex, are still being distributed all over the continent.

Romania is a free-malaria country since 1967 with about 300 000 clinical malaria cases per year before the eradication campaigns.

Our study was carried out between 2005 and 2009 in two former malaria – endemic areas (Comana - Giurgiu County and Razim – Sinoe lagoon complex, Danube Delta Biosphere Reserve) and intended to establish the composition of the anopheline fauna as well as the longevity of the various species in order to evaluate the risk of malaria re-emergence in Romania. About ten thousand mosquitoes belonging to *Anopheles maculipennis* complex were collected and identified. The presence of the former malaria vectors was pointed up: *Anopheles atroparvus*, *Anopheles messeae* and *Anopheles maculipennis* sensu stricto.

The comparative analysis of the present situation of the anophelism from the two former endemic malaria areas in connection with the historical data gathered up during the endemic malaria period indicate a low risk of malaria re-emergence in Romania.

Biology of *Anopheles multicolor* in OUARGLA (south of Algeria).

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A re-emergence of *Plasmodium vivax* malaria has been reported in Ouargla, a district of south of Algeria, in the 1990s where migrants from Sub Saharan countries increased these last years for economical region increasing its vulnerability in term of malarial potential. The aim of this purely entomological study was to determine which *Anopheles* species are living in this area and their vectorial capacity meaning the receptivity to malaria in this defined area. Adults and larvae were collected from June to December 2005 in Aïn Moussa, a small village at the northeast of Ouargla. Larvae were collected by classical dipping; adults were captured using CDC light traps only inside houses, by regular human landing catch (both inside and outside human houses), and by morning collection of females in resting sites, inside (houses and animal shelters) and outside. Information on monthly dynamics, feeding preferences, and biting cycle were collected. *Anopheles multicolor* Cambouliu (Diptera: Anophelinae) was the only one anopheline species collected. Our results indicated a significant anthropophily (64 %) and great monthly variation in parity rates (21 % and 57 % during dry and rainy months) and vectorial capacity (0.41 in dry months and 10.73 during the wet months for *P. vivax*). These observations support the assumption that this species could be considered as the potential vector of malaria in this region which is therefore both receptive and vulnerable.

Characterization and distribution of *Anopheles maculipennis* s.l. (Diptera: Culicidae) breeding sites in the Valencian Autonomous Region, Spain

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The Valencian Autonomous Region is a coastal region located in the East of the Iberian Peninsula whose climatic and environmental conditions are highly influenced by the Mediterranean Sea. Historically, it is one of the Spanish regions which has most suffered from malaria episodes until its eradication, in 1964. Since then, it is considered as an endemic area free of malaria (OMS, 1964), but after a few years without incidences, the tendency shows an increase in the number of imported cases. This trend, coupled with the presence of disease-born vectors, raises the need to establish preventive measures based on spatial and temporal monitoring of these efficient vectors.

The purpose of this study is to find out the distribution, evolution and behaviour of main malaria – transmissible anophelines species present in the Valencian Autonomous Region. Some species of *Anopheles maculipennis* (Meigen, 1818) play an important role in malaria transmission due to their particular biology, host preference and ability to breed in urban areas.

A sampling study on water collections located throughout the Valencian Region has been carried out for five consecutive years (2005 – 2009). The collection of samples was performed using the “dipping” technique (Service, 1993) on immature stages. The sampling effort was standardized in 10 minutes including the time for finding and collecting samples (Bueno Marí et al., 2008). Captured larvae were transported to the laboratory into cold – acclimatized bottles to maintain their viability. In the laboratory, samples were kept in a 70% alcohol solution and identified under binocular and optical microscope in accordance with taxonomic criteria of Encinas Grandes (1982), Romi et al. (1997), Schaffner et al. (2002) and Melero Alcibar (2004).

Each positive focus has been photographed, characterized, georeferenced and mapped using GPS and transferred to a distribution map divided into 10 Km² grids. Breeding sites have been characterized

employing a multi parameter analyzer that measures water quality based on physical & chemical parameters (temperature, conductivity, salinity, TDS, pH and RedOx potential). Each species has a tolerance range to these factors so small variations can help or hinder the establishment of some or other mosquito species.

Anopheles atroparvus (Van Thiel, 1927), the main malaria vector in Europe, shows more flexibility to environmental changes. Its ecological plasticity causes its presence both in rural and in urban areas. *Anopheles maculipennis* s.s. (Meigen, 1818) has less tolerance to variations in such factors so its distribution is limited to natural environments far away from human activity.

Contribution to the study of the biology of *Anopheles L. labranchiae* Fellorini, a malaria vector in Algeria

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Background : Before the 70's, malaria was one of the most important vector-borne disease and the main public health problem in Algeria. In the northern areas of the country, *Plasmodium vivax*, was prevalent and transmitted by *Anopheles labranchiae*, the main vector. After the malaria eradication program launched in 1968, the incidence of the disease has dropped significantly from 100 cases per 100,000 inhabitants in 1968, to 0,5 case per 100,000 inhabitants in 1976. In 1991, a *P vivax* epidemic was declared in the locality of Sidi M'Hammed Ameziane, North-West of Algiers, where 12 autochthonous cases were registered. This reminds once again that malaria could reappear in the north of the country when the transmission factors will be present and suitable.

Since the remarkable works of the Frères Sergent and coll on malaria and its vectors in Algeria, very few studies were realized, particularly in the East of the country where malaria constitutes a real threat.

The aim of this study is to report the main biological features of *An labranchiae* in the Tonga lake, a natural reserve, North-East Algeria, in which a significant eutrophication is noted since ten years. The impact of the eutrophication of this lake on the *An labranchiae* population's dynamics was also analyzed using satellite imagery and water pollution analysis.

Methods: Larvae of *An labranchiae* and adults mosquitoes were collected bimonthly, from March to November 2007 in and surrounding two lakes (Tonga lake and Oubeira lake, as control) using standard dipping method, light traps and human landing catch. The temporal and spatial growth of the lake's eutrophication were highlighted by satellite imagery taken from 1987 to 2006.

Results: *An labranchiae* was the only species of *Anopheles maculipennis* complex found in this area. It was less abundant in Tonga lake than in Oubeira lake. The seasonal dynamics of *An labranchiae* larvae shows two peaks in Tonga lake in May and in the end of September. However, in the Oubeira lake one peak is observed in June. In other hand, a clear trend of deterioration of water quality was observed in Tonga lake compared to Oubeira lake. The values of organic matters, nitrogen and phosphorus are significantly higher in the first one than in the second.

This study shows a possible association between the aquatic invasive plants such *Eichhornia crassipes* and other aquatic weeds with the decline of the density of malaria vector in Tonga lake.

Behaviour and social characterization of a human population from a malaria risk area in southwestern Europe

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In the forecasting and control of vector-borne diseases, understanding the behaviour and customs of humans living in risk areas becomes crucial. The aim of this study was to analyze and characterize the human population of an area of the Spanish Mediterranean coast, the Ebro Delta (Tarragona), which was a malaria endemic area until the mid-twentieth century. The presence of the vector *Anopheles atroparvus*, with a current density and distribution similar to that it showed during the malarial period (almost 10,000 *An. atroparvus* were captured in the entomological study developed in 2005), the increase of imported malaria cases in Spain (between 2005-2008 the study area received 5,610 immigrants from Africa, Asia and America) and future climate conditions confer this area a potential risk for the recurrence of malaria. Two groups of people were differentiated for social studies, residents and tourists, with different behaviours and habits. Depending on the number of inhabitants and the entomological studies conducted in the study area, three villages were selected. A total of 219 residents and 102 tourists were interviewed. The questionnaire included information on the annoyance degree of mosquitoes and the type of protection used, the time and type of work or activity performed, the knowledge about the disease and whether they had travelled to a risk area in recent years. A descriptive study of absolute and relative frequencies was conducted by means of the SPSS program. A total of 54.8% of the persons who responded the questionnaire did not consider the mosquitoes as a negative aspect of the area, although 25.6% were bitten often and 23.4% regularly. Sunset was the day time when the highest level of discomfort was reported (82.6%). The use of protection measures is very widespread (85%), primarily including the use of repellents (56%). Agriculture and construction are the main activities of the residents, who spend little time on leisure. On the contrary, tourists spend most of their time outdoors, also at dusk and at night. A high percentage (75.5%) was unaware of mosquito-related diseases but nearly 78.5% had ever heard about malaria. Finally, just 8.5% of tourists who visit the Ebro Delta had travelled to an endemic area in recent years.

This work shows that people who live or visit the Ebro Delta take precautions to protect themselves against mosquitoes; however, they ignore their vector role, a situation that represents some risk for insect-borne diseases. Therefore, health information campaigns should be added to mosquito control efforts and entomological studies as a part of future joint strategies for the forecast of these diseases. Tourists represent a risk group since they usually visit the area during the period of major anopheline density, namely July and August, in addition to their ignorance of the characteristics of the area, their habits and travels to endemic areas. Ebro Delta residents form a lower risk group because they know the area, avoid the contact with the insect and do not usually travel to endemic areas, although they are exposed to all risk factors for a considerably longer year period.

The status of *Anopheles labranchiae* in northern Africa based on DNA

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group in northern Africa is as to the taxonomic status of its junior synonym, Rabat, Morocco), which reportedly exhibits a much paler egg than morphological and behavioural distinctions, it has been suggested that Moroccan populations have been isolated from and that Moroccan populations may represent

The only reported member of the *Anopheles (Anopheles) maculipennis* species *Anopheles labranchiae* Falleroni. However, some confusion exists *An. sicaulti* Roubaud (type locality: near *An. labranchiae*. Based on *An. labranchiae* in other Maghreb countries by the Atlas Mountains, *An. sicaulti*, a unique geographical variant of *An. labranchiae*.

Herein, DNA barcodes (658 bp of the mitochondrial cytochrome c oxidase I gene [COI]) obtained from 89 2007 and 2008 were used to assess if Moroccan populations are genetically isolated from other Magreb countries by the Atlas Mountains, and whether there is molecular evidence to support the presence of more than one member of the *Maculipennis* Complex in the region. There was no evidence for speciation detected between Moroccan and Algerian populations, nor within populations in northern Morocco. Moreover shared COI haplotypes between Algeria and Morocco indicate ongoing gene flow between populations in these countries, suggesting that the Atlas Mountains are not a boundary to gene flow in synonymy of the same species in these Maghreb countries is important for malaria control. *An. maculipennis* s.l. collected in Morocco (n=79) and Algeria (n=10) in *An. labranchiae*. The *An. sicaulti* with *An. labranchiae* is confirmed. That *An. labranchiae* comprises

Malaria risk and environmental changes in Morocco

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Although malaria has been eradicated in Morocco since 2004, risk of malaria resurgence is remaining because the vectors are still abundant and parasite's reservoirs are regularly introduced. This risk increases with the environmental change and international travel.

To estimate this risk and to assess the impact of environmental changes on its evolution, we have studied the entomological risk of two villages of Larache province in the North West of Morocco, Boucharen situated at 300 meters away from rice fields and Beggara at 100 meters away from permanent swampy zones. This area is a former focus of malaria where the last autochthonous case was reported in 1995. Results of this study show that the entomological risk to *Plasmodium vivax* in the two villages was high. But in rice cultivation area, this risk was three times higher. It was remarkable in July August and September. Meanwhile, we have conducted a retrospective landscape analysis using multi-temporal satellite images at high resolution to quantify change in land-use and particularly in breeding sites. For that, we have used a series of three Spot images of various dates: 1995, 1998, and 2007. Results of this analysis showed that before 1998 the only one breeding site present in the area was the swampy zones. The extent of breeding sites increased in 2007 with the addition of rice field and the increase of irrigated cultivations for the profit of the uncovered soil. We demonstrated that malaria risk in this region was increased consequently to environmental changes, mainly economical and sociological changes. This study showed, also, a modification in the distribution of the anophelines species of Morocco. *An. d’thali*, desert specie, was found in a humid zone of the Rif chain in the north of Morocco. The analysis of climatic data of the region for 40 years shows that there is a tendency to decrease of precipitations and to increase of temperatures. The dry period extends over years: it went up from 6 to 8 months during 40 years. These climatic changes led to changes in the Moroccan ecosystem which became favorable to the development of new species.

The imported malaria situation and the surveillance strategies applied in Albania

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Malaria was a hyper endemic disease in Albania with a spleen and parasite rate 60% in 1938. The insecticide DDT was introduced in 1946 and sprayed on the interior of the houses. The national plan of malaria eradication was prepared in 1957 according to WHO recommendations. The surveillance of imported cases was established at that period. Finally the carefully agricultural, economic multifaceted program succeeded eliminating the disease in Albania in 1967.

Among the anopheles species mainly *Anopheles maculipennis* (typicus) Maigen, *A. sacharovi* Faver and *A. superpictus* Grassi present in Albania as described by Hacet (USA), Dr. Ashta and Dr. Adhami, are known as the vector of malaria disease. The environmental changes after 1965 such as the drainage and land-filling was accompanied with the decrease of density and spread of *A. sacharovi*, the most common vector of malaria in Albania. The construction of drainages and the spread of rice fields from Buna River to Konispoli field, created the conditions of an increase of *A. maculipennis* rate at that time. Meanwhile, the main vector in hills or mountainous areas, near the rivers in fields was *A. superpictus*. During seventies, 15 years after ceasing the DDT house spray, as the result of environmental changes, earth erosions, alteration water salinity or organic matter, pesticide pollution and the disappearance of vegetation in many areas, reduced the populations of *A. superpictus*. Mosquito control is increasingly difficult because of development of insecticide resistance in many species of Anopheles. The first experience of anopheles sensitivity to insecticides was described by Dr. Jorgji Adhami, in the various sites of the costal and lowland areas of Albania.

The first imported malaria case in Albania has been registered in 1962, and the number of cases reached 42 in 2009. 4 Albanian citizens are among them. Mainly or 57.15% (24 persons) are infected in Asia, China, India; and 35.71 % (15 cases) in Africa; only 1 (2.38%) case was infected in South America and 2 (4.76%) cases (Albanian citizens) were infected in their traveling in Africa, Middle East and Asia. The cases were above 20 years old and mainly males. *Plasmodium vivax* accounted 66.67% of the cases while *Pl. falciparum* 30.95% of them. Only one case mix infection (*Pl. falciparum* and *Pl. vivax*) 2.38 % has been recorded. May and July are the months when the disease has been diagnosed. The diagnose has been completed within 3 days in the 52.63% of the cases, within 6 days in 84% of cases and only in one case with *Pl. vivax* after seven month.

Malaria is a mandatory reported disease in Albania. It is part of the National Mandatory Reporting System. The density of mosquito species is increasing, especially in some local areas. Therefore a Country Vector Strategy Control and Efficient Diagnosis need to be established.

Malaria Outbreak Among The Illegal Foreign Workers In Felda Rubber Plantations And The Use Of Anti Malaria Chemoprophylaxis In Jempol District

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Introduction: Jempol district is situated in the east of Negeri Sembilan which is one of the fourteen states in Malaysia. It is the largest district in Negeri Sembilan with more the 70 percent of land use is for commodity based agriculture such as rubber and palm oil plantations managed by Federal Land Development Authority (FELDA). The increase in commodity price in 2008 caused an influx of illegal foreign workers in the Felda scheme plantation. The illegal workers, who were mostly Indonesian from malarious area such as Lombok, entered the country without medical check-up. As they were considered as illegal worker, they usually lived in a simple wooden hut inside the plantation and often not sleeping in the hut to avoid being detected by the

immigration officers. Most of the plantations were not well maintained and often fully covered with jungle and poorly accessible. To avoid being caught by the immigration officer they often having a night shift to tap the rubber. This lead to the increase of malaria cases among the illegal foreign workers and subsequently malarial outbreak in the Felda scheme of Lui Selatan 1, Serting Hilir 3 and Palong 13. Mass treatment (mass drug administration) of all or a large section of the population whether symptoms are present or not) has been carried out in the past, usually in conjunction with insecticide residual spraying, as a way of controlling epidemics. Analysis of 19 mass drug administration projects during the period 1932–1999 did not draw definitive conclusions because study designs were so variable

Methodology: A total of 94 malaria cases were detected in 2008 and 71 cases (75.5%) were from outbreak localities. The problem with free movement of illegal foreign workers, attitude of the foreign workers and minimal cooperation from the local contractor in screening the workers have contributed to the malaria outbreak problem in Jempol district. Other factors were the difficulty to implement residual spray and impregnated bed nets program among the illegal foreign workers due the environmental factors and accessibility. Moreover their night-working hours made themselves exposed to the mosquitoes bite. To break the local transmission of malaria infection among the illegal foreign workers in the outbreak localities, we introduced the administration of anti malaria chemoprophylaxis among the contacts of the malaria cases in the Felda scheme of Lui Selatan 1, Serting Hilir 3 and Palong 13.

Result: Subsequently no new malaria cases were reported from the affected area and the outbreak were declared free by early January 2009. Looking into relevant study, reduced transmission was also seen in one study, in Vanuatu, where the population concerned was relatively small, well defined and controlled which is in the similar situation (1)

Conclusion

In conclusion, the influx of unscreened illegal foreign workers due to economic factor poses new health threat to the Felda settlement population. Factors such as the natural environments for mosquitoes infestation, night working hours and accessibility lead to the difficulty to implement residual spray and impregnated bed nets program among the illegal foreign workers. Thus the implementation of anti malaria chemoprophylaxis had successfully break the local transmission of malaria is proven successful in a relatively small, well defined and controlled population as what among the illegal foreign workers in Felda rubber plantations in Jempol district, Negeri Sembilan.

Impact of human activities on the presence and abundance of *Anopheles labranchiae* (maculipennis complex), in Maremma Plain (Tuscany, Central Italy)

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Background: Recently some European countries have experienced the introduction/re-introduction of some Mosquito Borne Diseases, including malaria. Climate and environmental changes induced by human activities have been recognised as the major factors that may influence arthropod range distribution and the diffusion of these diseases. Maremma region, a narrow coastal plain hyperendemic for malaria up to early 1950’s, was selected to assess a possible reappearance of malaria in Italy [1]. In this region the former vector, *Anopheles labranchiae* is still present, often with relevant abundance, due to a number of breeding sites made available by human activity (ricefields). Moreover, in this area an autochthonous induced *Plasmodium vivax* case occurred in 1997 [2]. This paper examines the impact of anthropogenic environmental changes on the *An. labranchiae* abundance in Maremma region. The results of the

investigations, focusing on rice-field areas in Grosseto and Siena Provinces, are presented and discussed also related to the local socio-economic factors.

Methods: Six study sites were selected: 1) Val di Merse, rice-field, maize cultivation and swine rearing area; 2) Monte Antico, intensive farming area; 3) Ampio, horse-rearing, extensive sunflower and maize cultivation area; 4) Principina, extensive rice field and farming area; 5) Alberese, a wild area with cattle and horse rearing; 6) San Donato, extensive rice cultivation and cow rearing area. In 2006-2009 *An. maculipennis* s.l. females were collected in indoor resting sites and human-bait night collections were also performed. For each site a representative number of specimens was identified by egg-exochorion ornamentation and by Multiplex-PCR. Data on human activities, spatial and temporal distribution, demographic data, seasonal variation of inhabitants, tourist and immigration movements were also acquired. An analysis of 2000-2008 imported malaria cases in Grosseto and Siena Provinces was carried out. In 2007-2008 to assess the level of awareness of the resident and tourist population, about the presence and nuisance of mosquitoes and the malaria issues, a social inquiry was performed in site 3, 4, and in Grosseto town.

Results and discussion: Our findings suggest that the human activities by modifying the natural environment, may allow the colonization and the massive reproduction of *An. labranchiae* (i.e. rice-fields) or in other cases they may prevent its spread (reclamation and land management). Nevertheless the environmental profile of the study area is changing due to a gradual replacing of rice by corn and vineyards cultures, and to an increase of tourism activities. The inquiry showed that mosquito nuisance is considered as a serious local problem and the prevalent protection against bites are screened windows and doors. Agricultural labours, tourists and hunters resulted particularly exposed to mosquito bites. The knowledge of the respondents about malaria transmission is widespread. Immigration workers are mainly from East Europe and North-Africa. Imported malaria cases in Grosseto and Siena Provinces represented 10% of the all Tuscany cases (6% of the national cases).

Major conclusion: *An. labranchiae* surveillance has to go on, especially along the coastal Maremma Plain, where the epidemiologically relevant abundance of this species still represents a concern about the possible reappearance of malaria in Italy.

This research was founded by EU grant GOCE-2003-010284 EDEN

Fine structure of the female reproductive accessory gland and the spermatheca in anopheline mosquitoes (Diptera, Culicidae).

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Much of what we know about the ultrastructure of mosquito female genitalia is based on an *Aedes* model, while anophelines, the most important vectors of parasites to humans, appear to deviate from that model in several ways.

Although there are studies on the ultrastructure and physiology of the three spermathecae with their basal glands and ducts of *Aedes*, no data is available on the ultrastructure of the only one spermatheca and small female accessory gland of Anophelines. The accessory gland is a highly specialized organ opening into the apical portion of vagina with unpaired oviduct. It shows a number of functions in several dipterans, but in mosquitoes its ultrastructure and specific function has yet to be determined. Because it is believed to be secretory, one measure of its activity has been a change in the size of the gland that reflects the increased activity of the largely secretory cells that comprise it. Although the increase in its size correlates with certain periods of the gonotrophic cycle in *Aedes* and *Culex*, any significant increase in size that correlated with the reproductive cycle of *Anopheles gambiae* was identified. On the contrary, Detinova (1962) reported that the *Anopheles maculipennis* gland increased in size during ovarian development and decreased during oviposition.

In order to characterize the anofeline female reproductive system and to correlate morphological data with physiological events, we proposed to describe the possible differences among reproductive biology aspects of *An. maculipennis* ss and *Anopheles labranchiae* in comparison with *Anopheles stephensi*, by using of light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

At SEM, in all three species there are two ovaries, two lateral oviducts, a single spermatheca and a single accessory gland. As showed by LM, the spermatheca consists of spherical shell of pigmented cuticle, bounded by a layer of very thin epithelial cells and bearing a plaque of thicker glandular cells at the posterior pole. The cuticular shell contains many small understained areas which give it a perforate appearance. The gland is globular in shape and has a thin duct which empties into the vagina near the opening of the spermathecal duct. *An. stephensi* shows the smallest gland, *An. maculipennis* the largest, while *An. labranchiae* has intermediate size gland. The effect of bloodmeal on the size of this organ during the gonotrophic cycle of *An. labranchiae* was also investigated. For LM, the glands from females at different times before and after blood-feeding (unfed, engorged, half-gravid, gravid and after oviposition) were surgically removed. A significant growth of the accessory gland was observed immediately after the bloodmeal, but not at subsequently digestion steps. After deposition the accessory gland decreases in size, but remains bigger than in nulliparous female. At TEM, the cellular and sub-cellular organization of accessory glands of the three species was also described in detail.

In order to clarify the morphology of the glandular epithelium and to elucidate the functional significance of the secretory process in the reproductive biology of Anophelines, the present study provides the first detailed descriptions of spermatheca and accessory gland ultrastructures.

This research was partially founded by EU grant GOCE-2003-010284 EDEN

Environmental and climatic analysis for mapping the potential malaria vector, *Anopheles labranchiae* (Diptera: Culicidae) in Maremma Plain

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Although the WHO officially declared Italy free of malaria in 1970, the re-emergence of this disease remains a potential risk in some areas of the country, related to the presence of former vectors (mainly belonging to the *Anopheles maculipennis* complex) and to the presence of yearly imported malaria cases and, consequently, of gametocyte carriers. The autochthonous introduced case in Grosseto province occurred in 1997 transmitted by an indigenous *Anopheles labranchiae* (Baldari et al. 1998), together with the predicted climatic and environmental changes, has increased the concern of health authorities over the possible resurgence of this disease in our country, and in particular in Maremma Plain. In this area, that extends from Tyrrhenian Sea to the internal hilly area, including a narrow coastal plain between Tuscany and Lazio regions, malaria was hyperendemic up to 1950's. There, epidemiologically relevant densities of the former vector, *An. labranchiae*, are still observed. A wide variety of habitats, mainly rice-cultivations, but also wetlands, marshes, ponds, irrigation and drainage canals provide favorable breeding sites for reproduction of this species.

To characterize the spatial distribution of *An. labranchiae* and to assess how the environmental and climatic factors may influence its occurrence and its seasonal fluctuations in Maremma Plain, remote sensing techniques were used.

In addition, in order to explain the *An. labranchiae* population dynamics and to forecast their densities with climate changes a statistical model able to correlate entomological field data with several climatic variables was developed.

In order to assess the presence and abundance of anopheline vectors in the study area, longitudinal surveys were carried out: in four selected sites located in Tuscany during 2005-2006 and in eight sites in Lazio during 2007-2008. Geostatistical analysis through the use of GIS software and its module of Spatial Analyst were carried out on shape and raster data of land use, hydrography, presence of livestock and *An. labranchiae* collection data in order to identify the potential breeding sites and to assess the abundance of adults mosquitoes. Maps with spatial distribution of larvae and adult presence were produced following the approach of Tran et al. (2008). The final map of adult distribution shows that maximum abundances are strongly related to the presence of the most relevant breeding sites (rice fields and marshes) and livestock density. Furthermore a multi-category statistical model based on logistic regression and driven by climatic

predictors was computed to describe the yearly absence/presence of *An. labranchiae* around the main trapping sites, attempting good performances. The statistical model can be directly fed by weather numerical output data to form an operative chain for the categorical forecasting of adult anopheline. Our findings aim to demonstrate that the development of statistical models and spatial distribution maps of malaria vectors are essential prerequisites to assess the risk of malaria re-emergence in Italy and to plan suitable control strategies.

This research was partially founded by EU grant GOCE-2003-010284 EDEN

Studies on the anopheline fauna (Diptera: Culicidae) in order to evaluate the risk of malaria re-emergence in Romania

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Romania had about 300,000 malaria cases per year before the eradication campaign and became a free-malaria country since 1962 but the former vectors of the *Anopheles maculipennis* group are still distributed all over the country.

The study was carried out in 2005 – 2009 in sites located in three former "malaria stratification" areas: Comana area in Romanian Plain with *An. atroparvus* as main vector; Razim-Sinoe lagoon complex in area with *An. sacharovi* and *An. atroparvus* as main vectors; Danube Delta known as "anophelism without malaria" area.

The identification of about ten thousand mosquitoes of *An. maculipennis* group put in evidence the presence of former malaria vectors: *An. atroparvus*, *An. messeae* and *An. maculipennis* s.s. The absence of the *An. sacharovi* was confirmed.

The annual and seasonal dynamics and the main elements of the vectorial capacity of the anopheline populations were investigated in order to evaluate the risk of malaria re-emergence in Romania.

Laboratory-reared *Anopheles arabiensis* males, reduction of the sexual maturation period.

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The sexual maturation of *Anopheles arabiensis* males requires the rotation of the genitalia. This phenomenon has been described to occur at least 24 hours after the emergence. However, visual observations of male genitalia rotation, spermatheca dissections and oviposition trials showed that males from our laboratory *An. arabiensis* Dongola strain (Sudan) need much less than 24 hours to be sexually mature and to be able to inseminate females. This reduction of time is commonly reported in laboratory reared flies, the rearing would favour the selection of the first sexually mature males. However such a phenomenon has never been documented for *An. arabiensis*. This new observation is of high importance for mating and competitiveness experiments and for mosquito mass-rearing.

Turkish studies in the scope of eden project

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Among the malaria vectors, *Anopheles sacharovi* is the most important malaria vector in Turkey and also in the study area which locates in the Southeastern part of Turkey and is still one of the largest focuses of malaria in the country. *An. superpictus* ranking next in importance to *An. sacharovi*. In EDEN Project studies, seasonal population dynamics of these species in 7 different breeding sites have been carried out. Bio-ecological parameters such as biting habits, periodicity and vectorial capacity have also been studied. *An. superpictus* samples sent to Radboud University, Nijmegen for experimental transmission studies and the results showed 5/17 *An. superpictus* tested positive for oocysts or and salivary gland sporozoites. According to the questionnaire carried out among app. 10.000 inhabitants, the most significant factors in malaria incidence are ceiling material, the presence of a well, watertank or watersource as physical factors and education, poverty and age as social factors. Migration is also important for the study area and this movement is governed by the activities including most importantly agriculture, then tourism and construction. Data management and preliminary GIS studies have also launched in the study area as the first time in vector studies.

Outbreak simulation of malaria in mainland Portugal

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Recent studies have demonstrated the existence of several applications that can be designed to simulate the spread of infectious diseases. One of the increasingly adopted technologies used in epidemiological simulation models are cellular automata and multi-agents, both based on computational modeling. The use of these models in epidemiological studies allows a multiplicity of applications such as exploring the space-time dynamics between vectors, hosts and the determination of interactions between heterogeneous agents and the area, making it a useful tool for integrating multi-disciplinary knowledges. Currently these models are increasingly applied to risk assessment and to the implementation of strategies for disease control. The techniques used in simulation studies of malaria transmission in non-endemic areas, like Portugal, are aimed at drawing up different interaction scenarios between the various agents of the malarial biological system. Evidences related to the development of this cycle of autochthonous transmission of the disease, show a high on the interaction between the vectors of malaria: people with gametocytes (infected) and non-immune people (susceptible to infection).

With the creation of these model-generated scenarios it becomes possible to simulate spatial and temporal variations of the contact rate and infection between the uninfected vectors and gametocyte carriers, and infectious vectors and the healthy population, evaluating the risk of re-emergence of malaria, limiting possible outbreaks of the disease in mainland Portugal.

Preliminary data on vector competence of *Anopheles labranchiae* from Maremma Plain (Grosseto, Tuscany), a former malaria hyperendemic area in Central Italy

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Background: In Maremma, a coastal plain in Grosseto Province, malaria was endemic until 1950's. Hyperendemic levels were maintained by *Anopheles labranchiae*, the more competent vector species of the maculipennis complex, still present in the region. In 1997 an autochthonous-introduced *Plasmodium vivax*

case, reasonably transmitted by indigenous *An. labranchiae*, occurred in a rural locality of Grosseto [1] demonstrating Maremma suitable area for malaria reintroduction. To assess a possible risk of malaria resurgence in this region in the light of the predicted climatic and environmental changes, an entomological study to analyse in more detail vector bionomics was performed. The findings showed the high receptivity of the study area, *An. labranchiae* occurring at different densities related to climatic parameters and anthropic activities [2]. Moreover, while the competence of *An. labranchiae* from Maremma for exotic *P. vivax* strains has already been shown [1], to assess the susceptibility of this vector to *P. falciparum* a first attempt of artificial infection with a long established afro-tropical strain was performed in 2007. The results showed a potential vector competence of *An. labranchiae* as indicated by the development of oocysts in three specimens [3]. In 2008 and 2009 further experimental infections were performed and the preliminary results were reported. Methods: *An. labranchiae* females were collected in animal shelters in Principina, an extensive rice field area in Grosseto Province. Field-caught female cages were secured inside polystyrene boxes with moistened cotton and shipped to the Institut Pasteur, Paris (France). Artificial blood infection were performed with gametocyte-containing cultures of the *P. falciparum* NF54 African strain by a parafilm membrane feeding system in P2 security insectarium (26±1 °C; 75% RH). Laboratory colonies of *Anopheles gambiae* Ngousso strain from Cameroon were used as positive control. Dissections were made 8 or 15 days after infection to observe the oocysts in the digestive tube. Head and thorax of 15th day dissected mosquitoes will be analysed for sporozoite detection in the salivary glands. Legs of fed females will be used for species identification by Multiplex PCR. Results: In 2008 about 1500 *An. labranchiae* females were collected and three infection experiments were performed. In total 25 out of 96 surviving mosquitoes (until 8th day), showed a range of 1-4 oocysts, resulting in a mean infection prevalence of 29.3% and a mean number of 0.4 oocysts/mosquito. Specimen identification results were 24 *An. labranchiae* and 1 *An. melanoon*. In 2009 about 4500 *An. labranchiae* were collected and four infection experiments were performed. Nine mosquitoes out of the 130 restants, dissected in 15th day, were found infected (1-2 oocysts/mosquito). The mean infection prevalence was 7% and the mean number of oocysts/mosquito was 0.08. In one specimen was observed a possible mature oocyst. Infected mosquitoes were identified as *An. labranchiae*.

Major conclusion: Although, some technical difficulties should be taken into account (specimen high mortality and poor membrane feeding adaptation), the results of all experiments shown a very low susceptibility of *An. labranchiae* from Maremma, to the tropical strain of *P. falciparum*, at least at the laboratory conditions. Nevertheless, sporozoite detection analysis is in progress.

This research was founded by EU grant GOCE-2003-010284 EDEN

A study on Anopheline mosquito species in command area of Lunkaransar Tehsil of Bikaner district in Rajasthan, India

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Malaria continues to remain a major health problem worldwide including India. It is endemic in almost all parts of the State of Rajasthan. The introduction of Indira Gandhi Canal in Bikaner district has changed the micro-ecology of command areas including Lunkaransar Tehsil and compounded the problem as mosquito-genic conditions with respect to breeding and survival have improved due to water logging at number of places resulting from canal seepages and also because of increase in vegetation. Further, the species which were not earlier reported from the region have also established themselves.

Paper deals with the study on species composition, density, adult habitats and blood feeding preference of Anopheline mosquitoes in command area of Lunkaransar Tehsil of Bikaner district, Rajasthan during 2006-2008. Study also looks into the malaria magnitude status during the same period.

Three species viz., *An. subpictus*, *An. annularis* and *An. stephensi* were recorded from the region, *An. subpictus* being the major form. Adults were documented mostly near households, fodder rooms and cattle water points. *An. subpictus* was found to prefer bovine blood, *An. stephensi* human blood while, *An. annularis* was found to feed exclusively on cattle blood. Both, Pv as well as Pf cases were recorded from the region, although the former cases outnumbered the later ones.

Bacterial toxin and Botanical Pesticides for the control of Malarial vector, *Anopheles stephensi* at different Agro-Climatic regions of Tamil Nadu, India.

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Vector-borne infectious diseases are emerging or resurging as a result of changes in public health policy, insecticide and drug resistance, shift in emphasis from prevention to emergency response, demographic and societal changes, and genetic changes in pathogens. Effective prevention strategies can reverse this trend. Research on vaccines, environmentally safe insecticides, alternative approaches to vector control, and training programs for health-care workers are needed. Malaria is a vector-borne infectious disease that is widespread in tropical and subtropical regions. It causes disease in approximately 400 million people every year and is the cause of between one and three million deaths annually, mostly among young children in Sub-Saharan Africa. The most serious forms of the disease are caused by *P. falciparum* and *P. vivax*. *Anopheles gambiae* is the main malaria vector in Africa that carries *P. falciparum*. Environmental conditions play an important role in the transmission dynamics of malaria, as the parasite has to pass its developmental cycle in the mosquito. Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development. The parasites, transmitted by the female mosquito when she sucks blood, multiply within red blood cells, causing symptoms that include symptoms of anemia, as well as other general symptoms such as fever, chills, nausea, flu-like illness, coma and death. The three main climatic factors that affect malaria transmission and distribution are temperature, precipitation and relative humidity. Climate predicts, to a large degree, the natural distribution of malaria. Laboratory investigations have been made on the effect of *Leucospora axillaris* leaf extract and *Bacillus sphaericus* on the larvicidal and pupicidal properties against malarial vector, *Anopheles stephensi*. There was considerable mortality was evident after the treatment of (Bs) and it suggest that the bacterial toxins might have affected the larvae. Mortality was increased after the combined treatment and *Bacillus sphaericus* revealed that the multiple effects on malarial vector. In the present study, *Leucospora axillaris* treated larvae of *Anopheles stephensi* showed prolonged development. Lethal concentrations (LC50 and LC90) were worked out for the treatment. Combined treatment had profound inhibition on growth of pupae and the treated mosquitoes did not emerge from the pupa as adults. Field trials were also conducted to test on the effect of *Leucospora axillaris* and *Bacillus sphaericus* in different Agro-Climatic regions of Tamil Nadu, India, breeding habitats of *Anopheles stephensi*. Observations were made on the efficacy of plant extract on the mortality of larval populations was noted at 24, 48 and 72 hrs. Percentage of larval reduction was further reduced after the combined treatment. It reveals that the active compounds present in the *Leucospora axillaris* used to control mosquito vector effectively by emitting active substances in the breeding habitats of *Anopheles stephensi*. In the review of current state of knowledge on larvicidal and pupicidal activity, extraction processes, botanical pesticides effects on non-target organisms for the control of malarial vector *Anopheles stephensi*.

Surveillance of potential malaria vectors in a past endemic area of Spain

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As in other European countries, malaria was an endemic disease in Spain until the second half of the twentieth century. Furthermore, the disease showed an important heterogeneity in transmission rates due to different aspects related with vector abundance and its specific richness, as well as anthropogenic factors

(agronomic practices or socioeconomic questions) that occurred in our country. In this context malaria was an important catalyst for mosquito studies, but since the disease's eradication in 1964, knowledge on Spanish mosquito populations has decreased dramatically. As a consequence of this and bearing in mind current topics such as globalization or climate change, in 2005 we started intensive larval sampling in Eastern Spain in order to establish possible scenarios of the reemergence of malaria. Our area of study was the Valencian Autonomous Region. The few historical data available in this Region indicated that *Anopheles atroparvus* was the main vector of the disease in the past. Moreover, in the south of the study area malaria was mainly transmitted by *Anopheles labranchiae* and *Anopheles hyrcanus* was probably involved in sporadic cases around the Valencian rice fields.

Until now, our samplings have allowed the capture of 7 *Anopheles* species, among a total of 30 Culicidae species collected. Our findings suggest that *An. atroparvus* is currently the only anopheline likely to play a role in malaria transmission in the area of study. Among other anopheline species recorded, *Anopheles algeriensis*, *Anopheles claviger* and *Anopheles plumbeus* are rare and accidental vectors, and *Anopheles maculipennis*, *Anopheles marteri* and *Anopheles petragrani* due to their high degree of zoophily are considered insignificant transmitters.

Finally the "malariogenic potential" of the investigated area will be discussed according to the following three factors: receptivity, infectivity and vulnerability. The lack of findings of common anophelines such as *Anopheles melanoon*, *An. labranchiae* or *An. hyrcanus*, during the first half of the past century will be also presented.

Development of vegetable farming: a cause of malaria transmission and the emergence of insecticide resistance in populations of *Anopheles gambiae* in urban areas of Benin.

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Poverty, food insecurity and malnutrition have become critical urban issues due to population growth in sub-Saharan Africa. Meeting this challenge, peri-urban and urban agriculture plays constitutes a major economic activity in the cities. However, agricultural practices in vegetable farming retains water that provides suitable breeding sites for the development of mosquitoes and contributes to the selection of insecticide resistance genes in the major malaria vector, *Anophele gambiae*.

To explore these two assumptions, three vegetable farms were monitored in Benin. The protocol was based on sociological data by interviewing vegetable farmers about their various agricultural practices and the types of pesticides they use and by collecting Anopheline mosquito larvae in breeding sites. Laboratory bioassays were conducted in reared adult mosquitoes to assess the susceptibility status of malaria vectors to various insecticides. Molecular analysis was also carried out to characterize the type of insecticide resistance genes present. Alongside the insecticide testing, adult mosquitoes were collected in households situated far away (with no vegetable farming) or just across the vegetable farms for a comparative analysis of the rate at which malaria is transmitted under both contexts. By using CSP ELISA to investigate on the annual Entomological Inoculation Rate (EIR),

This research showed that:

(1) The rapid development of urban agriculture is related to the strong unemployment among young men living in the cities, rural exodus and the desire for a balanced diet by urban populations ;Urban agriculture increases the farmers' household income and their living standard;

(2) Molecular studies revealed the presence of three sub-species of *An. gambiae* (*An. gambiae* s.s., *Anopheles melas* and *Anopheles arabiensis*) and the two molecular M & S forms within the *An. gambiae* s.s. complex.

(3) Insecticide susceptibility tests showed strong resistance to permethrin at Porto-Novo (24% mortality), Cotonou (17%) and to a lesser extent Parakou (76 % mortality)

(4) The knock down resistance gene (*kdr*) conferring cross resistance to pyrethroids and DDT was recorded in all samples from the three sites (>70%).

(4) The determination of the annual Entomological Inoculation Rate (EIR) using ELISA technique was significantly higher in household close to vegetable farming (16.5) than those located far (5.3).

This study showed that urban malaria transmission is ongoing in many cities of Benin and that the EIR seems increased in urban areas where irrigated farming takes place. In addition, the urban setting seems to pose a number of unique challenges to malaria control that need further investigation, e.g. anophelines may be adapting to more polluted larval habitats, may be more exophilic than in rural settings thereby decreasing the efficacy of IRS and the use of insecticide in an improper manner to control vegetable pests, thus exerting a huge selection pressure on mosquito larval population, which resulted to the emergence of insecticide resistance in malaria vectors.

Influence of the anthropogenic activities on the potential risk of malaria reappearance on the spanish mediterranean coast

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In the last century, ancient malaria areas on the Spanish Mediterranean coast have underwent important environmental modifications, due to the introduction of rice cultures, modification of natural flooding events, disappearance of natural areas and massive urbanization. The absence of actual entomological studies in the Mediterranean coast, the possible spread of African *Anopheles* to the Iberian Peninsula, the human activities and the different man-made environmental changes recommend the appropriateness to evaluate the influence of these anthropogenic changes in Malaria reappearance risk.

Two traditionally malarious areas in the eastern Mediterranean coast of Spain were studied: Ebro Delta (Tarragona) and Albufera of Valencia and surrounding areas (Valencia). The study included the characterization of climate and territory, entomology and genetics of the *Anopheles*, history of the disease (review and malaria reported cases) and human activities and their influence on the evolution of Malaria in both areas. In the past, epidemic Malaria was recorded in both areas, and two *Anopheles* species were present, *An. atroparvus* and *An. melanoon*, transmitting mainly *P. vivax* and *P. falciparum*. Ecological and social conditions were behind the high malaria endemicity in both areas as rice culture fields, poor health conditions and movements of people, as well as lack of coordination between public health services. Nowadays, the two studied areas have a large expanse of rice fields, natural wetlands and similar climatic conditions. However, our entomological and molecular results confirm the presence of *An. atroparvus* as a potential vector in the Ebro Delta, whereas in Valencia no *Anopheles* species was found.

During 12th century, several anthropogenic changes occurred in the Albufera of Valencia. The modification of the natural flooding process and the introduction of rice cultures allowed the introduction and establishment of *An. melanoon*, which began to compete with autochthonous *An. atroparvus*. The increase of cultivated rice area progressively allowed *An. melanoon* to exceed *An. atroparvus*. During the second half of the last century, other anthropogenic changes occurred, as the worsening of the irrigation water of the rice fields and the agricultural treatments, making *Anopheles* larval development difficult. The introduction of machinery in the rice culture process, the urbanization of natural areas and the disappearance of animal farms affected the feeding and resting of *Anopheles* female mosquitoes. In the Ebro Delta, *An. atroparvus* was also present. The introduction of the rice took place at the beginning of 20th century, allowing the establishment of *An. melanoon*. However, the man-made changes in rice culture, the agricultural treatments and the high salinity of the land of this area prevented the adaptation of *An. melanoon* and were probably the causes of its extinction. The absence of a direct competitor, the lower human activity in the rice fields and the maintenance of the animal farms have allowed the surviving of *An. atroparvus* in the Ebro Delta.

These findings indicate that anthropogenic changes can affect the evolution of the anophelism and the factors conditioning their evolution and recrudescence in a ecosystem and, thus, should not be underestimated when dealing with Malaria and other mosquito-borne disease.

GIS in prediction and mapping of mosquitoes and their breeding sites

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GIS provides excellent means for visualizing and analyzing epidemiological data, revealing trends, dependencies and inter-relationships. GIS is a combination of hardware and software for capturing, storing, checking, integrating, manipulating, analyzing, and displaying data that relate to each other by location on the earth's surface. Mosquitoes are the most single group of insects in terms of public health significance. Despite many attempts to successfully eradicate them, they still co-exist with man and animals and transmitting dreaded diseases like malaria, filariasis and dengue etc., Hence, the present paper is to develop new and improved detection and control systems for mosquito vectors, new methods for forecasting mosquito distribution, and new methods for defining target areas and buffer zones for the containment and eradication of indigenous and exotic mosquito vectors and the pathogens they transmit. In the present work the presence, abundance and species composition of immature mosquitoes were characterized in time and space and were amended to a Geographic Information Systems (GIS) geodatabase comprising feature classes for environmental variables (physiography, edaphic factors, hydrology, vegetation, land use, host presence, climatic variables, demographic factors, and virus incidence in sentinel livestock). The candidate larval development sites in the field were developed using image analysis methods (in the lab) and the sites were characterized by photographs, digital images, and/or satellite-acquired imagery. Descriptive (pictorial) analysis of changes in this relationship through time was also made using serial editing (versioning). Hence, the present work will be best fit model(s) for accuracy and can be adapted for use in an operational system for the location of larval developmental sites for treatment with new and improved mosquito control technology.

Impact of climate change on biopesticides, mosquito vectors and mosquito borne diseases at the different agroclimatic zones of India.

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Of the all insects that transmit diseases, mosquitoes represent by far, the greatest menace. World Health Organization (WHO) has declared the mosquito "Public Enemy Number One" because mosquitoes are responsible for the transmission of various dreadful diseases (malaria, filariasis, dengue/chikungunya and Japanese encephalitis). In view of the above, the biologically-active plant materials integrated with other biological insecticides are attracted in recent years, because of their biodegradable nature and being relatively safer to human, target and other non-target organism in the Environment. Botanical pesticides when combined with microbial insecticides will help to kill the spectrum of mosquito larvae in the breeding habitats, allelochemicals present in the botanicals affect the biochemical and physiological processes of insect system and thereby increase the pathogenicity of microbial pesticides. In the present investigation we have studied the following: (a) Anti-ivectorial plant compounds with microbial insecticides for the control of mosquito larvae (b) Anti-malarial plant compounds and their impact on malarial parasites and (c) Impact of climate change (temperature) on the mosquito population and bioefficacy of biopesticides at different agroclimatic zones. Mosquito breeding sites have been made at different temperature regimes of Tamil Nadu, Southern India., viz., Ooty (25° C) with the elevation of 2,623 m., Coimbatore (34.7°C), Madurai (37.5°C), Sivakasi (39. 2° C), Chennai (43.2° C). Biopesticides such as

neem seed extract and fungal pathogen, *Beauveria bassiana* have been used to control the larval populations of malarial vector, *Anopheles stephensi*, filarial vector, *Culex quinquefasciatus* and dengue vector, *Aedes aegypti*. Laboratory lethal toxicity as well as field applications has also been done in the different larval stages of mosquitoes. Mosquito larval populations were higher and bio-efficacy of biopesticides was diminished at the breeding sites located at the higher temperatures such as Chennai, Tamil Nadu, India. The similar kinds of studies were also done at the Tezpur, Assam, North Eastern India with different temperature, humidity and rainfall pattern. The percentage reduction of larval populations during different period of time (24 hrs, 48 and 78 hrs) were also noted after the biopesticides applications.

Malaria poses a serious threat to the population in the tropical and sub-tropical regions of the world and malaria has been a problem in India for centuries. The appearance of drug-resistant strains of the malaria parasite has materially worsened this danger. Laboratory bioassays have been done to test some of the indigenous plants on the antiplasmodial activities. Aqueous and organic solvent extracts obtained from specific parts of the plants *Azadirachta indica*, *Ocimum sanctum*, *Cassia occidentalis*, *Jatropha curcus*, and *Andrographis paniculata* showed antimalarial activity against *Plasmodium falciparum* strains were tested against malaria strain *Plasmodium falciparum* FCK 2 in vitro. Visual evaluation of the antimalarial activity of the plant extracts on thin blood smears was followed by quantification of the activity by use of [³⁵S]-methionine incorporation into parasite proteins to determine the value that inhibits 50% (IC₅₀). Among the different plants tested, Neem (*Azadirachta indica*), *Cassia occidentalis*, *Andrographis paniculata* had significant inhibitory effect on *P. falciparum* in vitro.

Malaria soon in our cities? Contribution to a micro-landscape analysis to malaria transmissions risk in urban area

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Since *Plasmodium Falciparum* was first discovered in 1880, many important discoveries have been made and tools have been developed that have enabled France to eliminate malaria. But climate changes and *Anopheles* adaptation (major vector of the disease) may cause its recurrence. Isolated in France by vector control measures (like swamp draining up), it seems that *Anopheles* (still present in Camargue) might be able to adapt to urban setting.

Even if malaria was a time considered as a rural disease, urban malaria appears as a “new” and paradoxical phenomenon. Long considered as imported, malaria is currently seen as endogenous. In Dakar city, recent studies have proved this hypothesis. The malarial risk is nevertheless defined in very specific places and involves a very heterogeneous spread of the disease.

If we consider that this West African context could be transposed to our region in a hypothetical future, our study aims at quantifying this urban malarial phenomenon. It roughly deals with chance and the presence of spatial markers analyzed as indicators of epidemiological risk. The human aspect must be considered in this reflection by the population’s capacity to affect and transform the environment. We will implement risk evaluation, which considers hazard (the contaminating potential) as modulated by the perceptions and behaviors of potentially infected people.

As we know that the environment determines the presence or absence of the pathogen complex, we postulate that spatial factors significantly condition the structure of living. Metapopulations, i.e. “a population made up of populations which is based on colonization-extinction equilibrium” and among them vector mosquitoes are submitted to the same process of structuring.

The general problematic of the project consists in elaborating a methodology to identify environmental structures that enable metapopulations carrying Plasmodies to live. Therefore, the goal is to set up a reading grid adapted to urban environment in order to develop an expert knowledge of the eco-geography of the vector in a heterogeneous environment. This implemented methodology must enable us to get concrete and exploitable results in a context first judged as imprecise and uncertain.

In order to develop our method, we will adopt several stages including a landscape analysis coupled with entomologic data and with a malaria prevalence map based on 3000 households in Dakar city. (ACTU PALU data).

The landscape analysis will consist in a landscape typology (in two or three dimensions with the help of a Digital Evaluation Model morphometric analysis). The aim is to identify some eco-landscape specificities with some discriminatory elements in the way of living of the target metapopulation.

These results will be confronted to citizen's perceptions of those landscapes. We also include a reflection on people's development patterns and the environmental home management of the local populations (waste management, water management, representation of mosquitoes…)

This study in Dakar city aims at developing a generic methodology which can be transposed to another epidemiological and geographical context where scientific research improvement and reflexion on urban malaria is needed.

Effect of seaweed *gracillaria verrucosa* and bt toxin for the control of dengue vector- *aedes aegypti* in the coastal regions of Tamil Nadu, India.

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India is one among 12 mega-biodiversity countries and 25 hotspots of the richest and highly endangered eco-regions of the world. Among the Asian countries, India is perhaps the only one that has a long record of inventories of coastal and marine, mangroves, backwaters, salt marshes, rocky coasts, sandy stretches and coral reefs, which are characterized by unique biotic and a-biotic properties and processes. The Gulf of Mannar Marine National Park is a unique marine biosphere reserve, located in the Tuticorin and Ramanathapuram districts of Tamilnadu. Seaweed is a loose colloquial term encompassing macroscopic, multicellular *Gracillaria verrucosa* (Hudson) weed is collected from Gulf of Mannar and subjected for insecticidal property that inhibits mosquito population. Mosquitoes are the single group of insects in terms of public health significance. It breeds in a varied aquatic eco-systems ranging from clean water, stored and sewage water system, The yellow fever mosquito, *Aedes aegypti*, a mosquito Chemical insecticides to control mosquito species, That emergence and spread of insecticide resistance, environmental pollution, and unresolved issues pertaining to their toxicity to humans and non-target organisms hamper progressive use and broad acceptance of these tools. In recent years, biopesticides having attributes of activity and efficacy, specificity and selectivity, low mammalian toxicity, environmental acceptability. Moreover, the phytochemicals derived from marine sources sea weeds have provided numerous beneficial uses ranging from pharmaceuticals to insecticides. *Bacillus thuringiensis* (Bt toxins) also effectively kill mosquito larvae and relatively safer to the environment. In the present study is to evaluate the effect of seaweed extract *Gracillaria verrucosa* and bacterial toxins (Bt) for the successful control of Dengue vectors at the coastal region of Tamil Nadu, India. Field trial was also conducted at the coastal areas and studies the efficacy on the larval and pupal mortality during 24h, 48h and 72h treatment by using the seaweed extract and bacterial toxins in different breeding habitats of dengue vectors. The results revealed that the combined treatment of *Gracillaria verrucosa* extract and bacterial toxins had higher efficacy than the individual treatments. Prolonged larvicidal efficacy was found to be exhibited after treatment with the combined treatment of *Gracillaria verrucosa* extract and bacterial larvicides's. It reveals that active chemicals combined with microbial insecticides will help to kill the spectrum of mosquito larvae in the breeding habitats. The results of our study will throw more light on the right combination of seaweed extracts in conjunction with bacterial pesticides for the formulations potential of larvicides's for the different mosquito vectors in their respective breeding habitats at coastal regions of Tamil Nadu, India. This paper discusses on the use of natural products from the marine ecosystem for eco-friendly mosquito vector management at the coastal areas.

West Nile virus

Analysis of mosquito fauna in South Moravia in an area endemic for West Nile virus

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In the years 2007 and 2008, adult mosquito collections in CDC light traps using dry ice and in pigeon-baited traps were carried out in south Moravia (Czech Republic). The study was conducted at two study sites: Nesyt fishpond near the village of Sedlec (1 collection site) and in the Soutok region (2 collection sites).

In 2007, collection was begun on 28 May and eleven two-day captures were carried out in 2-week intervals, and 1,490 female mosquitoes of 9 species were caught. In 2008, 15 two-day trappings of mosquitoes were carried out at these locations and under the same conditions, and 6,778 females of 19 species of mosquitoes were trapped. The numbers of captured female mosquitoes of individual species were: *Anopheles claviger* 53, *An. hyrcanus* (var. *pseudopictus*) 57, *An. maculipennis* s. l. 76, *An. plumbeus* 48, *Aedes cinereus* 83, *Ae. rossicus* 37, *Ae. vexans* 5050, *Ae. cantans* 412, *Ae. caspius* 28, *Ae. cataphylla* 15, *Ae. excrucians* 4, *Ae. flavescens* 9, *Ae. sticticus* 356, *Culex modestus* 138, *Cx. pipiens* 1753, *Culiseta annulata* 108, *Cs. morsitans* 2, *Coquillettidia richiardii* 33, and *Uranotaenia unguiculata* 5. The results showed marked differences in abundance and species composition of mosquitoes between both study sites, and between the trapping methods were confirmed. In the alluvial floodplain forest ecosystem of the Soutok study area, the species *Aedes vexans* predominated. While the abundance of mosquitoes at the Nesyt location was lower, the species composition was more varied and no species was significantly predominant. At this study site, the presence of thermophilic mosquito species *Anopheles hyrcanus* and *Uranotaenia unguiculata* was noteworthy.

The largest capture of mosquitoes was in traps with CO₂ placed at a height 1 m above the ground. The capture of mosquitoes in the pigeon-baited traps as well as in the traps with CO₂ placed at a height of 5 m in the canopy of trees generally was markedly lower in both study sites, with the predominant species being *Culex pipiens*.

The study was funded by EU grant GOCE-2003-010284 EDEN.

Serologic survey of wild birds for West Nile virus and other mosquito-borne viruses in Czechland and Poland

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In Moravia (Czechland), 391 wild birds representing 28 migratory and resident species were tested for Flavivirus West Nile (WNV) antibodies using a 90% plaque-reduction neutralization microtest (PRNμT) on Vero cells with the Eg-101 topotype strain of WNV. The birds were sampled in freshwater reedswamp habitats (fishpond ecosystem) during 2004–2006. Antibodies to WNV were detected in 23 (5.9%) birds of 10 spp.: common coot (*Fulica atra*), kingfisher (*Alcedo atthis*), reed warbler (*Acrocephalus scirpaceus*), sedge warbler (*A. schoenobaenus*), marsh warbler (*A. palustris*), Savi's warbler (*Locustella luscinioides*), reed bunting (*Emberiza schoeniclus*), blackcap (*Sylvia atricapilla*), penduline tit (*Remiz pendulinus*), blue tit (*Parus caeruleus*), and starling (*Sturnus vulgaris*). When 14 of the 23 sera

reacting with WNV were tested in parallel with Flavivirus Usutu (USUV), 12 of the birds had specific antibodies to WNV (i.e., at least 3.1% of total), one coot (*F. atra*) had a higher titer of antibodies against USUV than to WNV, and another one could not be attributed to any of the two viruses. In addition, 178 of the birds (14 spp. of Passeriformes) were also examined for haemagglutination-inhibiting (HI) antibodies to Alphavirus Sindbis (SINV: 0.7% positive), and bunyaviruses *Tahy* (TAHV: 14.0% positive) and Batai (BATV: 6.8% positive). The serosurvey indicates circulation of mosquito-borne viruses TAHV and BATV while a low activity of WNV and a very low, if any, USUV and SINV activity in Moravia in the period. A similar serosurvey (PRNμT) for WNV was carried out in 97 wild birds belonging to 10 spp. from different areas of Poland. Specific antibodies were detected in five juvenile (hatching-year) birds collected in 2006: three white storks (*Ciconia ciconia*) and one hooded crow (*Corvus corone cornix*) in a wildlife rehabilitation centre, and one free-living mute swan (*Cygnus olor*); i.e., the overall seropositivity was 5.2% among wild birds sampled in Poland. The WNV activity in Poland was obviously limited during the years 2004-2006, but the data (especially the seropositivity of storks with high titres of antibodies) are a respectable trace demanding further investigations. The studies were funded by EU grant GOCE-2003-010284 EDEN.

Serologic Survey of Wild Birds for West Nile Virus in the Danube Delta Biosphere Reserve and Dobrudja Tableland (Romania)

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West Nile virus (WNV) research in the Danube Delta Biosphere Reserve (DDBR) and Dobrudja Tableland (DT) involves the study and monitoring of landscapes, vectors, hosts and animal reservoir research. In this abstract will be treated only the serologic survey of wild birds during 2005-07. For small birds, the blood was taken from jugular vein, for larger birds from wing or leg veins. We used for small birds insulin syringes of 0.5 ml (0.33 mm gauge, 29 G), for larger birds syringes of 1 ml (25 G). Serum was separated using micropipettes and stored at -20°C. All 1,210 samples were tested (ELISA, IgG) by National Institute of Research and Development for Microbiology and Immunology, Bucharest, Romania.

Between 2005-07 we took 1,210 samples from 21 wild bird species. The total WNV seroprevalence during 2005-07 was 15.8% (40% in 2005, 16.4 in 2006 and 10.7 in 2007).

Considering relevant only species with at least 20 samples, during 2005-07, the highest WNV seroprevalence was found on Hooded Crow (25%), followed by Great Reed Warbler, Coot, House Sparrow and Reed Warbler.

We found 9.7% WNV seroprevalence in birds breeding outside of Romania, 35.1% in possible breeding birds and 14.6% in birds breeding in the DDBR; 18.25% WNV seroprevalence in resident birds, 19.7% in migratory birds and 8.2% in partial migratory birds; also, 8.9% WNV seroprevalence in medium and large waterbirds, 17.3% in Passeriformes excepting *Corvus* ssp. and 26% in *Corvus* ssp. Almost no difference by age, 15% seroprevalence in juveniles and 13.5% in adult birds. We found positive birds in most of the habitats (terrestrial, aquatic and mixed) among all sampling locations.

A first conclusion is that all migratory category of birds are involved in WNV transmission. Whether the migratory birds could be infected outside of the study area and their chicks by maternal transmission through eggs, the positive resident birds and the seroconverted birds represent the evidences of local circulation of the WNV. Even in our survey the WNV antibodies seroprevalence was lower than in former studies, yet, the WNV prevalence was higher than in other European countries, excepting Spain.

Our study has confirmed the local circulation of WNV in the DDBR and DT. The evidence is provided by positive antibodies against WNV in resident birds (18.25%) and by two seroconverted birds. Even that in

our survey the WNV antibodies seroprevalence was highest within Europe, yet, was lower than in former studies in Romania.

Approaches to the risk of establishment and transmission of the West Nile Virus in the Valencian autonomous community

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The West Nile virus (WNV), like Dengue or Yellow Fever, belongs to the Flavivirus genus, and has acquired great importance during the past decades due to its enormous process of invasion and expansion across new geographical areas. It is transmitted, fundamentally, through a natural cycle between birds (especially wild migratory birds), and ornithophilous mosquitoes, mainly belonging to the *Culex* genus, although it could also affect other animal species such as mammals, reptiles, and amphibians, and occasionally, human beings. In this last case, 1% of the affected victims develop neurological symptoms, such as encephalitis, meningitis, and/or acute flaccid paralysis; the mortality index is around 15% of these victims.

Cases of epidemics of birds belonging to the genii *Corvus*, *Phoenicopterus*, *Phalacrocorax*, *Haliaeetus*, *Columba*, *Streptotelia* and *Pica* have been reported, in addition to birds with a marked migratory behaviour, such as those belonging to the *Anas*, *Anser* and *Ciconia* genera. The presence of all of these species in our country, the level of interaction that they establish with human beings, and the presence of adequate vectors involve a critical stage in the transmission of this disease.

Furthermore, due to the proximity of the African continent and being this an obligatory crossing point for migratory birds and people, along with its characteristic climate conditions (close to the areas with transmission of vectorial diseases) and the presence of the transmissive vectors of the disease, Spain is defined as a country in which this vectorial disease, could present processes of emergence or re-emergence.

The large number of Natural Parks, Protected Wetlands, and areas declared Z.E.P.A zones (Zonas de Especial Protección para las Aves – Special Zones for the Protection of Birds) included inside the frontiers, which act as a passing and nesting point for a great number of migratory birds, along with the presence of numerous species of dipteran culicids belonging to the *Culex* genus, indicate that nowadays, this Spanish Autonomous Community is an area at risk of this disease. This study analyzes the possibilities of establishment and starting of transmission cycles of the West Nile virus in the Valencian Autonomous Community.

Prevalence of West-Nile fever virus, sandfly fever Toscana virus and sandfly fever Sicilian virus in Cyprus

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West Nile virus (WNV) is a mosquito-transmitted flavivirus widely distributed in Africa, the middle east, southern Europe and today also in North America. Sandfly fever viruses, genus *Phlebovirus* (family *Bunyaviridae*), are transmitted by phlebotomus flies. Three distinct serotypes are prevalent in Europe and

the Mediterranean basin: Toscana, Sicilian and Naples. Even though usually associated with non-specific febrile illness, they can also cause severe infections of the central nervous system manifested as meningitis or encephalitis. Previous studies showed a high prevalence of antibodies to sandfly fever viruses among Swedish tourists and also Cypriots (1,2). Recently, an outbreak of febrile syndrome among Greek soldiers stationed in Cyprus was reported and attributed to a Cypriot strain of sandfly fever Sicilian virus (3). The aim of this study was to determine the prevalence of arbovirus infections in Cypriots.

For this purpose 250 sera from random samples were examined for IgG antibodies to sandfly fever Toscana virus and West-Nile Virus (from Diesse, Italy, and Focus, USA, respectively) using an the ELISA method. The percentage of seropositives for IgG were 6% and 13.4%, for WNV and TOSV, respectively. 26.7% of the seropositives for WNV were also positive for TOSV, as compared with 12.1% among the WNV-seronegatives. It was noted that for TOSV the prevalence of IgG antibodies increased with age, while WNV antibodies were mostly observed in people less than 20 years old, indicating that WNV, in contrast to TOSV, is an emerging pathogen in Cyprus. The results indicate that arboviruses are prevalent in Cyprus and are probably responsible for many cases of non-specific febrile illness in this region. For this reason, clinicians should consider sandfly fever and West Nile fever in the differential diagnosis of such cases.

West Nile Virus surveillance in Germany - serological and virological studies for monitoring the West Nile status

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West Nile Virus (WNV) is a flavivirus that is transmitted by blood-suckling mosquitoes. WNV is an important zoonotic virus with its major reservoir in the wild bird population. Some bird species like ravens, falcons and jays are highly susceptible and develop deadly encephalitis while others undergo only a subclinical infection. A wide range of mammals (infections of humans can cause encephalitis with fatal outcome) and reptiles are also susceptible and can develop deadly diseases.

The emergence of WNV in New York in 1999 resulted in its rapid dissemination to the whole United States and other countries in central and South America. In Europe WNV has been found in France, Italy and Austria recently. A first study of the WNV surveillance in Germany demonstrated the presence of WNV-antibody reactive migratory birds which have flown into Germany.

The main objectives of this study were the surveillance in migratory birds as possible means of virus spread and the role of resident birds as an indirect indicator for the endemic presence of the virus including domestic poultry and horses. We have developed diagnostic procedures based on the detection of virus genome copies and on the presence of antibodies in the animal. Viral genome sequences are detected in field samples (blood, tissue samples) by using inhouse realtime RT PCR systems which are capable of amplifying both WNV-subtypes as well as the closely related Kunjin-virus. For the serological investigations, immunofluorescence techniques and a microtitre virus neutralization test are used in combination with an inhouse indirect ELISA based on recombinant domain III of the E-protein.

We have investigated more than 2.700 samples (serum/plasma/tissue) from migratory and resident birds (with emphasis on Passeriformes (incl. crows) and storks) and about 1.000 serum samples from domestic poultry and nearly 1.200 serum samples from horses in Germany.

In essence we found antibody carriers in migratory birds, but not in resident birds/domestic poultry or in local horses. Similarly, WNV-specific nucleic acid could not be found in any of the samples. There is no indication at presence for WNV infections in Germany. However, given the potentially increasing infectious pressure in regard to arbovirus infections a state-of-the-art monitoring system for WNV infections in animals in Germany is mandatory to be continued.

Molecular detection of West Nile virus lineages 1 and 2 using two new sensitive quantitative real-time RT-PCR assays

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West Nile virus (WNV) is an arbovirus, and belongs to the genus *Flavivirus* in the family *Flaviviridae*, which is transmitted by birds and *Culex* spp. WNV was isolated for the first time in Uganda in 1937 and was only found in the Old World until recently. However, in 1999 WNV emerged in the New World, when it was detected during an outbreak of encephalitis in New York City. Since the year 2000 WNV has spread across North America and into Central America. In Europe only spatially (and in most instances also timely) limited WNV outbreaks happened in southern and eastern European regions up to now. WNV has two genetic lineages: lineage 1 has been isolated in North America, Europe, Africa, Asia and Australia. Lineage 2 comprises WNV strains from sub-Saharan regions of Africa and from Madagascar and more recently in Austria.

Two new quantitative real-time reverse-transcriptase PCR (qRT-PCR) assays for the detection of WNV strains from lineages 1 and 2 were developed and compared to 3 previously published qRT-PCR assays. In the new assays two different primer and probe sets are used, which annealing at different regions of the WNV genome: in the highly conserved 5' NTR segment (assay 1) and in the NS2 region (assay 2). The analytical sensitivity of all assays was evaluated by testing 10-fold culture supernatant dilutions of 4 WNV strains, consisting of lineage 1a (NY99), 1b (Kunjin) and 2 (Uganda, Sarafend), which were passaged on Vero cells. Under these conditions the here presented new qRT-PCR assays were generally more sensitive and in contrast to the previously described methods detected both lineages equally well. Moreover, we have designed a novel external calibrator for the determination of WNV genome copy numbers which is based on synthetic RNA, harboring the target sequence of assay 1. The synthetic RNA includes specific guanine/cytosine exchanges and can be detected by three different probes targeted to either the synthetic calibrator RNA sequence, the original WNV RNA sequence or to both templates.

In summary, we have established two new quantitative real-time RT-PCR assays with comparable and excellent analytical sensitivity that performed well for WNV viral RNA. The parallel use of two primer and probe sets, which anneal at different regions of the WNV genome enables the independent, highly sensitive and specific detection of lineage 1 and 2 WNV viruses. The spread of West Nile Virus in the western hemisphere after its detection during an outbreak in 1999 in New York requires the establishment of a high-throughput detection system, which rapidly detects acutely infected animals or individuals. A validated and field-tested assay could therefore have great utility to prevent transmission and disease.

Feeding preferences of WNV vectors and diversity of trypanosomatids of *Culex* mosquitoes

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Changes in the distribution of vector-borne disease are one of the most important results of climatic changes. In the Czech Republic, human cases of WNF as well as WNV infected mosquitoes were reported from Southern Moravia. To evaluate the role of *Culex pipiens* and *Cx. modestus* as potential vectors of WNV, a mosquito surveillance program was conducted from 2004 to 2008. Mosquitoes are known as vectors of various pathogens all over the world including viruses (e.g. WNV spreading recently through USA and Europe) and parasites (e.g. filaria and animal trypanosomes). In addition to this, monoxenous kinetoplastids have been described from several groups of invertebrates, including bloodfeeding insects (mosquitoes, biting midges, fleas and sand flies). However, information about prevalence and diversity of trypanosomatid species in mosquitoes is limited.

Mosquitoes were captured by CO₂-baited CDC traps at five localities in the Czech Republic. Females were placed in pools of one to 50 specimens (sorted by species, locality and date) for isolation of DNA and/or RNA and subsequently tested for various pathogens by specific nested or semi-nested PCR assay. Part of females was dissected and those who were positive for trypanosomatids were used for parasite cultivation. Source of blood were determined by sequencing of cyt b gene.

Altogether 81.757 mosquitoes of 23 species and 5 genera were identified; two species (*Anopheles hyrcanus*, *Culiseta fumipennis*) were recorded for the first time in the Czech Republic and originally rare Mediterranean thermophilic species *Cx. modestus* become common in wetland areas in Bohemia during the last years and nowadays represent one of the dominant species in many places. Our findings correspond with a theory of vector spreading to new localities as a result of climatic changes. To evaluate an infection risk, transect lines of CDC traps were used. Both mosquito species *Cx. pipiens* and *Cx. modestus* are not retracted to reeds only but could search for hosts in distant biotopes up to several hundred meters faraway. The role of both *Cx. pipiens* and *Cx. modestus* as important bridge vectors was supported by our results related to WNV infection (six positive pools of *Cx. pipiens* and four of *Cx. modestus*) and by feeding preferences, since willingness to feed on both birds and mammals plays an important role in the WNV cycle. Finding of the Rabensburg virus, a member of WNV family, in Bohemia and Moravia demonstrate spreading of the virus into new localities.

Altogether, 6.595 mosquito females (152 pools) were tested by PCR and 365 specimens were dissected for presence of kinetoplastids. Obtained isolates represent eleven groups/species of kinetoplastids, four dixenous of the genus *Trypanosoma* and seven monoxenous belonging to four different genera.

Mosquitoes (Diptera: Culicidae) from the Danube Delta Biosphere Reserve and their involvement in West Nile virus (Flaviviridae) circulation

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The mosquito fauna in relation to the presence of West Nile virus circulation in the Danube Delta Biosphere Reserve have been investigated in 2005 – 2009.

A number of 26 mosquito species have been recorded and their annual and seasonal dynamics in the study sites both inside the delta and near lagoons have been observed in relation to the climate evolution.

Culex pipiens, *Coquillettidia richiardii*, *Ochlerotatus caspius* and *Anopheles maculipennis* s.l. have been found positive for West Nile virus by RAMP test for this virus in parallel with the confirmation of its circulation in the area by sero-conversion in sentinel birds and the presence of antibodies against this virus in local populations of birds and horses. The passing of West Nile virus over the winter in Romanian climate seems to be possible since the presence of this virus in over-wintering mosquito females has been detected. In addition, the detection of West Nile virus in *Culex pipiens* males suggests the vertical transmission of the virus.

The aspects of the involvement of the mosquito fauna in the persistence of West Nile virus in the Danube Delta Biosphere Reserve are evaluated.

Mosquito vector species in Belgium

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Spatial distribution of adult mosquitoes is associated with various environmental and climatic factors. These factors can produce significant differences in the distribution of adult mosquito populations which may have important implications for current risk on (re)emergence and epidemiology of vector-borne diseases and the formulation of control strategies. Knowledge of the taxonomic and functional biodiversity and spatial distribution of both endemic and invading mosquito species as well as the factors driving change is missing in Belgium. Therefore, the project MODIRISK started in 2007 to inventory endemic and invading mosquito species in Belgium, to assess the population dynamics of selected endemic and invasive mosquito species and their interrelationship and to develop spatial mosquito distribution models.

In total 22 species (26,990 specimens) were collected during sampling campaigns in 2007 and 2008 of which 20 endemic and 2 exotic species. The most omnipresent species was *Culex pipiens* s.l. which was observed in almost all collection sites. Other important vector species include amongst others *Aedes vexans*, *Anopheles maculipennis* s.l., *An. plumbeus*, *An. claviger* and *Coquillettidia richiardii*. This poster will focus on the identification of « vector community zones » i.e. zones where vector communities could be identified based on different ordination methods taking into account presence/absence, density and main habitat characteristics. The term « community » is here defined as an assemblage of species which occur together in space and time. The results will be discussed in the light of possible risk of disease transmission and vector species monitoring.

Domestic birds – main vertebrate hosts of West Nile Virus in anthropic ecosystems in Romania

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The circulation of West Nile flavivirus (WNV) takes place in cycles between mosquitoes and wild and domestic birds as main vertebrate hosts. The domestic birds have been investigated in 2005 – 2009 for their involvement in WNV transmission and amplification in anthropic environment in 112 rural and urban localities of 15 districts and urban Bucharest. The presence of the IgG antibodies has been investigated on 8333 sera of domestic birds by epitope-blocking ELISA for the detection of serum antibodies to WNV in multiple avian species (Blitvich et al. 2003). The general seroprevalence mean value was 13.6 %. Several micro-foci of very high enzootic WNV circulation in localities of Teleorman, Prahova, Dolj, Tulcea and Giurgiu districts have been put in evidence since almost all the birds in one or in 2 – 3 neighbouring households were sometimes positive in comparison to the rest of the locality. The sentinel chickens installed in April in Bucharest Metropolitan area (urban and rural locations) and Tulcea

showed an increasing rate of seroconversion until July & August followed by a gradual and continuous decrease of the seroprevalence of the antibodies against WNV until the next spring when the seroconversion appeared again. This is the evidence of the disappearance of WNV antibodies in some domestic birds over the winter and a new seroconversion in the next year probably because of the new contact with mosquitoes. The mortality among domestic birds linked to WNV has not been detected in Romania. Our results showed that the domestic birds are very important hosts in the WNV enzootic cycles in anthropic environment in Romania where the transmission to humans takes place. They are widespread in the dwellings of rural type existing not only in rural environment but also in urban areas together with vector mosquitoes, and a high rate of viral amplification is realized. The serological investigations on domestic birds showed their significant involvement in intense and extended circulation of WNV in Romania.

The involvement of wild birds in enzootic circulation of West Nile virus in Romania

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The circulation of West Nile flavivirus (WNV) takes place in cycles between mosquitoes and wild and domestic birds as main vertebrate hosts. The wild birds have been investigated in 2005 & 2008 for their involvement in WNV transmission and amplification both in natural and anthropic environment in 39 rural and urban localities of 7 districts and urban Bucharest. The presence of the IgG antibodies has been investigated on 3376 sera from 43 different species of wild birds by epitope-blocking ELISA for the detection of serum antibodies to WNV in multiple avian species (Blitvich et al. 2003). The general seroprevalence mean value was 15.2 %. The presence of the antibodies to WNV in several species of migratory birds captured in the Danube Delta or in other places in Romania suggests their possible involvement in the occasional introduction of WNV strains from Africa into Romania. The presence of antibodies to this virus was also detected in wild resident birds even in those areas and periods of time when they were not detected in domestic fowl and the disease cases were not registered in humans. This could be a proof of the permanent maintenance and amplification of West Nile virus in local enzootic cycles with the involvement of wild resident birds. The seroprevalence in synanthropic birds (crows, pigeons, doves, sparrows) seem to have an important role in the introduction and circulation of virus in anthropic areas. Many of these birds (especially crows and pigeons) have daily movements between localities and their surroundings because climatic conditions and feeding needs. The investigation of wild birds showed their involvement in WNV enzootic circulations both in natural and anthropic environment.

The horses – indicators of the level of West Nile virus circulation in anthropic ecosystems

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The horses are tangential hosts of West Nile virus in anthropic ecosystems. They represent a good indicator of the level of WNV circulation in the human vicinity. The presence of the IgG antibodies has been investigated in 2005 – 2009 on 6991 sera from 97 rural and urban localities of 9 districts and urban Bucharest by epitope-blocking ELISA for detection of WNV antibodies in domestic mammals (Blitvich et al. 2003). The general seroprevalence mean value was 23.7 %, and the presence of the antibodies was detected over all the investigated territories. The highest seroprevalence mean value (36.6 %) has been detected in Tulcea district (where the virus is introduced by migrating birds in the Danube Delta) comparatively with the areas in the rest of the country. Values even over 60.0 % of the seroprevalence registered especially in the localities situated near the Danube and lagoons and inside the Danube Delta. The values of seroprevalence decrease to the western part of the country, and in the middle of Romanian Plain the values were around 15.0 %. Neurological disorders and mortality in horses because of WNV have not been yet recorded in Romania. The level of seroprevalence of antibodies against WNV in populations of horses of different ages in different areas shows the general level of the WNV circulation in these areas for quite long periods of time. The surveillance of horses can lead to the detection of the territories with a general higher WNV circulation.

Studies on the virus-host cell interaction of West Nile virus

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Species which are naturally infected by West Nile Virus strains show differences in the susceptibility to the virus and in the severeness of disease. Most of infected birds show no symptoms of a disease normally but some species, e.g. crows, are heavily affected meaning the death of the animal in a short time after infection in most cases. Humans as well as horses are ‘dead-end-hosts’. Infection of horses results in a fatal outcome for 40% of infected animals. Infections in humans are mostly subclinical. However, about 20% of infected humans develop the so called West Nile Fever and less than 1% show clinical signs of encephalitis. Differences in the transmission efficiency and outcome of infection may originate in the differences in the molecular interactions of the viral infection. First of all the immunological status of the infected animal plays an important role. The second step is the entry of the virus into cells including the binding of the virus to a cellular receptor. Eventually virus needs to replicate in the infected cell and be released to infect other cells. During the viremic stage differences in all the mentioned steps (immunological status, virus entry, replication and release) can be responsible for differences in the course of the disease.

The aim of our studies is to identify factors determining the susceptibility of vertebrate species to West Nile Virus, therefore we started to investigate the role of the cellular receptor. The cellular receptor was described by Chu and Ng (2004) to be the integrin alpha v beta 3. We have established cell culture models comprising wildtype cells and embryonic mouse fibroblasts which are deficient for the integrin subunits alpha v, beta 3 and beta 1, respectively. These cells were infected with three different WNV strains, namely NY99 (Lineage 1), Uganda (Lineage 2) and Sarafend (also Lineage 2). Results from infection experiments comparing the three strains in their binding and replication capacities in the cell culture models mentioned above are presented.

Vectors of West Nile virus in the Rhone Delta (France): seasonal dynamics and host preferences

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The camargue plain (Rhône Delta, France) is a reserve for wildlife, known for its peculiar landscape of wetland, ponds, marshes and rice fields, and its richness in birds. Outbreaks of equine West Nile fever were also reported in 2000 and 2004. We monitored mosquito populations in different areas where the virus circulated, using carbon-dioxide ice baited traps, bird baited traps and human landing catches. *Culex pipiens*, mainly ornithophilic, and *Culex modestus*, more opportunistic, are the main supposed vectors in south of France. We draw up their seasonal dynamics and host preferences. We observed differences in the pattern of host-vector contact rates and between the different dynamics. The influence of human activities on breeding sites and hosts distribution leading to modifications of the risk of transmission is discussed.

Identification of mosquito species in the Province of Alessandria, Piedmont Italy - a high-risk ecosystem for species composition of mosquitoes

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Exact identification of mosquito species is essential for control of those species which serve as vectors of human and animal infections. A special problem is to identify the representatives of the species complexes, such as *Anopheles maculipennis* and *Culex pipiens*. The species within these complexes are almost or totally similar in morphology of larvae and adults. However, they show considerable differences in ecological and physiological characters, including food preferences. Their epidemiological significance is also different.

The aim of the present work was to identify cryptic species of the *Anopheles maculipennis* and *Culex pipiens* complexes and to study the genetic structure of *Ochlerotatus caspius* in the Region of Piedmont, NW Italy. The mosquitoes, adult and larvae were collected in Alessandria province neighbouring to huge untreated ricefields biotops (these for 100 % *O. caspius*, most of *C. spp.*)

PCR-RFLP assay and identification based on sequence data were used for analysis.

The mtDNA gene COI was amplified using primers LCO and HCO (Folmer et al., 1994) and *Culex* COIF and *Culex* COIR (Shaikovich, 2007). The ITS2 region rDNA was amplified using primers 5,8S and 28S (Porter, Collins, 1991).

After analysis of *Anopheles* spp. with the help of the PCR-RFLP analysis four members of the *An. maculipennis* complex were identified: in Ovada *An. maculipennis*, in Alluvioni Cambio *An. maculipennis* and *An. messeae*, in Rivarone *An. sacharovi* and in Alessandria *An. atroparvus*.

The results of research of *C. pipiens* complex mosquitoes with PCR-RFLP assay (Shaikovich, 2007) based on polymorphism of the 5' end of the mitochondrial gene for COI from 11 gathering (Almost all *C. pipiens* have as breeding site cans, water container, sewage water from urban area) have shown that all of the 48 investigated individual mosquitoes correspond to anthropophilic mosquitoes of form *molestus*.

Sequences of 563 bp of the mitochondrial COI gene (Barcode fragment) of *O. caspius* were obtained for specimens from four populations (Alessandria, Bosco Marengo and Castellazzo Bormida – almost 100% from ricefield area). Three haplotypes were identified. The studied *O. caspius* vary less than by 1 %. The difference between the studied mosquitoes and those from Iran is about 3%. In addition, sequences of ITS2 rDNA *O. caspius* were analyzed from Alessandria, Bosco Marengo, Valle S. Bartolomeo, Alluvioni Cambiò and Castellazzo Bormida population samples. The size of the PCR fragment of ITS2 region was approximate 370 bp. Pairwise distances between samples from different populations varied from 0 to 3 %.

The obtained results indicate that Piedmont is a high-risk ecosystem, since the mosquitoes there may act as primary or secondary vectors generating favourable conditions for development of the vector-borne diseases.

Spatial analysis and molecular studies of mosquito vectors and West Nile Virus in Piedmont, Northern Italy

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West Nile Virus (WNV) is an arthropod-transmitted flavivirus that commonly circulates between mosquitoes (mostly *Culex* sp.) and birds. Horses and humans are considered accidental hosts, and may exhibit symptomatic disease. In Italy, WNV outbreaks were registered in 1998, 2008 and 2009, totaling 16 human cases and 3 deaths. These outbreaks occurred along the Po River, and appear to be expanding geographically. The aims of our study were to identify risk areas for WNV transmission by using spatial analysis of the distribution of *Cx. pipiens*, *Ochlerotatus caspius* and *Cx. modestus*; and to determine the presence of WNV infection in collected mosquitoes.

The study was performed in Eastern Piedmont Region, where both migratory bird routes and extensive rice fields overlap. Adult mosquito collections were performed weekly in 36 trapping stations using CO₂-baited traps operated overnight during May-September for the years 2000-2009. Weather data (temperature, rainfall and relative humidity) were collected from three weather stations within the study area. Modis satellite imagery was employed for landscape characterization. We used the Getis-Ord *G_i** spatial statistic to compare the degree of clustering of mosquito abundance between and within years, as well as to quantify the association between mosquito distribution and weather conditions. Insecticide-based mosquito control measures were also considered in the analysis.

WNV infection was assessed by molecular analysis on pools of 10 mosquitoes collected in one session in mid-July and in two sessions in August 2009. Forty pools of *Cx. pipiens* and 40 of *Oc. caspius* were collected on each session (total, 240 pools) and tested. WNV-RNA presence was investigated by one-step RT real time PCR, identifying NS1-NS2b region. So far, only samples from the July session have been analyzed, with no positive results.

In all years, traps with high catch of *Cx. pipiens* clustered in a zone between the Po River and surrounding rice fields, while low catches of *Cx. pipiens* were clustered on hills. *Cx. modestus* and *Oc. caspius* were each clustered only in two of the ten years. Our GLMM model provided a good fit to the data and underlines the role of weather variation in explaining the spatio-temporal distribution of all species and of the effectiveness of control strategies against *Oc. caspius*.

Our analysis identified high risk areas for WNV transmission in the immediate vicinity of the Po river, and suggest that specific weather conditions and year periods can increase mosquito abundance and WNV infection risk in our study area. Potential changes in weather patterns (e.g., higher summer temperatures, increased frequency and level of extreme rainfall events) may enhance conditions for transmission of West Nile virus (and other arboviruses).

Spreading of *Culex modestus*, Vector of West Nile Virus, in the Dombes, France

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Unlike of what has been witnessed in the American continent where the West Nile virus has increasingly extended over almost the whole continent, there is as yet no evidence that the WN virus is spreading in France. However, it might be interesting within the framework of risk assessment to study the status of *Culex Modestus*, the main vector of WN virus in the Camargue, France [1], in the Dombes, a region still free of WN virus up to now. Indeed, the Dombes area (Rhône-Alpes region, France) is in many ways very similar to the Camargue, a seasonal endemic focus of WN virus. The Camargue and the Dombes are linked

by an important corridor for migratory birds crossing France. About 300 km north-east of the Camargue, the Dombes is a continental mosaic of wetlands with hundreds (about 1400) of fish farming ponds offering an extensive area conducive to mosquito development and suitable for the reproduction, resting and overwintering of migratory birds, putative carriers of arboviruses like WN virus. In addition, the Dombes and Camargue are linked by exchange flows of birds and by the « rhodanian corridor »; where influences of the Mediterranean climate wind up northward. All of these indicate the Dombes as an area potentially at a risk of introduction and circulation of WN virus. Our goal in this study was to estimate *Cx. modestus* abundance in the Dombes and to compare it with the 1970's [2]. In doing so, we aimed to assess the preliminary risk of WN virus to spread out of the Camargue to the Dombes.

Material and methods: Using the pond as the ecological statistical unit, larval entomological survey was conducted to assess the prevalence and abundance of *Cx. modestus* in the ponds. We carried out an ecological screening test consisting in determining whether ponds were positive or negative for the presence of *Cx. modestus* larvae. Our study dealt with a sample of 39 ponds scattered over the Dombes area and for which owners' or managers' authorizations were obtained.

Results: One hundred fifty-one sites from 39 ponds were sampled from April to September 2007, compared with 79 sites from 49 ponds from March to October between 1972 and 1974. Apparent *Cx. modestus* prevalence rates were 85% in 2007 and 22% in 1970s, varying from 11% to 33% between 1972 and 1974 [2].

Conclusion: We found that *Cx. modestus* was more widely distributed over the Dombes area in 2007 than it was in the 1970s. To understand the possible reasons of *Cx. modestus* extension in the Dombes, we discuss the impact of meteorological and anthropic factors on the presence and persistence of larval habitats and, therefore, on population abundance and distribution of *Cx. modestus*.

EuroWestNile collaborative research project: an innovative response to the HEALTH.2010.2.3.-3 call of the 7th Frame Program

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West Nile virus (WNV) is one of the most evident examples of emerging/re-emerging pathogens one can put forward, which is characterized by occasional virulent epizootic outbreaks. Despite intensive research done since its first appearance in the Americas in 1999, many aspects of its molecular biology, epidemiology, ecology, pathogenesis and life cycle are still poorly understood. Being a generalist pathogen par excellence, its eco-epidemiology is extraordinarily complex, involving hundreds of different vectors and hosts, which differ between locations. In addition, as other RNA viruses lacking proofreading replication, its genome is highly variable and consequently of extraordinary plasticity, which means huge evolutionary and adaptation capacities. As a result, many WNV variants have evolved independently in different parts of the world. As the virus moves from one area to another, either by nature, through migrating birds, or by human influence (commerce and/or other activities), different WNV variants (lineages) from different origins can coexist and co-evolve in a particular area. This is the case in Europe, with at least five of seven WNV lineages identified to date. This situation is peculiar to Europe, and clearly different from that of North America. However, most studies on WNV currently come from North America, biasing the knowledge available not only toward the virus there -a serious bias with important consequences influencing, for instance, diagnostic methods- but also to the WNV ecology in hosts and vectors.

In the EuroWestNile project we have submitted to the HEALTH.2010.2.3.3-3 topic of the 7th FP, we propose to complement the work of the EDEN-next initiative undertaking comprehensive studies on the WNV situation in Europe and surrounding affected countries bearing in mind the peculiarities of WNV eco-epidemiology in this region. Moreover we will cover knowledge gaps on its ecology, epidemiology and pathogenesis in Europe. Finally, the project aims to develop new tools and strategies for research on treatment and prevention of WNV disease, as well as to produce new diagnostic methods, taking into account the Euro-Mediterranean peculiarities.

Surveillance for West Nile virus in wild birds from Northern Europe

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A total of 1935 migratory birds from 104 different species were captured in southeastern Sweden in 2005–2006 and tested for antibodies against West Nile virus (WNV). Overall, 46 birds (2.4%; binomial confidence limits, 1.8–3.2) were positive by blocking-ELISA, but only 2 (0.10%; binomial confidence limits, 0.0–0.4) had antibodies detectable by both blocking-ELISA and WNV neutralization test. ELISA-positive birds included long- and short-distance migrants likely exposed to WNV while wintering in or migrating through areas enzootic for WNV. Exposure to a cross-reactive Flavivirus was suspected for short-distance migrants of the Turdidae family, but no cross-neutralization with tick-borne encephalitis and usutu viruses was observed.

Longitudinal surveillance of the exotic species *Aedes j. japonicus* in a second-hand tire company in Belgium

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In 2009, a national mosquito survey (MODIRISK, www.modirisk.be), aiming to study the taxonomic and functional biodiversity of both endemic and invasive mosquito species has reported the introduction and establishment of an invasive mosquito species *Aedes j. japonicus* in Belgium. *Ae. j. japonicus* specimens were first collected in 2002 on the premises of a second-hand tire company in the village of Natoye (Namur). New specimens have been sampled using different sampling methods during two consecutive years (2003–2004). Finally, in 2007 and 2008, its presence as adults and larvae was confirmed based on morphological and molecular identification. In 2009, a longitudinal survey of *Ae. j. japonicus* adults has been launched in and around two second-hand tire companies of Natoye using three types of traps: Mosquito Magnet Liberty Plus traps (Woodstream Corporation, Lititz, PA), BG sentinel trap containing the BG-Lure® attractant (Biogents AG, Regensburg, Germany) and CDC gravid trap (model 1712, John W. Hock Co., Gainesville, FL). Sampling for immature mosquitoes was also conducted on the both sites during four visits in May, August, September and October 2009 including potential breeding sites (all water-holding sites) in a perimeter of 500 meters around the sites. The aim of this survey was to verify the establishment, to study the phenology of the species and the dynamics of its population (e.g. the population built up, the peak activity, the number

of generations per year and the seasonality). *Aedes j. japonicus* was only collected in one of the two companies. First appearance of *Ae. j. japonicus* larvae and adult was recorded in June. The majority of specimens were collected in July, August and September. Gravid traps appeared to be the most effective for collecting *Ae. j. japonicus* compared to Mosquitoes magnet and BG sentinel traps. Larvae have been collected from a wide variety of artificial habitats but rarely in natural ones (tree holes and one time in a puddle in the forest). In artificial breeding sites, *Ae. j. japonicus* larvae were frequently found with *Culex pipiens* larvae. To date, it seems that *Ae. j. japonicus* is well-established at least on one site. There are currently no indication that this species present since at least six years in Belgium on that site had spread in the surroundings.

Combining Climate Projections and Dispersal Mechanisms for the Future Development of Aedes Species in Europe

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Aedes vexans and *Ae. cinereus* which are native mosquitoes in Europe are considered to be competent vectors of arboviruses there (Tahyna Virus, Snowshoe Hare virus and only *Ae. Cinereus*: Sindbis Virus). Feeding on birds and mammals, *Ae. vexans* and *Ae. cinereus* are also known to be bridge vectors for West Nile virus (WNV) in other parts of the world such as North America. There WNV lead to an epidemic outbreak ten years ago. The virus conquered the North American continent within a few years after introduction.

New introductions of WNV and an increasing activity in Europe (Austria, Hungary, Italy, Romania and Turkey) were observed. This raises the question whether native mosquitoes of the genus *Aedes* may play a future role as WNV vectors, establishing a natural European foci. Beside biological and genetic factors in the transmission cycle of arboviruses like virus load above threshold, presence of non immune hosts, there are several ecological components which determine the process. Climate change alters thermal and hydrological conditions throughout Europe and will therewith influence also pathogen, vector and host distribution. In case of WNV modified bird migration behaviour may play an interesting role. Furthermore intensity and quantity of extreme climatic events are expected to increase. Human outbreaks of WNV seem to be triggered by above-average and below-average rainfall.

On the other hand, non native, invasive aedine mosquito species (*Ae. albopictus* and *Ae. japonicus*) are of medical importance in Europe, too. *Ae. albopictus* is a competent laboratory vector of at least 23 arboviruses. Both, *Ae. albopictus* and *Ae. japonicus* were detected as highly susceptible to WNV infection in North America and they are in rapid extension of their dispersal area. This spread is strongly driven by human activities, such as transport, travelling and increased connectivity of potential habitats across regions. Beyond, even continents are connected nowadays by fast transport facilities. Introductions happen by chance, but the establishment of viable populations depends on climatic and further environmental habitat restrictions respectively. Some species, however, do not depend on large-scale naturally moist ecosystems for reproduction, but can establish offspring even in small accumulations of water like small containers filled with water. The establishment and adjacent dispersal of *Ae. albopictus* in Southern (since 1986) and Northern America (since 1985) and in Italy (since 1990) and the recent emergence of *Ae. japonicus* in Switzerland demonstrate that these vectors can hardly be controlled. As a consequence early warning systems are needed.

We interlink current and future suitable climate conditions in Europe for aedine species with possible vector invasion pathways like harbours, airports and highways. Moreover introductions of pathogens for example by infected returning travellers as well as by migratory birds are considered. Combining climate projections for vector and pathogen and their dispersal mechanisms for the future development may contribute to the identification of risk areas.

Comparative analysis of sialome and salivary gland proteome from vectors of tropical diseases: selection of protein candidates to evaluate travelers exposure to mosquito bites.

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Exposure to vectors of infectious diseases has been associated with antibody responses against salivary antigens of arthropods among people living in endemic areas. This antibody response could be used to evaluate the human exposure to vector bites, and therefore the protective efficacy of antivectorial devices and the risk of pathogen transmission. The existence and potential use of such antibody responses in travelers briefly exposed to malaria or arbovirus vectors in tropical areas has been little investigated. Thus, we analysed the IgG antibody responses of French soldiers against saliva from two mosquito species belonging to the Anopheles and Aedes genus. A specific immune response to the mosquito genus was described and an immunoproteomic analysis allowed the identification of antigenic saliva proteins which could be considered as markers of travelers exposure to mosquito vectors. To evaluate the inbreeding effect on mosquito salivary protein repertoire, a comparison of saliva and salivary gland proteins from three *Ae. aegypti* colonies (Rockefeller, Paea, and Formosus) was performed. Forty-four and 120 proteins were respectively identified in saliva and salivary glands. Finally, to characterize immunogenic proteins specific to the mosquito species and /or genus, salivary glands proteome from *Anopheles* (*An. gambiae* s.s., *An. arabiensis*, *An. stephensi*, *An. albimanus*), *Aedes* (*Ae. aegypti*, *Ae. albopictus*, *Ae. caspius*) and *Culex* (*Cx. pipiens*, *Cx. quinquefasciatus*) genus were compared using two complementary approaches: (i) 1D SDS-PAGE coupled to MS, (ii) iTRAQ® technology. Based on these results, several mosquito salivary proteins were selected based on their species specificity. These mosquito salivary proteins could be candidate markers to evaluate travelers exposure to vector bites.

Relationship between exposure level to mosquito bites of southeast French individuals and their respective antibody response against mosquito salivary gland extracts.

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The Camargue is an extensive wetland in the southeast of France, which provides abundant breeding sites for mosquitoes. The high mosquito density is responsible of nuisances for people and animals, and is a potential threat to human health. Although entomological methods are well adapted and have proven their efficiency to measure mosquito population "dynamics", these methods are not designed for estimating individual exposure level to mosquito bites. Recently, we have shown that antibody responses against mosquito saliva proteins could be useful to estimate the exposure of individuals transiently exposed to arthropod vector bites. To confirm these results, we have compared the kinetic antibody responses (before, during and after the mosquito exposure peak), against mosquito salivary gland extracts of southeast French individuals living in 3 areas with distinct mosquito density and species (Camargue area: high density of *Aedes caspius* and *Culex pipiens*; Fos-sur-mer city: intermediary density of *Ae. caspius* and *Cx. pipiens*; Marseille city: low density of *Cx. pipiens*). Antibody responses correlate with mosquito exposure level taking into account mosquito density (i.e., living area) and time sera sample collection (i.e., before, during or after mosquito peak exposure). Moreover, we have demonstrated that antibody response seems specific to mosquito genus and species. Collectively, these data suggest that it could be conceivable to use human antibody response to assess individual exposure to bites of mosquitoes that are endemic in France.

Development of a quantitative RT-PCR and Evaluation of replication kinetics of a Cameroon isolate of Yellow fever virus

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Yellow fever (YF) is one of the most significant public health problem in many tropical areas of sub-Saharan Africa, South America, Central America, and the Caribbean. There is an annual incidence of 2,00,000 cases, including 30,000 deaths. Yellow fever virus, the causative agent is the prototype member of genus *Flavivirus* and is transmitted primarily by *Aedes aegypti* mosquitoes. The early and specific detection plays a crucial role in the surveillance of endemic and non endemic areas.

In the present study, a SYBR Green I-based real-time RT-PCR assay for rapid detection as well as quantitation of YFV was developed by targeting the 5'UTR and capsid gene. This assay was found to be 10-fold more sensitive compared to conventional RT-PCR. The specificity of the assay was established through melting curve analysis. The applicability of the assay for screening of YFV infected mosquitoes were initially carried out through spiked mosquito samples. The quantitation of the viral load in samples was done using a standard curve obtained by plotting cycle threshold (C(t)) values versus virus concentration. The detection limit of this assay was found to be 0.05PFU.

The replication kinetics of a Cameroon isolate of Yellow fever virus in *Aedes aegypti* (Rockefeller strain) was examined through the developed RT-PCR. Following oral infection, the replication of virus was monitored daily till day 14 post-infection (pi). The amount of virus varied at different days pi, with the virus titre initially declined up to day 7 pi, followed by an increase. The titre in individual mosquitoes varied greatly with a high of 2×10^3 pfu on day 14 pi. This study clearly demonstrates the potential usefulness of real-time assay for quantifying virus replication and to study vector competence and transmission of YFV in potential vectors from endemic and non endemic areas.

AFR-OTHER MBD

West Nile virus in Tunisia: sero-epidemiological survey in equids

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West Nile virus (WNV) is a mosquito-borne virus that may be responsible for a zoonotic infection. In most cases, human infections remain asymptomatic. However, humans can experience flu-like symptoms (20% cases) and/or neurological conditions (

Yellow fever and its vectors in the Valencian Autonomous Region: Spatial distribution and temporary follow-up of its populations

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Yellow fever was the first hemorrhagic fever to be transmitted by mosquitoes and linked with port cities and international commercial activities, from which Spain has suffered diverse epidemics during its history. The first of them dates from 1701, when the disease was endemic for more than a century, especially in Andalucía where it killed 120.000 people. In 1821, the disease was appeared in Barcelona due to maritime exchange with Cuba, causing the death of a sixth of the population. This, along with the risk of transmission to frontier countries, caused the establishment of barriers as a form of quarantine along the Pyrenees. In the Valencian Autonomous Region, yellow fever attacked the city of Alicante in 1804, causing the death of 17,71% of the population. The last known epidemic outbreak took place in Madrid in 1878.

At the moment, in spite of the minimum frequency of imported cases, the presence of recognized potential vectors added to the continuous increase of spreading to endemic countries (business, leisure, family visits due to immigration…) makes Spain a country at risk of the reoccurrence of this disease.

Even though human cases of yellow fever are usually associated with population increases of *Aedes albopictus* and *Aedes aegypti*, the existence of a third vector with the capacity to transmit the illness to humans, *Aedes vittatus*, promotes even more, if possible, the disease's power to spread. This is why an effective preventive program must be planned according to the distribution, behaviour and evolution of its vectors.

The purpose of this study is the design of a map of distribution and the follow-up of the possible vectors that could transmit yellow fever in the Valencian Autonomous Region (Spain).

The methodology used responds to continuous larval samplings from 2005 until the present day throughout the whole Valencian Region every season of the year. To establish behaviour guidelines and population fluctuations on those positive points for any of the potentially transmissive species of the virus would help,

if necessary, to take control measures at the right moment. Every focus detected is characterized (type of focus, moment of capture, larval density) and is georeferenced by GPS coordinates. The captured samples are kept in a 70% alcohol solution and are identified by binocular magnifying glass and optic microscopes according to the criteria described by authors such as Encinas Grandes (1982) or Schaffner et al. (2002).

As a result, the presence of *Aedes albopictus* and *Aedes vittatus* has been detected in various localities of the province of Alicante and Castellon, respectively. Both are recognized transmissive species, being *Aedes albopictus* the principal and most effective vector responsible for the maintenance of the transmissive urban cycle. *Aedes vittatus* actively participates in jungle cycles in African countries.

Anti-mosquito saliva antibodies as a tool to quantify exposure to vectors bites in domestic animals

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Epidemiological surveys of vector-borne diseases are still in need of accurate tools allowing to monitor the pressure of exposure to the pathogens. Entomological methods are the gold standard but are difficult to implement at a satisfactory scale. As an alternative tool, our team has already explored the anti-mosquito saliva antibody response of exposed humans as a potential marker of exposure to bites and to the resulting morbidity. Since domestic animals can interfere with the epidemiology of such diseases if their vectors are zoophilic, we decided to investigate if anti-mosquito saliva antibodies can be markers of exposure also in animals. Seric IgGs from different species (bovines, equines, caprines, ovines and dogs sampled in Senegal) have been screened for specific antibodies against *Anopheles* salivary gland extracts using western-blotting. Surprisingly, only horses revealed as positive at a high rate (70%). Seven distinct protein groups were recognized with the 72, 115 and 175 kDa bands being immunodominant. The same equine sera were then screened against salivas collected from other mosquito genus. 30% recognized *Aedes* antigens and 60% *Culex*. 25% of the sera reacted to a single extract whereas more than half were reactive to at least two antigens and one quarter to the three of them. Only two protein bands, of 32 and 72 kDa, were recognized in *Aedes* and *Culex* extracts suggesting the existence of cross-reactive antigens. But five other antigens recognized in *Anopheles* extracts appeared to be *Anopheles* species-specific. Season and animal location were factors influencing the antibody profiles. In conclusion, horses develop specific antibodies against mosquito saliva at a high rate. Preliminary data indicate that these antibodies could be a valuable tool to estimate the level of exposure to vectors bites.

Monitoring of *Aedes albopictus* (skuse, 1894) in eastern Spain

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Since de first detection of *Aedes albopictus* in Spain in 2004 (Sant Cugat del Vallès [Barcelona], NE Spain) the expansion of the species has been constant throughout three coastal provinces of the Catalanian region. However, in 2005 *Ae. albopictus* was recorded in the south of the province of Alicante (Orihuela [Alicante], SE Spain), around 400 kilometers south Catalonia. After 4 years with no information available on the status of the southern distribution of *Ae. albopictus*, in 2009 we caught a few larvae of tiger mosquito in the neighbouring town of Torreveija, just 30 kilometers from Orihuela.

Taking into account that the spreading of mosquitoes along the Mediterranean Highway (A7) is well documented, we started the surveillance of *Ae. albopictus* in several cities surrounding the A7. The optimal connection that A7 offers between Spain and other Mediterranean countries with a high presence of Asian tiger mosquitoes as well as Italy or France, justifies the decision to give these monitorings priority. Our catching methods were based on larval samplings in small recipients and adult captures using the BG-Sentinel trap.

Until November 2009 the presence of *Ae. albopictus* was only recorded in Orihuela and Torrevieja. According to the European Centre for Diseases Prevention and Control (ECDC) these findings imply the most southern distribution of *Ae. albopictus* of non-insular Europe. Consequently there are many exciting aspects to study which will be discussed such as the duration of the activity periods and the number of generations that the Asian tiger mosquito can display in this hot and dry area.

a biometeorological population dynamic model for *Aedes albopictus* (Skuse) adapted to urban italian (Emilia-Romagna Region) context

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Aedes albopictus had surely appreciable impact among new introduction species in European Mediterranean area and in the last twenty years *Aedes albopictus* has been having an extraordinary diffusion. *Aedes albopictus*, beyond giving discomfort to community, is an important vector of tropical virus, ethiological agents of many infectious diseases (e.g., Dengue and Chikungunya fever), increasing health risk even in European countries across Mediterranean sea. This alarm contributed to form in 2007 a multidisciplinary working group directed by the Public Health Department of Emilia-Romagna Region in order to study and improve the knowledge about the mechanisms of adaptation and colonization by *Aedes albopictus*, characteristics of breeding sites and vector control strategies. For this purpose, a biometeorological population dynamic model developed by Otero et al. (2005) from the University of Buenos Aires has been investigated, adapted and modified to Italian urban context. The model, called BiTE (Biometeorological Tiger mosquito Extimator), can be fed either with observed meteorological data or directly from weather forecasting numerical models. A statistical calibration algorithm has been created for a better representation of water temperature of a standard catch basin that is the main responsible for the urban dynamic of *Aedes albopictus*. This dynamical model takes into account 5 age classes (eggs, larvae, pupae, female adults not having laid eggs and female adults having laid eggs) originating from a finite number of breeding sites with equal physical characteristics. The number of breeding sites is the fundamental environmental parameter regulating population dynamic. The 5 age classes are described with deterministic equations, each modelled by a stochastic Poisson process. Some model settings are based on appropriate monitoring survey carried out on four Emilia-Romagna cities through the use of ovitraps, breeding sites detection (pupal demographic survey) and taking into account specific type of vector control strategies. Also the diapause is modelled because it is one of the most important adaptation strategies of this specie at temperate climate.

The model has been tested and validated over two years (2007 and 2009). The results confirmed that the BiTE model represents a good support to carry out vector control strategies as well as an *Aedes albopictus* infestation forecasting model.

Epidemic in Italy during summer 2007

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During the 2007 summer, Italy experienced an epidemic episode caused by the Chikungunya virus (CHIKV), which has been the first Chikungunya outbreak documented in a temperate climate country. *Aedes albopictus* was the proved vector for this outbreak.

The aims of this study were: 1) to investigate the transmission potential of the CHIKV; 2) to evaluate the effectiveness of the vector control operations performed during the outbreak in limiting the epidemic.

In order to achieve such goals, a mathematical model of CHIKV transmission dynamic was developed. Basically, this model accounts for hosts (humans) and vectors (*Aedes albopictus* adults), which are both divided into epidemiological classes (e.g., susceptible, exposed, infective and immune agents). The epidemic transmission process is modelled by an ordinary differential equations system assuming homogeneous mixing among different classes. As one can expect, a crucial role in the spread of the epidemic is played by the density of the vector at each time. For this reason a biometeorological population dynamic model for *Aedes albopictus* has been used. This model, an adapted version of the model developed by M. Otero and H.G. Solari (2005), can be fed either with observed meteorological data or variables obtained from weather forecasting numerical models (temperature and relative humidity). This dynamical model takes into account 5 age classes (eggs, larvae, pupae, females not having laid eggs, and females having laid eggs) originating from a finite number of breeding sites with equal physical characteristics. The 5 age classes are described with deterministic equations, each modelled by a stochastic Poisson process. The model run with different numbers of breeding sites (100, 150, 200 / hectare) to describe a densely populated environment, either taking into account the control measures, or without.

Simulated adult mosquitoes densities in different model configurations were assimilated in the epidemiological model in order to describe the impact of control measures in the epidemic's evolutions.

Our results suggest that the transmission potential of CHIKV in Italy was similar to the one observed in tropical regions where larger Chikungunya epidemic occurred (e.g., Reunion Island).

Moreover, our analysis demonstrate the efficacy of the vector control actions performed during the outbreak, which drastically reduced the final size of the epidemic.

Therefore, our analysis shows that, even if the transmission potential of CHIKV in a temperate climate country can be sensibly high, an epidemic outbreak can be controlled by performing a timely intervention to reduce the local vector density.

Nuisance and health risk: improvement of the monitoring system of *Aedes albopictus* in Emilia-Romagna

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Monitoring by means of ovitraps is an indirect surveillance method that can provide information about the development of the adult population.

Since the first detection of Asian Tiger mosquito in Emilia-Romagna, the system of entomological surveillance has been improved. The outbreak of Chikungunya fever occurred on August 2007 in Emilia-Romagna made a significant contribution to this development. In the second half of 1990s, after the first detection of *Aedes albopictus* in Italy, few ovitraps were placed in their preferred breeding grounds (tyres)

in order to detect the vector. In 2005 the Public Health Department of Emilia-Romagna promoted the Regional plan for the Asian Tiger mosquito control and the prevention of Chikungunya and Dengue fever with the aim of reducing the nuisance to citizens. A telephone survey, conducted in 2005, showed that 47% of respondents had been forced to change their habits spending less than an hour a day in their home garden. In 2006, the Regional Working Group promoted the use of a better standardized strategy among the Municipalities and Local Health District.

In the monitoring plan of 2007 (before the outbreak of Chikungunya fever) about 1,600 ovitraps were placed in Emilia-Romagna region.

After the epidemic monitoring was extended, with the aim of collecting homogeneous data that could provide quantitative information over large areas such as all the provinces and the major urban centers.

Besides the entomological surveillance system, health surveillance was strengthened. Before 2007 outbreak every suspected case had to be reported to Health Authority and a case was defined suspected if the person had the clinical symptoms and had travelled, in the previous 15 days, abroad in a country where Chikungunya is endemic. In 2008 the system of mandatory reporting of suspected cases, during the seasonal activity of the vector, was based on the only presence of clinical symptoms without taking in account previous travels.

Besides the resources employed to manage the clinical cases of disease, the health impact of 2007 Chikungunya outbreak included the surveillance of blood donations and organs and tissues donations. Since 2008 the monitoring has been conducted with a standardized methodology and well-defined criteria in each phase of the project. In 2008 and 2009 were placed in 2,741 and 2,606 ovitraps, respectively. The overall data for the region show a substantial stability of population density in the 2008-2009. The surveillance and the tiger mosquito control has been supported over the years by a widespread communications campaign with the aim of increasing public awareness and encouraging citizens and local community participation.

Aedes albopictus population density threshold for the transmission of Chikungunya virus in Italy

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Aedes albopictus was responsible for a Chikungunya epidemic which took place in Northern Italy in the summer 2007.

During the season 2008, in four cities of the Emilia-Romagna region, we analyzed the correlations between the eggs density obtained by standard ovitraps, the Pupal Demographic Survey (PDS), the Human landing Collection (HLC) and the number of bites/human declared by citizen in a survey.

The optimal sampling plans for ovitrap monitoring were defined in each city by applying the Taylor's power law model with a level of accuracy $d=0.2$. The PDS was conducted by inspecting a random sample of 3,509 premises, during which the owners were asked for the number of bites supported the day before (2,357 valid answers).

In 20 green areas HLC sessions (15 minutes in the period 6.30-7.00PM) were conducted three times during the season (June, August, September) by 5 technicians.

Good correlations were obtained between the number of pupae/ha (PDS) and the number of eggs collected with ovitraps one week later ($R=0.86$ and p

Comparison of surveillance methods for the adult Asian tiger mosquito, *Aedes albopictus*.

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Mosquito surveillance may have different aims, ranging from obtaining a comprehensive species overview to the early detection of invasive species and finally to the surveillance of vector populations in the disease risk assessment. Depending on these aims, different tools and methods can be used either alone or in combination (i.e. sampling mosquito eggs, collecting larvae and pupae and catching adults). One of the most important potential disease vectors in Europe is the Asian tiger mosquito, *Aedes (Stegomyia) albopictus*, an invasive species that is spreading across much of Europe. While the results of ovitraps monitoring give a good indication of the current distribution of this mosquito, capturing adults is especially meaningful in the study of population dynamics and the assessment of the disease risk. The efficacy of the trapping technology for adult Asian tiger mosquitoes (and other members of the subgenus *Stegomyia*) is improved greatly with the development of the BG-Sentinel, a trap that can be used without carbon dioxide (Meeraus et al., 2008; Roiz et al, 2010).

In our presentation, we compare ovitrap, human catching collection and BG-Sentinel surveillance data from two studies on the effects of mass-trapping and/or larviciding on *Aedes albopictus*. The studies were performed in the Italian cities of Cesena (Emilia-Romagna), and in Montecatini Terme (Tuscany). While ovitraps are cheap, easy-to-use, needing neither electricity nor much time in the field, both the human landing rate and the BG-Sentinel traps were most sensitive indicators for the adult *Ae. albopictus* density at a given point in time. They allow for a fine comparison of population dynamics, for example with weather data and site-specific characteristics.

A comparison between the capture rates showed that the BG-Sentinel without carbon dioxide collected about as many mosquitoes in 24 hours as human volunteers in 0,5 to 1,5 hours during the times of maximum *Ae. albopictus* activity. The traps captured both female and male mosquitoes. In areas without mosquito control measures, they caught 40% nulliparous, 30% parous and 30% gravid *Ae. albopictus* females (Cesena) and 8% nulliparous, 52% parous, 40% gravid females (preliminary data, Montecatini), while human volunteers captured 56% nulliparous, 37% parous and 7% gravid females (Cesena) and 35% nulliparous, 50% parous and 15% gravid females (preliminary data, Montecatini). About 10% of the mosquitoes captured with BG-Sentinels in Montecatini were bloodfed. Besides *Aedes albopictus*, the traps also collected significant numbers of *Culex*.

(The study in Montecatini was made possible with support from Entomox (Ditta di disinfestazione Srl, Pisa), I.N.D.I.A (Industrie Chimiche S.P.A, Padova), “Società Terme di Montecatini”, “Consorzio di Bonifica del Padule di Fucecchio”, and citizens and hotels owners in the study areas.)

Aedes albopictus (Skuse, 1894) (Diptera: Culicidae) in France: gonotrophic cycle, fecundity and longevity

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Aedes albopictus, the Asian tiger mosquito, is colonising Mediterranean countries and is established in the French Riviera since 2004. Being a vector of arboviruses, it is already accountable of an outbreak of chikungunya in Italy (Reza et al., 2007). In order to better understand the vectorial risk, it is necessary to study the life history traits of local populations of the species, which is characterized by a wide variability and a strong adaptation capacity. This study concerns the gonotrophic cycle length, the fecundity and the longevity of *Ae. albopictus* (Strain AM), three parameters having a major impact on the vectorial capacity. The length of the first gonotrophic cycle is 5.7 days and 3.9 days for the following five cycles. The first oviposition is more voluminous than the followings ones, although the fecundity doesn't seem to be affected by physiologic age. Adult mortality increases with age (survival type I). To conclude, *Ae. albopictus* AM is characterised by a prolific fecundity, a gonotrophic cycle of medium length and a high longevity. All these characteristics strengthen the Asian tiger mosquito's vectorial capacity, and confirm its potential role as a vector in metropolitan France.

Distribution of the genus *Culicoides* (Diptera: Ceratopogonidae) along the Scheldt and its tributaries in Flanders with special attention for the pest species *Culicoides riethi*.

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Culicoides or biting midges are worldwide best known as vector of viruses pathogenic for various mammals, such the bluetongue virus (BTV). As they often occur in swarms, they can be a nuisance to humans, especially for people that react allergic to their bites.

At 85 sites along the Sea Scheldt (maximal distance from the river 9 km) a Mosquito Magnet Liberty Plus (MMLP) operated one week during the period May-October of 2007 and 2008. These samples of bloodsucking insects (mainly *Ceratopogonidae* and *Culicidae*) were taken in the framework of the MODIRISK-project; Mosquito vectors of disease: spatial biodiversity, drivers of change and risk (2007-2011) (see www.modirisk.be). In this project a network of MMLP traps was used throughout Belgium in a grid-based (10 x 10 km) sampling approach in which different habitats (urban, agriculture and natural) were sampled in each of the grids.

Additionally, a more detailed study on the biology and ecology of *Culicoides riethi* has been done in Ghentbrugge, which locally has been the cause of severe nuisance since the summer of 2005 (Versteirt et al., 2009).

In 48 of the 85 sites along the Scheldt and its tributaries, one or more *Culicoides* species were found. A total of 1244 *Culicoides* individuals were counted in these samples and 17 of the 45 Belgian known *Culicoides*

species (Deblauwe et al., 2009) were found. Hence, we can conclude that the MMLP seems to be an efficient trap to collect a large range of Culicoides species.

The following 5 species were the most abundant: *C. chiopterus*, *C. dewulfi*, *C. riethi*, *C. obsoletus-scoticus* complex and *C. punctatus*. Some species are candidate vectors of bluetongue virus.

The effect of contrasting water management regimes on mosquito population dynamics in a wetland habitat in the United Kingdom

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The United Kingdom is presently ill-equipped to monitor, predict and mitigate the impacts of climate, socio-economic and land use change on the distribution and incidence of mosquitoes (Diptera: Culicidae) and mosquito-borne pathogens (MBPs). Numerous predictions regarding the introduction and establishment of alien mosquito species and the emergence or re-emergence of MBPs in Britain have been published. These studies largely relied on ecological and epidemiological data from elsewhere because recent information on the distribution and abundance of mosquito species and MBPs in Britain is patchy or non-existent. Within the UK plans are afoot to increase the area covered by wetland habitat for biodiversity enhancement and conservation as well as flood management. Such habitats are likely foci for the maintenance and introduction of MBPs since wetlands support abundant populations of mosquitoes (putative vectors) and migratory birds (potential reservoir hosts).

We are undertaking a three-year study at the largest wet grassland in the UK, the Somerset Levels and Moors (SLMs). We aim to contribute baseline data on culicid distribution, to identify significant biotic and abiotic factors related to mosquito population dynamics, and also to assess the effect of contrasting hydrological management regimes on these factors. A government-funded scheme, established in 1987 at the SLMs, pays farmers to manage water levels at one of three tiers. Crudely, tier 1 is drier and tier 3 is wetter and more like natural wetland conditions.

SLMs habitat types include coastal grazing marsh, reed beds and wet woodland. Our study focuses on coastal grazing marsh which represents the most common lowland wetland type in the UK. Eighteen sites maintained under two contrasting water management regimes, tier 1 (n=9) and tier 3 (n=9), were selected and sampled for adult mosquitoes using CDC CO2 black light traps. Within each site, four randomly selected stream plots were surveyed for aquatic mosquito life stages using a standardised dipping methodology. Details of emergent and submergent vegetation, potential predator taxa, as well as a range of physicochemical factors were recorded. GPS coordinates were taken to allow ground based and remotely sensed climate and vegetation measurements to be related. All sites were visited in mid-May, at the end of June and end of August.

During the first field season in 2009 over 4600 immature and adult mosquito specimens were collected and identified to species level. A total of 11 species were identified. The most abundant species during all three sampling periods on both water tiers were *Culex pipiens* (47%) and *Culiseta annulata* (38%), both involved in MBP transmission in other countries. Other putative vectors found on the SLMs include *Anopheles claviger* (5%) and *Coquillettidia richiardii* (4%). Multivariate statistical analysis of associations between abundance of mosquito species and biotic and abiotic factors will be presented.

Which is the role of the ticks in the maintenance of the virus West Nile in Senegal (new assumption about the maintenance of the virus in West Africa)

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Studies brought back in the different geographical zones where manifests the virus West Nile lead to the notion of cyclical or temporary activity of the virus with sometimes evolution towards a visible disappearing of activity. There is not evidence of the possible maintenance of a latent virus between seasons in a vertebrate guest reservoir and the regular reintroduction every season by migratory birds always remains a hypothesis. They assumed that in Europe the reservoir was entomological with the persistence of the virus in mosquitoes falling into hibernation, but their infectiosity must be deficient to restart the circulation of the virus in the absence of vertebrate reservoir. In the Senegal, we isolated sequences of basic pairs belonging to the virus West Nile from two lots of ticks of the cattle (*Hyalomma marginatum rufipes* and *Rhipicephalus evertsi eversti* larval stadiums of which are ornithophilic), at the end of the dry season where the activity of culicid fauna is quite void and in a region very distant from the delta of the river Senegal where meet birds migrants. Moreover, researches on serological prevalences at the horses show rates of IgM against the virus West Nile well height at the end of the dry season, before the arrival of rains and mosquitoes. Given the rarity of vectors mosquitoes during this period, they can envisage safekeeping in all seasons and space dispersion of the virus

West Nile by the adult ticks by the herds of cattle, which circulate, between the distant watering places during

transhumance. Then, in rainy season with strong culicid density at the level of the resting place which are pools where the cattle and the horses come to drink, then the virus will be able to outbreak itself with mosquitoes and ornithophilic larval stadiums of these ticks who will bites birds. The results of these observations in the Senegal offer new research orientations with an inter-seasonal cycle of assertion of the virus West Nile in the ticks. It will be necessary to look what is indeed the role of ornithophilic larval stadiums of these kinds of ticks potentially vectorial and that is the role of that ornithophilic mosquitoes in the rain season. On the other hand, it will be necessary to look for if it exists a trans-stasial and trans-ovarial transmission of the virus West Nile in the ticks in nature.

Validation of an Immuno-Epidemiological Tool Assessing Human Exposure to *Aedes albopictus* and *Aedes aegypti* Bites

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Aedes mosquitoes which are among the main vectors of mosquito borne diseases are currently expanding. Both *Aedes* mosquitoes and mosquito borne diseases that they transmit are currently expanding in Europe. That situation stresses the need for accurate monitoring of these vectors populations.

We aim to develop new methods to evaluate Human/Vector contact by immuno-epidemiological tools complementary of entomological methods.

In this way, we have shown that the evaluation of human antibody response to mosquito salivary proteins is a valuable tool to measure exposure to these vectors. In this study, our purpose is to evaluate human IgG response to *Aedes* salivary proteins, in order to get an insight of the population exposed to *Aedes* bites. In addition, this biomarker could be useful to discriminate exposure to *Ae. aegypti* and *Ae. albopictus* bites both present in ultraperipheric areas of European Union and mains vectors of arbovirus.

Our results indicate that assessing human IgG anti *Aedes* whole salivary proteins can be used to detect individual exposure to vector bites and by this way can help to evaluate the risk of pathogens transmission. The sensibility of the used biomarker shows that it could be possible to detect low level of exposure to *Aedes* bites. This propriety can be useful in European environment where, mosquitoes density is low and difficult to detect by traditional entomological technique.

In addition, we notice no systematic IgG cross reaction between *Ae. albopictus* and *Ae. aegypti* salivary proteins. This particularity may be used to discriminate exposure between these two vectors.

Further investigation will focus on identification of specific immunogenic salivary protein of *Ae. albopictus* and *Ae. aegypti*. This biomarker could be used for monitoring emerging *Aedes* borne diseases and to evaluate efficacy of vector control program in European environment.

Quantitative analysis of spatial transmission for bluetongue virus serotype 8 in western Europe in 2006

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In 2006, NW-Europe was surprised by an introduction of a bluetongue virus serotype 8 (BTV-8), which spread effectively among sheep and cattle herds, in The Netherlands, Belgium and Germany. To learn more about the dynamics of this epidemic, we quantified the spatial transmission by estimating a spatial kernel. Because outbreak control measures were not uniform in the different countries, we estimated the kernel for the total outbreak, but also for the different countries separately.

It appeared that the transmission of BTV-8 occurred over a much larger distance than found in previous analyses, i.e. the Foot and Mouth Disease (FMD) 2001 epidemic and the Avian Influenza (AI) 2003 epidemic in The Netherlands. Furthermore, long distance transmission was far more important in Belgium than it was in the other two neighboring countries. During the BTV-8 epidemic the control measures on animal transport were more restrictive in Germany and The Netherlands in comparison with Belgium. During the earlier FMD and AI epidemics they were even more restrictive.

Linking those observations with the differences in applied control measures, it seems that more restrictive spatial control measures in the infected area kept the transmission at a more local level.

Behaviour of *Aedes albopictus*, population from La Reunion Island

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Aedes albopictus is present worldwide, from Asia to Europe, the Indian Ocean region, South Africa and North America. This mosquito is the major vector of dengue and also the major vector of Chikungunya virus outbreak which occurred in 2005, 2006 and 2009. It is also the vector of other diseases. Its rapid spread in Europe is a major concern. It has been recorded in Italy 10 years ago, and after invading the whole of Italy, *Ae. albopictus* is now in most of the northern mediterranean countries from Italy to Spain. It has been observed in the French Alps where it has established a colony in 2009. It is able to invade most of Europe despite vector control measures. The use of insecticides has no effect on its establishment in Europe. A research project assessing the feasibility of a sterile insect technique as an alternative vector control

measure is under progress at La Reunion Island. This presentation includes the first results on the biology of this invading species, and focuses on the fecundity of males and their ability to disperse.

Their mating ability has been tested with several batches of females under laboratory conditions. Tests were conducted in large and small cages with 2, 10 and 20 females. Females were used during all the experiment or removed every 24h. All females were desiccated in order to observe their spermathecal capsules and assess whether they were mated or not. In regard to the different methods used during this experiment, results showed that *Ae. albopictus* male was able to inseminate an average of 9-10 females with a maximum of 16, with a constant mating rate during the first 7 days. A decrease of their mating ability was then observed until day 10. After this date, males did not mate females.

In the SIT program, before any hypothetic release, flight behaviour, dispersal of released mosquitoes and population of wild mosquitoes must be estimated. The second part of the study focused on the dispersal of males and females *Aedes albopictus* in a semi-urban area which is the most common case on the island. The size of the wild population of *Ae. albopictus* was also estimated during the different seasons. Mice-baited BG-sentinel traps were used to catch mosquitoes in mark-release-recapture experiments carried out every 3 months (March, June, September and December 2009). An overall 5% of released males were recaptured (15% for the females). The dispersal behaviour and the distribution of *Aedes albopictus* showed high disparities. Wild population was estimated between 60 000 and 25 000 on the same released site (40 000m²), depending on the season. Females seemed to disperse more than males but when males were released, they covered more distance. The relationship with their physical environment is also discussed.

Characterization of *Aedes albopictus* breeding sites and evaluation of sampling methodologies for population size estimation in Italy

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Starting from the beginning of the '90s *Aedes albopictus* has intensively colonized Italy.

In the summer 2007 an epidemic of Chikungunya virus started in a small village in the Ravenna province, with secondary small foci in Ravenna, Cervia, Rimini, Cesena and Bologna.

A study to characterize *Ae. albopictus* breeding sites and to evaluate population density estimation indices was conducted in 2008 in four cities (Cesena, Forlì, Ravenna and Rimini) of the Emilia-Romagna region. Each town was divided in 6-7 zones, and in each zone 50 premises were randomly chosen and inspected, for three times during the season (June-August-September). Data collected were: the Premise Condition and Shadow Indexes, breeding sites characteristics and pupal density. Public and private road drains were also sampled.

In 20 green areas, in parallel timing with larval sampling, 5 technicians conducted the human landing collections (HLC) for 15 minutes during the peak of activity (6.30pm-7.00pm).

A standardized monitoring plan by ovitraps was also organized in the four study cities.

The number of ovitraps/city was calculated using the Taylor's power law considering a precision coefficient $d=0.2$.

Cumulatively 3,509 premises were inspected, finding 27,836 potential breeding sites, of which 1,583 resulted active at the inspection, with a total of 44,184 pupae.

Results show that public road drains and private catch basin are the main breeding site typology of *Ae. albopictus* in the area, hosting 94.14 % of the total sampled pupae. In average the premises resulted in good conditions, with only a 4 % showing poor management or abandoned. In this 4 % premises a mean of 2 active breeding sites/premise with 38 pupae/premise were censused.

In this study correlation between the Traditional Stegomyia density indexes (House Index, Container Index, Breteau Index) were significant, while no correlation was obtained between the indexes and the number of pupae/hectare.

Good correlations were observed between the pupal demographic survey data and the mean number of eggs/ovitrap when considering a one week shift of the two data sets, and between HLC and the number of eggs collected in ovitraps the previous week.

Dengue Fever Vector *Aedes aegypti* Control in Brazil: some suggestions for Europe

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Epidemiological data from the Brazilian Ministry of Health, reported 406883 cases of dengue in Brazil up to October 2009. There were several dengue fever epidemics in Brazil in 2008. There was a reduction of 46.3% of dengue cases during 2009 in comparison to 2008.. The objective of this report is to show the history of dengue prevention and control in Brazil. For this purpose the medical literature was consulted. Results: The first registered cases of dengue occurred in Brazil in 1923. During the early 1950 and 1960 *Aedes aegypti* was eliminated from Brazil. The national campaign then employed was centralized, vertical with military similar structure. Reintroduction of *Aedes aegypti* was observed during 1976 to 1977, via the port city of Rio de Janeiro. 1981 to 1982 there occurred a dengue epidemic in Boa Vista in the state of Roraima. Since then, dengue epidemics have occurred regularly in Brazil. The Ministry of Health has launched several programs for the prevention and control of dengue epidemics. The coefficient of incidence of dengue was 70 cases per 100.000 habitants a for 2006; the coefficient jumped to 100 cases per 100.000 habitants in 2007. The results point out less than desirable results of the Brazilian National Program for the Prevention and Control of Dengue. Analysis of spatial distribution of incidence of dengue showed that the urban settings of Brazilian cities and towns, especially the peripheral areas, with deficiencies in all socio-economic, educational and cultural settings are more vulnerable for the growth of mosquitoes. A study of the association socio-demographics factors and the dengue fever epidemic in 2002 in the state of Rio de Janeiro, showed higher dengue incidence in areas characterized by growing urbanization and deficient running water supply. Family Health program with the presence of mid level health agents was an important facilitator of vector control. At times the lack of strong political will and continuous, adequate financial funding were identified as important drawbacks for the vector control program. Sincere efforts were taken by the health authorities for the dissemination of health promotion programs to combat dengue at the federal, state, and municipal levels including communities programs and promotion of dengue prevention at school levels. However the existence of sever social, cultural, educational, economical and infrastructure inequalities did not permit the desired success of vector control programs in Brazil. These factors affected negatively the human behavior for dengue control. Conclusion: The European nations could learn lessons from the Brazilian efforts that in spite of having enough technical entomological knowledge, good insecticides and technology for their applications, sufficient epidemiological data, it is the form of the human behavior that will decide the potential establishment of the vector in Europe and its subsequent control. The imminent climate changes, the globalization of persons, commercial products, air traffic, movement of containers and the lack of adequate public health policies may facilitate *Aedes aegypti* to establish itself as a potential vector in Europe.

Application of Integrated Ecohealth and Adaptive Management Approach in Dengue Prevention and Control in Bangladesh

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Since 1964 there were some sporadic reports of dengue incidents in the territory of Bangladesh. A WHO collaborative study with the Integrated Control of Vector-Borne Diseases (ICOVED) in 1996-97 confirmed dengue infection in the country (13.7% of a 225 sample), which also indicated potential large scale outbreaks. The major cities of Bangladesh, a country of more than 150 million people crowded in an area of 143,000 sq km, have experienced a resurgence of dengue, particularly since 2000. In view of the persistent trend of dengue infections in the country, particularly since 2000, two major concerns emerged among the health practitioners and policy makers: 1) improvement in disease management to reduce morbidity and mortality, and 2) understanding dengue disease transmission processes to determine factors that enable dengue viruses to affect human health. Dengue disease management aspects have received some attention in Bangladesh's public health policy and practice community in recent years; nonetheless, little attention has yet been paid to understand the dengue transmission processes in the country. Prevention and controlling dengue infection through interventions, especially through controlling the vector or intervening into the ecological, biological and social factors, have been generally ignored. This research aims to develop and adopt an innovative approach (i.e., Integrated Ecohealth and Adaptive Management approach) whereby local communities will directly participate in the decision-making and implementation of dengue prevention and control activities in a manner that will adapt to changing eco-bio-social factors. A multi-scale analysis of the correlates of dengue incidence in tropical countries like Bangladesh, and a test of a new approach, would prove an excellent example for understanding and addressing the climate-eco-bio-social complexity of disease emergence and for improving community health and development not only in Bangladesh but also in South and Southeast Asian region.

Predictive and quantitative models for vector-borne diseases

Priority Setting of Emerging Zoonoses in the Netherlands : Focus on emerging arthropod borne zoonoses

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The highly urbanized Netherlands with its intensive livestock sector, high international passenger traffic and trade activities faces many threads of emerging zoonoses is threatened by a wide variety of pathogens transmitted from vertebrate animals to humans (zoonoses). Effective and efficient policy-making for control and prevention and choices for research and surveillance of these zoonoses requires focusing on the most relevant ones.

92 emerging zoonoses relevant for the Netherlands (Europe) were identified as relevant to prioritize. For the priority setting, an innovative method was developed, as existing methods often based on current disease burden in a country fail for 'emerging zoonosis' only present as a 'threat'. Priority setting is a multi-dimensional problem, in which technical information is often intertwined with value judgments. To prioritize emerging zoonoses more transparently, a quantitative method, multi-criteria analyses (MCA), was chosen. Seven criteria were selected defining the most relevant aspects of risk in as few criteria as possible using natural numbers for the categories of a criterion instead of arbitrary numbers for scoring of criteria. Decision trees were developed to support scoring of zoonoses if data were scarce or unavailable. The weight of each criterion in the final score is determined through several panel sessions prioritizing hypothetical zoonoses. In our method, newly identified zoonoses or additional information for a zoonosis already in the system can be added or modified without the necessity to completely redo the analyses. The final prioritized list indicates which emerging zoonotic pathogens on the list pose the largest threat, expressed in its normalized score (ranging between 0-1 with 1 being the highest threat when introduced). The normalized score itself is a measure of threat independent of other agents on the list.

The Netherlands faces many potential threads especially arthropod borne ones. Nearly half (46%) of the emerging pathogens in the database are vector-borne (41 out of 92), of which 66% were viruses (27 out of 41). Three arthropod viral zoonoses (CCHF, JE and WNV) were prioritized in the top ten.

This quantitative dynamic method is especially valuable in the priority setting of emerging zoonoses (or infectious diseases), where information is uncertain or even absent, and changes constantly. This method is more transparent and objective and can form the basis of future knowledge management systems.

Vector surveillance in the Netherlands; a new centre is borne.

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In response to increased importance of occurring vector-borne diseases in the Netherlands, and increased risks of new vb-diseases incursions, a Centre for Vector Surveillance was established in 2009. The main aims of this centre are to:

- develop and carry out nation-covering vector surveillances

- carry out species identification
- map endemic and invasive arthropod species (with an emphasis on mosquitoes, Culicoides and ticks), including predictive species distribution modelling
- to identify high risk areas (of introduction and/or infection of vectors and vector-borne pathogens)
- provide input and data for risk-assessment studies
- advise and assist policy makers regarding vectors and vector-borne diseases (e.g. contingency plans, eradication/control strategies)
- participate in international vector-surveillance related programs

Disease Risk Modelling and Mapping in the Philippines

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The poster will outline and illustrate the various steps and processes involved in evaluating and obtaining data from multiple sources, preparing data for analysis, modelling procedures and mapping the predicted risk of disease occurrence, focusing on *Trypanosoma evansi* (Sura) and Fasciolosis (Liverfluke) in the Philippines.

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.

A web-based GIS for *Aedes albopictus* and Chikungunya virus surveillance in Emilia-Romagna region

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The *Aedes albopictus* survey group of Emilia-Romagna Region, Italy, collaborate in multidisciplinary context (entomology, epidemiology, informatics, public health) to develop a Web-Based Geographic Information Systems (Web-GIS) that enable storage, processing, and analysis of spatial monitoring and public health data, managed together with other external data in the open source Relational Database Management System (RDBMS) PostgreSQL.

A PostgreSQL session consists of the following cooperating processes: a server process, which manages the database files, accept connections to database from clients application, and performs actions on database on behalf of the clients; the user's client (frontend) application to perform database operations. PostGIS open source software was implemented to add support for geographic objects to PostgreSQL relational database. PostGIS "spatially enables" the PostgreSQL server, allowing it to be used as a backend spatial database for Web-GIS. The Regional Web-GIS, dedicated to professionals and to public health care decision-makers, provide specific web interface that includes maps, legends, controls for executing queries on relational data, for creating thematic maps (choropleth maps), time series and layers (shape files), reports, and Excel data extraction for vector control program and for Chikungunya virus (CHIKV) survey and control in Emilia-Romagna Region.

Furthermore the Internet application was implemented using open source technology that offers some advantages over commercial softwares like the low cost or even free of charge, high adaptation to users requests, a large open source community, which helps in turn to build up a market for support and customization of the software.

Can monitoring of mortality cases in wildlife help to detect emerging diseases?

Petit E1, Artois M1, Mastain O2, Calavas D3

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*. WildTech FP7 consortium: workpackage 6, epidemiology and risk analysis

Since 1986 France, as a country, is covered by a wildlife mortality monitoring system, called SAGIR. A network of technicians from the federations of hunting and the National Hunting and Wildlife Agency (ONCFS), collect carcasses found by hunters and the general public, and submit them for investigation by veterinary laboratories, in each département. The collected data are analyzed and results reported annually. More than 50.000 cases of mortality in wildlife were recorded between 1986 and 2007, representing 244 species of terrestrial mammals and birds, and were attributed to 220 different causes of mortality. Our aim is to use the data collected by the network to study the feasibility of early detection of unusual health events we call abnormalities.

We first analyzed the functioning of the network, in order to evaluate its ability for the early detection of health events affecting wildlife. Syndromes of interest were then defined by a statistical typology of the lesions observed on the carcasses, using factorial analysis in order to identify the information which best explains the observed variability, followed by clustering techniques. We then carried out trend analyses on selected historical data, to establish the "background noise" of occurrence of these syndromes, characterized by multiple features (time, space, nature (species, habitat, etc.)). Existing abnormality detection methods will then be adapted. Variation of occurrence of a syndrome should be detected by the methods as an abnormality and will lead to an alarm signal. The poster will describe the mortality monitoring system and the data collected; it will then illustrate some of its characteristics regarding detection of unusual health events. Then, it will detail the syndrome definition and trends, and finally present the abnormality detection methods chosen.

The poster gives insights on detection of syndromes by comparison with mortality cases and use algorithms for early detection of events which are not "usual";

Mosquito borne-disease vector isolated neurons: a new biological model for optimizing insecticide/repellent efficacy while reducing adverse effects on ecosystem.

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The mosquito has four distinct stages in its life cycle: egg, larva, pupa, and adult. Mosquito larvae that have an aquatic stage are vulnerable to natural predators (insects, fishes, batrachians and birds). In this context, the effect of ecosystem perturbation on the role of predation and its consequence for the control of mosquito-borne disease is essential. An important parameter that produces negative effect on ecosystem, which thereby affects predators involved in the control of mosquito populations, is the use of pesticides that are put into water to kill mosquito larvae and pupae before they become adults. Their massive utilisation affects predators and aquatic ecosystems and contributes to the emergence and selection of mosquito borne-diseases vectors. The major weakness of current pesticides is their relatively low selectivity combined to their long residual effect. To reduce adverse effects on ecosystem and particularly on predators, alternative insecticide strategies adapted on mosquito are urgently needed.

For optimizing the use of insecticides and/or repellents in public health, neuronal mosquito model appears to be an important tool to investigate i) repellents and insecticides mode of action and ii) mechanisms involved in insecticide resistance at molecular and cellular levels. In this context, we have developed a novel biological model of *Anopheles gambiae* neuronal cells from the mosquito head which is considered as a powerful platform of biotechnology offering all facilities for the design of specific tests to study the mode of action of insecticides and repellents, used alone and in combination. By using electrophysiology, molecular biology and biochemical assays well-adapted on isolated adult mosquito neurons, we will define precisely the mode of action of insecticides and repellents together with the characterization of the molecular determinants involved in the regulation of the pesticide efficacy. Using RT-PCR, we have demonstrated that isolated *Anopheles gambiae* neurons express a voltage-dependent sodium channel (AgNav) and two acetylcholinesterases (AChE; ace-1 and ace-2). Furthermore, electrophysiological patch-clamp technique has revealed that AgNav and AChE are functional and sensitive to the commonly used insecticides, the type I pyrethroid, permethrin (10-5M) and the repellent DEET (10-7M), respectively. The effect of the carbamate propoxur has been tested using a colorimetric method. The residual activity of AChE has been measured on these *Anopheles* neuronal cells for increasing concentration of propoxur. The IC50 value estimated for propoxur is $1.7 \times 10^{-7} M$.

These results indicate that the development of this neuronal mosquito model has practical applications in the development of highly specific strategy for disease vector control, by offering the perspective to develop more specific insecticides/repellents and by reducing negative effects on ecosystems.

Products, Services and Research Initiatives of the U.S. Armed Forces Pest Management Board, relevant to EDEN objectives

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The U.S. Armed Forces Pest Management Board (AFPMB) provides information services on Vector-Borne Diseases and their control, with online access to 180,000 publications via the <afpmb.org> website. On behalf of the U.S. Department of Defense (DoD) and allied agencies, the AFPMB mission is to ensure that environmentally sound and effective programs can be implemented against disease vectors and pests of public health importance. Specialist professional committees guide the AFPMB to produce training and technical aids backed by a wide variety of library services. To improve the scope for vector control, in partnership with the Agricultural Research Service of the U.S. Department of Agriculture (ARS-USDA), the AFPMB administers the Deployed War-Fighter Protection (DWFP) research program for developing new pesticides, new application equipment, and new methods of personal protection. Together with representatives of other Federal Agencies, experts from the AFPMB and ARS-USDA are preparing a 'white paper' on public health pesticide implications of environmental change, especially climate change. This poster will display some highlights of DWFP research, and portray the AFPMB range of products and services relevant to EDEN objectives.

Elimination of a primary filariasis vector population at an endemic field site: Assessing the vector competence of an incompatible strain of *Aedes polynesiensis*

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Field evaluation of a novel vector control strategy is underway in French Polynesia. In this approach, it is hypothesized that inundative releases of incompatible *Ae. polynesiensis* male mosquitoes infected with *Wolbachia* will result in the sterilization of wild female populations at a field site endemic for filariasis transmission. Prior to the field trial, experiments have compared the incompatible (CP) colony and field (APM) colony in their fitness, population dynamics and genetic structure, mating competitiveness, and vector competency. Although only males are to be released, the competency experiment is an appropriate precaution to exclude the risk of an increased vector competence through introgression crosses or due to unnatural *Wolbachia* infection that might have occurred in the CP colony. Vector competence of the incompatible colony was compared with the parental colony from which it was derived. Female mosquitoes of each cohort were fed with blood drawn from *Wuchereria bancrofti* positive donors containing either low or high densities of microfilaria (mf densities: trial 1= 990 mf/mL, trial 2=7000 mf/mL) respectively. To assess the vector competence, *Wuchereria* parasite development was monitored in both groups. Females were dissected and screened for microfilariae ingestion immediately after the infectious bloodmeal and for the presence of infective larvae at the end of the parasite developmental cycle. The study showed no significant differences in the number of females displaying infective L3 larvae 14 days after exposure to low or high densities of microfilariae. Although no difference was seen with low mf levels, there was a significant difference in the mean number of infective L3 larvae per female (9.67 vs 5.74 mean L3/female in APM and CP colonies respectively) that each cohort had permitted to develop following exposure to high microfilariae levels. While CP females are permissive towards filarial worm infection their degree of infection appears reduced when compared to natural populations of *Ae. polynesiensis*. Furthermore, related laboratory studies of adult female longevity show reduced CP female survivorship relative to wild type females. With a reduced longevity, fewer CP females would be expected to accomplish the extrinsic incubation period required for dengue and LF transmission, relative to wild type females. Thus, in the unexpected event that CP were to become established in the field, their vectorial capacity may be lower than that of wild type females. In addition to reduced female adult longevity, CP individuals also show lower egg hatch and immature survivorship, relative to wild type *A. polynesiensis*.

Immature and adult female fitness have the potential benefit of reducing the risk of accidental population replacement resulting from accidental CP female releases. If successful, this strategy could adequately complement ongoing mass drug administration and augment ongoing efforts to eliminate Lymphatic Filariasis in one of the overseas Countries and Territories (OCTs) of the European Union in the South Pacific region.

Towards a European expert network to map invasive mosquito species: The example of *Aedes albopictus*.

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TigerMaps is a study funded by the European Centre for Disease Prevention and Control (ECDC), and which aimed at developing risk maps on the distribution and spread of *Aedes albopictus* in Europe. Its objectives were (i) to produce a map of the precise current distribution of *Ae. albopictus* in Europe and (ii) to map the risk for establishment of *Ae. albopictus* in Europe, in the event of it being introduced. Vectorial risk assessment related to *Ae. albopictus* (Chikungunya, Dengue) needs precise information on the vector's distribution and biology. Thus, a European expert network was established and strengthened in order to collect the required data. In this poster acquisition and mapping of current occurrence data is discussed. A brief overview of the obtained distribution model outputs is given in a second poster (Hendrickx et al. 2009). See also ECDC technical report (2009) for more details on methods used and results obtained.

Data provided by the experts was gathered in an entomological dataset including all confirmed *Ae. albopictus* observations made in Europe since its introduction, with (1) geo-referenced data on presence/absence and (where available) abundance of *Ae. albopictus*, (2) information on past and present survey/control activities on mosquitoes in general and *Ae. albopictus* in particular. The data was collected at the finest spatial reference, with details of the distribution patterns in space and time, and the levels of monitoring and control related to the last five years (2003-2007). Only confirmed data was used to develop GIS-based maps.

Data were collected from 52 'states' (countries, microstates or territories having an ISO/NUTS national code). These are members of European Union or located in or close to geographical Europe (e.g. Cyprus, Madeira, Azores). Major findings are:

- The presence or absence of *Ae. albopictus* could not be assessed for 24 states, due to absence of feedback from contacted scientists (6 states) or mainly due to absence of updated information on the local mosquito fauna (18 countries).
- Twelve countries have implemented specific surveillance for *Ae. albopictus* during the last five years.
- Ae. albopictus* has been observed at least once in 16 states, but information on its presence varies in quality from national and regular surveillance to a total absence of surveillance.
- It has been eradicated, albeit temporarily, from some of the introduction foci in Croatia, France, Italy and Switzerland, on the basis of preventive surveillance and rapid application of control measures and it has not established in Belgium after introduction.
- It has been observed once in 2007 in Germany, but its establishment is not yet proven.
- It is regularly (re)-introduced into The Netherlands but it has not yet been observed spreading outside glasshouses, therefore it cannot be considered as established in this country.
- It has homogenous populations and could be considered as spreading in 11 countries and micro-states: Albania, Croatia, France, Greece, Monaco, Montenegro, Italy, San Marino, Slovenia, Spain and Vatican City.
- Albania and Italy: data shows a scattered distribution, due to a scattered surveillance; species most probably as homogenous populations in all coastal areas, from the seaside up to 500m.

The quality of the available data varies strongly between countries. Based on these results and on the recommendations of the V-borne project, ECDC recently established VBORNET, a European Network for Arthropod Vector Surveillance for Human Public Health. The aim of VBORNET is a.o. to further develop state of the art tools to model the distribution of *Ae. albopictus* and other invasive mosquito species (mainly *Ae. aegypti* and *Ae. japonicus*), and to further build on the expert network and database. Priority is given to the collection of new data in countries bordering colonized areas and from where no or very little data on mosquito fauna is currently available. At a time where arboviruses are also spreading worldwide (i.e. Chikungunya, Dengue), such studies are essential to improve vector related risk assessments.

European Centre for Disease Prevention and Control (ECDC) 2009. Development of *Aedes albopictus* risk maps. ECDC Technical report, Stockholm, Sweden [cited 2009 Jun 19]. Available from: http://www.ecdc.europa.eu/en/publications/Publications/0905_TER_Development_of_Aedes_Alboipictus_Risk_Maps.pdf

Hendrickx G., Ducheyne E., Medlock J., Scholte E.-J., Schaffner F., and colleagues 2010. Towards modelling the spread of *Aedes albopictus* in Europe: The complementarity of quantitative and qualitative methods. Poster Eden annual meeting, Montpellier.

Mapping vector habitats with temporal Fourier transformed MODIS imagery

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The presence of disease-transmitting vectors is largely determined by environmental factors. MODIS satellite images provide recurring measurements of environmental conditions around the world. We have developed a method for analysing time series of MODIS imagery to summarise the seasonal dynamics of temperature, vegetation, and moisture. MODIS Collection 5 data are downloaded and screened for optimal pixel quality. Gaps in the time series are interpolated, and cubic splines are fitted to the series to allow sampling at 5-day intervals. A temporal Fourier algorithm transforms the sampled values into a set of component sine waves, or harmonics. The algorithm records the mean, minimum, maximum, and variance of the time series, the amplitude, phase, and variance of the first three harmonics, and the error associated with different stages of the transformation. Thus a multi-year MODIS series comprising hundreds of time periods is described in a set of 17 images. These images can be combined with field data in statistical models to identify the conditions that favour or inhibit disease-transmitting vectors. Our products cover the entire EDEN study area at 1-kilometre resolution, as well as much of western Europe at 250-metre resolution. Data collection and processing are ongoing; we currently have Fourier-transformed imagery from the years 2001-2009. The imagery serves as a valuable tool for identifying areas where vector-borne diseases have a higher risk of emerging.