EDEN

EDEN ext

forest
abundance
culicoides trap
plasmodium tick–borne
genome
parasite rna blood protein bird
prevalence vector natural
perniciosus
model vole phlebotomus
leishmania density focus
human infection zoonosis virus
horse presence
complex landscape environment rodent
deer borrelia ixodes–ricinus glareolus west–nile saliva
ecology emergence phagocytophilum
disease season system questing serology
dynamics identification dna outbreak
control fever mosquito host
risk spatial temperature
molecular
wild lineage mouse
incidence
phlebovirus spread diptera
pattern sequence
adult africa cycle map
phylogenomics anopheles
variation animal
distribution
climate dog

The impact
of a decade (2004-2015)
of research
on vector-borne diseases

bluetongue sandfly antibody
myodes
vector–borne
change nymph tick–borne–encephalitis
reservoir species pathogen hantavirus
europe infantum
endemic detection tick public–health
epidemiology transmission
circulation

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The impact
of a **decade** (2004-2015)
of **research**
on vector-borne diseases

Preface

British author L.P. Hartley famously wrote, "The past is a foreign country: they do things differently there." And while a literary introduction to a book focused on science may seem incongruous, it offers a neat summary of the changes we have seen in European research on vector-borne diseases since the launch of the EDEN (Emerging diseases in a changing European environment) project in 2004 and the end of its follow-up project, EDENext (Biology and control of vector-borne infections in Europe), in 2015. These major projects, bringing together a total of more than 50 partners from across Europe, North and West Africa, have changed the way research on vector-borne diseases is conducted. The creation of international teams working on the same problems using the same methods, albeit in different locations, the routine sharing of data between institutes, the increased use of sophisticated models and the training of a new generation of scientists used to working with each other all mark a break with the past.

EDEN set out to identify and catalogue the European ecosystems and environmental conditions which can influence the spatial and temporal distribution and dynamics of human pathogenic agents. It developed generic methods, tools and skills such as predictive models and monitoring tools that can be used by decision makers for risk assessment, decision support for intervention and Public Health policies. EDENext took up the baton with a focus on investigating the biological, ecological and epidemiological components of vector-borne disease introduction, emergence and spread, and the creation of new tools to control them. Vector groups focused on *Culicoides* midges, Phlebotomine sand flies, mosquitoes and ticks, while a fifth team examined rodents and insectivore-borne diseases. They were supported by modelling and data management teams, plus a team focused on ensuring the project had a real impact on improving Public Health.

In this book, we present the impacts of these two projects. We start with an interview with EDENext coordinator Renaud Lancelot (CIRAD, France) and examine the work of the vector and rodent groups, presented at the GERI conference (Genes, Ecosystems and Risk of Infection) in 2015, accompanied by updates of PUBLICise Health, EDENext's telegrams reporting on the project's research results with a particular relevance for Public Health. We also examine in greater detail the works of the data management, modelling and Public Health teams, interspersed with interviews with various leading figures who have been involved in the projects. In order to measure the academic impact of these projects, we also present a bibliometric study, encompassing more than 500 papers published in scientific journals over the past 10 years, and examples of other kinds of impacts of these projects.

We hope you find this book an interesting and stimulating example of what can be achieved when highly motivated teams are given the opportunity to work together for a long period of time. This opportunity would not, of course, have been possible without funding under the European Commission's Sixth and Seventh Framework programmes and we sincerely thank the Commission for the confidence shown in the EDEN and EDENext projects. We hope you find the book thought-provoking, too, with regards to the future. Ongoing environmental, social and economic changes mean that vector-borne diseases – and other diseases sensitive to these changes – will be high on the agenda for the foreseeable future.

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Introduction: 10 years, 58 partners, 23 countries

EDEN[1] (2004-2010) and EDENext[2] (2011-2015) were two large collaborative research projects funded by the European Commission (Directorate-General for Research and Innovation) in Framework Programmes 6 and 7. They were aimed at improving knowledge as well as surveillance and control tools regarding vector-borne infections in Europe and neighbouring territories. In EDEN, more emphasis was put on understanding the effect of environmental changes on the emergence of these infections, and characterising those ecosystems at high risk of emergence. In EDENext, the focus was more on understanding the biological processes, and preparing the transfer of research results to Public Health.

(a) Geographical reach

Temperate broadleaf & mixed forest
Temperate conifer forest
Boreal forest / taiga
Tropical grassland, savanna & scrub
Temperate grassland, savanna & scrub
Flooded grassland and savanna
Montane grassland and scrub
Toundra
Mediterranean forest, woodland & scrub
Desert and xeric woodland
Mangrove
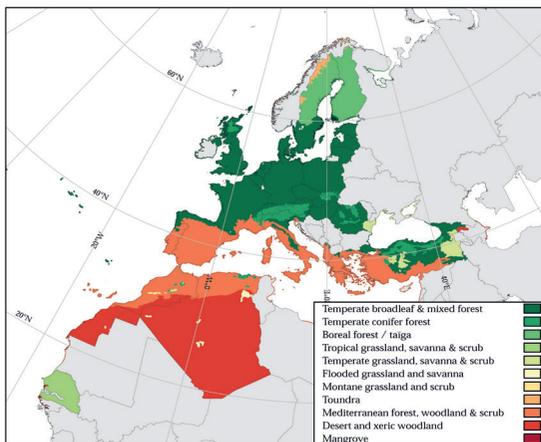
(b) Organisation of the work
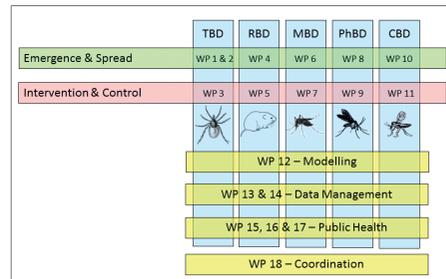
WP: subdivision of EDENext work programme into work packages

TBD, RBD, MBD, PhBD, CBD: vector or wild host groups for (i) ticks, (ii) rodents, (iii) mosquitoes, (iv) Phlebotomine sand flies and (v) *Culicoides* biting midges, respectively.

Figure 1. The geographical reach of EDEN and EDENext (a) and work organisation (b). The coloured layer on the map represents biomes covered by the two projects. The organisational diagram refers to EDENext (the EDEN diagram was very similar).

As a whole, EDEN and EDENext involved 58 partners from 23 countries in Europe, the Middle East, and Africa (Figure 1a). To implement and manage the work programme negotiated with the Commission, a matrix-like structure was adopted (Figure 1b) with vector groups as the 'columns', and integrative teams as the 'rows'. This structure was adopted to facilitate the harmoni-sation of field and laboratory work, as well as the dissemination of methods and tools.

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[1] Emerging diseases in a changing European environment

[2] Biology and control of vector-borne infections in Europe

Renaud Lancelot

Why conduct so much research on vector-borne diseases?

Renaud Lancelot (EDENext coordinator, CIRAD, France): *"The big question at the beginning of EDEN was to assess the possible role of environmental changes on the emergence of new diseases. We decided to work on vectors and vector-borne diseases because vectors, insects or ticks for example, are very sensitive to environmental conditions and therefore are supposed to be very sensitive to changes in these conditions. That is the basic reason we decided to work on vectors and vector-borne infections. The other reason was that at this time, there was a poor availability of scientists working on vectors and vector-borne infections. Moreover, they had few opportunities to work together. It was a neglected area in European research. EDEN allowed the gap to be filled and increased European capacity in this field."*

Can you tell us something about the impact of EDEN and EDENext?

Renaud *"In such huge projects it is quite difficult to make a list of all the possible impacts. I would say that there are three main domains. The first one is the impact on the scientific audience, via papers in peer-reviewed journals. During EDEN and EDENext we have published more than 500 papers in scientific journals and their average impact factor is above three. It is a very good score indeed, and we have had several outstanding papers in journals like* Nature.
The second domain is expertise and the impact on Public Health. Because the project was huge, involving lots of different partners from most European countries, we had the possibility to construct a network of all these scientists. This network was efficient at doing research, but it was also quite visible at the European level. It was used by the European Commission to form an expertise network to assess the risk of disease introduction and spread. The first-generation network, called VBORNET, ran between 2008 and 2013. Recently it was turned into a veterinary and Public Health network called VECTORNET. It brings together most of the European teams working on vectors and vector-borne diseases. It is used to answer specific questions asked by ECDC (European Centre for Disease Prevention and Control) and EFSA (European Food Safety Authority). The third domain is capacity building and we were able to implement and support more than 90 PhD theses. This means a whole generation of young scientists has been raised by EDEN and EDENext. We can consider that the impact of the project will be very large over the next 20 to 30 years because all these young people have used the same methods, the same network and they know each other. We can be sure that when applications are to be made for projects they will contact each other and enter into the projects together."

Renaud Lancelot is a French veterinary epidemiologist with a PhD in population biology and ecology. He is the deputy director, and epidemiology team leader, of the INRA-CIRAD Emerging and Exotic Animal Disease Control research unit (Montpellier, France, see umr-cmaee.cirad.fr). He has 30 years of experience in the epidemiology of animal, human and zoonotic diseases, including 20 years of field work in French Guiana, Mauritania, Chad, Senegal and Madagascar. He has been coordinating the large collaborative European projects FP6 EDEN (Emerging diseases in a changing European environment) and FP7 EDENext (Biology and control of vector-borne infections) since 2006 (www.edenext.eu). His current research interests are in the epidemiology and modelling of peste des petits ruminants, Rift Valley fever, and tsetse flies. He is the author of more than 160 scientific publications, communications, books and statistical software.

What about the future?

"In our quickly changing world, it is quite important that the Commission funds long-term research on issues related to environment and health. Vector-borne diseases are only a part of the problem. There are many other diseases which are not borne by vectors but are related to the environment and we know we will have many such disease emergences in the future. This makes it important for the scientific community to be ready to address the questions and, for the Commission, it is important to have large networks at hand, which can be reactive when there is a disease emergence and can be mobilised to find solutions to solve the problem when and where it occurs. I would also add that the geographical scope is much wider than Europe. In particular, we must help developing countries to control emerging diseases on their own, to protect local populations and limit the risk of spread across their borders."

SCIENTIFIC ADVANCES FOR BETTER PUBLIC HEALTH

Tarja Sironen[1], Heikki Henttonen[2] and the EDENext rodent and insectivore-borne disease group

[1] Department of Virology, University of Helsinki, Finland
[2] Natural Resources Institute, Finland

Many faces of ro de

INTRODUCTION

During the EDEN and EDENext projects, the rodent-borne and subsequent rodent and insectivore-borne disease groups have, in collaboration, assessed the current diversity, distribution and prevalence of important rodent-borne pathogens in Europe. Among the rodent and insectivore-borne diseases, hantavirus (family Bunyaviridae) infections pose a significant threat to public health in Europe and elsewhere. Some hantaviruses have a high case-fatality rate and many others represent a considerable disease burden. Hantavirus infections are emerging in new areas and outbreaks have been reported in established endemic regions such as Germany and Sweden. Beyond their role in transmitting infections directly, rodents and insectivores also serve as amplifying hosts of many arthropod-borne microbes, in particular tick-borne pathogens, many of which are spreading in Europe due to environmental change. What are the key characteristics in the life cycle of a rodent-borne virus that determine its persistence and ability to cause epidemics in humans? How are different rodent-borne viruses maintained in their reservoir host population? What factors affect spillover to humans? How do changes in the environment (for example, due to climate change or anthropogenic activities) affect the prevalence and distribution of pathogens? Can we model the dynamics and predict the behaviour of rodent-borne infections? Answers to these questions are sought at the level of (see Figure 1): the (1) virus, (2) individual host, (3) reservoir host species, and (4) host populations in different geographical regions

THE VIRUS

Rodent-borne viruses have a world-wide distribution with significant disease burden caused by hantaviruses and arenaviruses in particular (Goijenbier 2013). Both of these viral families are considered to be highly host-specific and almost exclusively rodent-borne. These viruses typically exhibit asymptomatic life-long infections in rodent hosts but can induce severe disease if they spill over into the local human population. Recent findings have suggested new host taxa for both viral families, for example, novel arenaviruses have been found in snakes and hantaviruses are now known to occur in shrews, moles and bats. Whether these new hosts carry viruses pathogenic to humans is unknown, and in most cases we are still lacking the tools to address this question.

Hantaviruses cause hantavirus cardiopulmonary syndrome (HCPS) in the Americas and haemorrhagic fever with renal syndrome (HFRS) in Europe and Asia (Jonsson 2010). In the Americas, hantavirus infections are rare but severe, and the case-fatality rate of Andes and Sin Nombre hantavirus infections is 30–40%. In Europe and Russia, Puumala virus (PUUV) carried by bank voles (*Myodes glareolus*) is the major hantavirus pathogen, causing thousands of infections every year with an emerging pattern in Central Europe (Heyman et al. 2011). The mild HFRS due to PUUV is often called nephropathia epidemica (NE). In Europe, Dobrava (DOBV) and Seoul (SEOV) hantaviruses also infect humans. SEOV is a global pathogen due to the worldwide distribution and movement of its host, the brown rat (*Rattus norvegicus*). During the winter of 2012–13, human cases of severe HFRS caused by SEOV were reported in the UK and France. Some of these infections were associated with pet rats, and SEOV has also been detected in domestic rats in Sweden (Lundkvist et al. 2013). In contrast to the persistent infection of rodent reservoirs, human hantavirus infection is transient and self-limiting. Humans typically inhale infectious aerosols of animal excreta. While capillary leakage is a hallmark of hantaviral diseases and it appears to be caused by direct viral effects and immunopathological mechanisms, specific details of pathogenesis are poorly understood (Vaheri 2013).

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Arenaviruses comprise a genus in the family Arenaviridae and have all been considered rodent-borne viruses with the exception of Tacaribe virus, which has been isolated from bats (Charrel et al. 2011). Similar to hantaviruses, arenaviruses are considered to be quite host-specific. Recent analyses have suggested that, on an evolutionary time-scale, arenaviruses have crossed the species barrier more frequently and easily than previously thought and that diversification might have been influenced by geographical proximity more than the relatedness of hosts (Irwin et al. 2012). Serological evidence for arenavirus infections is frequently reported for many different rodent species, but arenavirus genomes are rarely recovered. This might be due to low viral load in spillover infections or there might be a variety of arenaviruses circulating that are simply not detected with the current techniques. During EDEN and EDENext, lymphocytic choriomeningitis virus-like sequences have been detected at a low prevalence in bank voles (unpublished observations) and wood mice (*Apodemus sylvaticus*) (Ledesma et al. 2009).

Arenaviruses can be transmitted to humans primarily through aerosolized rodent excreta, but transmission via contaminated food or drink and through wounds or skin abrasions is possible (Moraz et al. 2011). Several arenaviruses are known human pathogens, with Lassa virus being responsible for most human infections. Of the known arenaviruses, lymphocytic choriomeningitis virus (LCMV) is found in Europe. Recently, we and others - to our great surprise - have detected arenaviruses in boid snakes where infections manifest as boid inclusion body disease (BIBD) (Hetzel et al. 2013). The prevalence of the arenaviruses that cause the disease in wild snake populations and the origins of BIBD remain unknown. It has been hypothesised that snakes caught the virus from rodents they had eaten, but the known lineages infecting these taxa are distantly related and inferences remain unclear.

Cowpox virus (CPXV) belongs to the genus Orthopoxvirus (OPV) and shares a cross-reacting immunoresponse with smallpox, monkeypox and vaccinia (VACV) viruses. As vaccinations against smallpox have been scaled back, the incidence of OPV infections seems to be rising. CPXV is transmitted to humans sporadically from its rodent reservoirs and a high CPXV seroprevalence has been observed in field voles (*Microtus agrestis*), bank voles and wood mice. In Finland, CPXV antibodies can be locally common in field voles, and the antibody prevalence shows a delayed density dependence in spring and direct density dependence in the autumn. In Europe, it seems that the bank vole has the highest seroprevalence although many other

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species are commonly infected. Unlike rodent-borne viruses with persistent infections (such as hantaviruses), CPXV has a short, transient viraemia and virus itself is therefore difficult to observe in natural populations.

Virulence is defined as the degree of pathogenicity within a species or group of parasites as indicated by case-fatality rates and/or the ability of the organism to invade host tissues. Different hantavirus species differ dramatically in their virulence and range from non-pathogenic to fatal infections. Virulence may also differ significantly among variants of a given hantavirus, and DOBV is a prime example of this. DOBV is a human pathogen that resides in several species of *Apodemus* in central and eastern Europe. The known strains are divided into four genotypes (Dobrava, Kurkino, Saaremaa, and Sochi) that show characteristic differences in their phylogeny, specific host reservoirs, geographical distribution and human pathogenicity (Klempa et al. 2013). A similar pattern of diversity has been reported for PUUV, with strains replicating to highly variable titers. It remains to be determined which genetic differences are responsible for this variation in virulence.

Hantaviruses are transmitted to humans via inhalation of aerosolized excreta from their rodent reservoir hosts. Knowledge of viral transmission patterns and survival of the virions outside the host is critical for assessing the human infection risk of PUUV and understanding how the virus is maintained in the host population. The infectiousness of PUUV is retained outside the host for at least two weeks at room temperature and, during EDENext, we showed that virions can survive for up to seven weeks in cold winter conditions. This may, in part, explain the higher incidence of NE in northern Europe where epidemics occur in winter, in contrast to the temperate zone where they occur during the summer following masting the previous autumn.

THE INDIVIDUAL HOST

Genetic variation among both viruses and hosts influences individual susceptibility to infection (Charbonnel et al. 2014). The field of immunogenetics aims to identify and understand the relationship between genetic factors and immunological phenotypes or immunity-related diseases. Research on the immunogenetics of wild rodents was conducted during EDEN and EDENext and we examined the association between immunity-related genetic variation (coding sequence and transcription) and the outcome of hantavirus infections considering the probability of infection and severity of the disease. These results have improved our understanding of hantavirus epidemiology by providing a more precise description of host switching and hantavirus transmission from the reservoir to the human population.

Immunogenetic studies have identified mechanisms linked with the persistence and clearance of hantaviruses in their reservoir hosts. Results have described genetic variation among rodent populations sampled from different regions of Europe. In endemic areas with continuously high PUUV prevalence (for example, Finland and the French Ardennes), strong co-adaptation of the host and virus could have selected for tolerance of PUUV infection more than in populations where prevalence levels are low (for example, Czech Republic and the southern Ardennes) (Guivier et al. 2014).

The bank vole (Myodes glareolus). © Erkki Oksanen (LUKE, Finland)

In the rodent reservoir, hantaviruses typically induce an acute infection spike before shifting to a persistent plateau. Yet variable patterns of infection have been observed among infected individuals within a given reservoir species, and profiles observed in laboratory infections also differ considerably from those in natural settings. In a monthly capture-mark-recapture study, we analysed wild bank voles in the field for the presence and relative quantity of PUUV RNA in the excreta and blood from two months prior to and up to eight months after seroconversion (Voutilainen et al. 2015). The proportion of animals shedding PUUV RNA in saliva, urine and faeces peaked

during the first month after seroconversion and continued throughout the study period with only a slight decline. The quantity of PUUV shed over time was constant. Our findings contradict the prevailing view of a decline in virus shedding after the acute phase, and of a short viraemia - assumptions widely made in epidemiological models of hantaviruses. Life-long shedding could enable hantaviruses to survive the population bottlenecks that are commonly observed in their rodent hosts, and promote virus dispersal in fragmented habitats where local host and/or virus populations face frequent extirpation. These findings suggest that the abundance of infective hosts, which is the most important driver of human hantavirus epidemics, may be underestimated in current simulation models. Whatever the underlying reasons for the discrepancy between experimental and natural infections, our results stress the importance of studying zoonotic infections in natural settings. Further investigation of hantavirus shedding by wild hosts in other virus-host pairs is urgently needed and the assumptions in transmission models need to be updated. Embracing these recommendations will lead to improved techniques to predict the threat posed by hantavirus to Public Health.

RESERVOIR HOST SPECIES

Rodent species exhibit different capacities to act as reservoirs of hantaviruses. Hantavirus infections are believed to be asymptomatic and chronic in their rodent hosts. Interestingly, hantavirus has yet to be detected in several rodent species that appear to be suitable reservoirs. This may be due to a lack of exposure to the virus or inadequate sampling. Indeed, hantavirus infections in new reservoir hosts have recently been reported in poorly sampled regions, for example, Sangassou and other hantaviruses in African rodents. A variety of new hantaviruses has been detected in shrews, moles and bats (Yanagihara et al. 2014), and these have been established as important reservoirs for hantaviruses. Non-reservoir species may also be those that die soon after infection, hence limiting the probability of detecting positive individuals in wild populations, or those in which hantavirus is unable to enter and/or replicate within cells. These would also include spillover infections in which the virus infects a non-reservoir host, as evidenced by an antibody response, but the virus is not transmitted further. Concepts of primary and secondary species, reservoir and spillover hosts are ambiguous, and detecting antibodies or RNA should not be considered as evidence of a role in the circulation of hantavirus.

Hantaviruses have been widely believed to have co-evolved with their reservoir hosts (Plyusnin and Sironen 2014). During active surveillance, numerous spillover events were observed and multiple hosts for a given hantavirus, or multiple hantavirus species in a single host species, have been proposed. The common vole (*Microtus arvalis*) is considered to be the reservoir host of Tula hantavirus (TULV). Since the first reports, TULV has been found in a number of *Microtus* species including the field vole (*M. agrestis*), the sibling vole (*M. levis*) and water vole (*Arvicola amphibius*) (Schlegel et al. 2012). The field vole might also host the recently detected

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Tatenale hantavirus (Pounder et al. 2013). These findings emphasise the need for further investigation of the host-specifity of hantaviruses and the potential for host-switching that may be associated with changes in the virulence of these pathogens.

Over the past decade a wide variety of hantaviruses have been detected in shrews and moles throughout the world. Four sori-comorph-borne hantaviruses circulate in Finland (Ling et al. 2014), including Boginia virus in the water shrew (*Neomys fodiens*) and Asikkala virus in the pygmy shrew (*Sorex minutus*). Common shrews (*S. araneus*) were found to harbour two different hantaviruses: Seewis virus (SWSV) and a new distinct and genetically divergent virus (Altai-like virus) which cluster with the most basal hantaviruses known. This was the first evidence of the coexistence of two clearly distinct hantavirus species circulating simultaneously in a single host species population. These results contradict earlier hypotheses concerning rodent-borne hantaviruses, and suggest an ancient host-switching event from a yet unknown host to *S. araneus*. These four insectivore-borne hantaviruses are reported from many European countries, with the addition of Nova virus in common moles (*Talpa europaea*).

In Finland, PUUV (carried by *M. glareolus*) co-circulates with SWSV (carried by *S. araneus)*. While PUUV causes 1,000 to 3,000 nephropathia epidemica cases annually, the pathogenicity of SWSV (or any insectivore-borne hantavirus) to man is unknown. To study the prevalence of SWSV antibodies in hantavirus fever-like patient sera, 486 samples (sent for PUUV diagnostics) were screened, but no evidence of SWSV infection was found among them (Ling et al. 2015). It is difficult to prove a negative result, but this data strongly suggests that insectivore-borne hantaviruses do not pose a threat to humans.

THE HOST POPULATION

Epidemiological patterns of human hantavirus infections are related to changes in the reservoir rodent population. Voles typically exhibit pronounced population cycles or more irregular outbreaks with high amplitude. Populations may also display prolonged but transient periods of weak cyclicity (Korpela et al. 2013), which may be reflected in the occurrence of zoonotic diseases. In temperate Europe, masting is an important driver of hantavirus epidemics (mostly due to PUUV) and warm summers are known to induce this effect (Tersago et al. 2011). In contrast, specialist predation is thought be the main factor affecting bank vole populations in northern Europe (Korpela et al. 2014).

During the EDEN and EDENext projects, one of our main aims was to understand the differences in the epidemiology of human hantavirus infections in the different climatic and biomic conditions of boreal and temperate Europe. Clear seasonal differences have been documented in the transmission dynamics of PUUV between the two biomes, and this is also reflected in the seasonality of human epidemics. Transmission of PUUV among bank voles in Finland is seasonal and peak seroprevalences are found in old overwintered voles in late spring-early summer and also in low-density years. Peak densities of positive voles are found in late autumn-early winter, one to two months prior to the peak in human infections. As a factor on which to gauge the imminent threat to Public Health, we recommend the abundance of positive rodents be used rather than rodent seroprevalence alone. Bank vole density, as such, is a reasonable proxy for infection risk.

The potential role played by landscape homogeneity and heterogeneity has been examined in rodent and virus dispersal and with respect to differences in the presence or absence of host threshold densities for PUUV. Landscape genetics have been used to analyse the dispersal of rodent hosts and viruses at different geographic scales, for example, indicating an efficient dispersal in non-fragmented boreal forests. Also, within geographic regions, higher genetic diversity is related to higher PUUV seroprevalence (Cosson et al. ms), reflecting the role of dispersal in homogenous landscapes. The quality of habitat in terms of resources, parasitism, predation and fragmentation may trigger trade-offs affecting the investment of individuals in alternative immunological pathways**.**

In nature, animals usually have multiple infections and the interactions among pathogens and their succession, including PUUV and CPXV, has been documented (Guivier et al. 2014). Landscape immunogenetics has been used to study immune gene variability among landscapes and its causes, and has provided critical information concerning parasite epidemiology. Several landscape features (for example, habitat quality, metapopulation dynamics) and helminth co-infection were identified and may participate in shaping the immunoheterogeneity of wild host populations (Guivier et al. 2014). In the future, the integration of such information in the analysis of PUUV epidemiology will improve our understanding of the impacts of co-infection on the evolution of PUUV tolerance in bank voles.

One of the main aims has been to develop predictive models of the spread or dispersal of a disease and a spatially explicit model for PUUV infection dynamics has been built. It was used to investigate PUUV infection patterns during vole population cycles, both in the vole (PUUV) and human populations. The model reproduced the natural dynamics observed in the field and showed that the temporal existence of maternal antibodies in the offspring is an important factor influencing the observed infection patterns and the consequent infection risk to humans. Also, it showed that the density dependent spatial behaviour of voles seems to play a less important role than one might expect (Rejniers et al. ms).

Small mammal communities vary geographically and the impact of community structure and diversity of small mammal species, often expressed via the so-called dilution effect, has been of interest. The presence of other species can affect intraspecific contact rates in the reservoir species and thereby viral transmission rates. This effect can be seasonal because breeding animals are typically more territorial and aggressive than nonbreeding individuals in autumn or winter. In boreal Finland, when we controlled for host density, a significant dilution effect was observed for breeding bank voles in early summer but not among non-breeding individuals in autumn. A similar effect was observed for the wood mouse (*Apodemus sylvaticus*) on PUUV transmission among bank voles in Belgium. These results suggest that the species composition of the small mammal community could indeed play a role in PUUV infection dynamics (Voutilainen et al. 2012).

CONCLUSION

A vast amount of new data has been gathered during EDEN and EDENext and is now being used to understand the different factors that influence the dynamics of rodent-borne infections and host-pathogen relationships. The new knowledge is improving our ability to predict the range and population dynamics of hosts and the pathogens they spread in relation to environmental change. Understanding these processes and changes at the ecosystem scale will provide the basis for more efficient disease monitoring and the development of early warning systems.

Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Makela S, Mustonen J (2013). Uncovering the mysteries of hantavirus infections. *Nat Rev Microbiol* 11, 539-550.

Voutilainen L, Sironen T, Tonteri E, Tuiskunen Back A, Razzauti M, Karlsson M, Wahlstrom M, Niemimaa J, Henttonen H, Lundkvist A (2015). Life-long shedding of Puumala hantavirus in wild bank voles (*Myodes glareolus*). *J Gen Virol* Feb 2015.

Voutilainen L, Savola S, Kallio ER, Laakkonen J, Vaheri A, Vapalahti O, Henttonen H (2012). Environmental change and disease dynamics: Effects of intensive forest management on Puumala hantavirus infection in boreal bank vole populations. *PLoS One* 7, e39452.

DETERMINANT OF HUMAN EPIDEMICS OF RODENT-BORNE ZOONOSIS?

Hantavirus particles. © Jussi Hepojoki, Pasi Laurinmäki and Sarah Butcher.
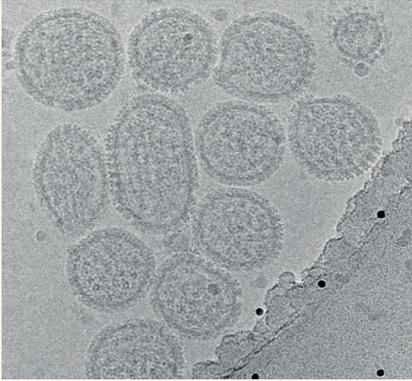
VIRUS

> Survival of the virions outside the host

> Virulence

> Ability to cross species barriers

INDIVIDUAL HOST OF INFECTION

> Persistence or clearance

> Susceptibility to infection

> Susceptibility to severe forms of infections (versus resistance)

Bank vole (Myodes glareolus). © Heikki Henttonen, LUKE.

RESERVOIR

> Longevity of shedding-natural versus laboratory settings

> Reservoir host versus spillovers hosts

> Co-divergence with the reservoir host

POPULATION

> Cyclic populations - predation in the boreal Europe and masting in temperate Europe

> Landscape structure

> Dilution effect

> Co-infections

> Changing environment

Bank vole distribution (Myodes glareolus). © EDENext DMT.

Habitat suitability index

PUBLICise Health Public Health Telegram: rodent-borne diseases

EDENext's Public Health Telegrams (PUBLICise Health) were established to inform interested individuals and institutions about research results on vector-borne diseases with direct or indirect impact on Public Health issues. Edited and produced by EDENext's Public Health team, each issue concentrates on the work of one of the project's five specialist groups. Here we present an updated version of the issue dedicated to the work of the team examining rodents and insectivore-borne diseases, first published in April 2013.

1. Detection of novel/emerging pathogens in new areas *Candidatus* Neoehrlichia *mikurensis*

The occurrence of the previously described novel human pathogen *Candidatus* Neoehrlichia *mikurensis* (CNM) was confirmed for the first time in trapped wild rodents in France during the course of an EDENext study (Vayssier-Taussat et al. 2012). CNM is an intracellular bacterium that belongs to the Anaplasmataceae family. DNA sequences had been found before in ticks and rodents originating from other European countries and from Asia. While the first human infection was reported from Sweden in 2010, followed by five cases described for Germany, Switzerland and the Czech Republic, and a canine infection reported from Germany, no infections in either humans or animals have yet been reported in France. Remarkably, the genotype of CNM isolated from the rodents, trapped in proximity to residential areas in the French Ardennes, was identical to those found earlier to cause disease in humans and animals. CNM infections might have hitherto been unrecognised or have been mistaken for other diseases displaying mild or unspecific symptoms (body aches, chills, sweats, fatigue and high fever) such as hantavirus-induced nephropathia epidemica, which is endemic in the French Ardennes. Because of the absence of serological tests and the gap in knowledge of medical authorities regarding this new pathogen, the relevance of CNM for Public Health is difficult to estimate. In order to provide fundamental data for a reliable assessment and evaluation of this Public Health risk, adequate diagnostic tools need to be developed and surveillance measures implemented.

CNM was discovered in French voles.
© LUKE, Finland.

Vayssier-Taussat M, Maaoui N, Le Rhun D, Buffet J-P, Galan M, Guivier E, Charbonnel N, Cosson J-F (2012). *Candidatus* Neoehrlichia *mikurensis* in bank voles, France. *Emerg Inf Dis* 18: 2063-5. Doi: 10.3201/eid1812.120846.

Seewis virus and other soricomorph-borne hantaviruses

Until recently, only rodent-borne hantavirus species were known in Central Europe. The development of advanced molecular techniques has led to the identification of numerous new soricomorph (insectivore)-borne hantavirus species in Asia, Africa, North America and Europe. In 2007, a Soricinae-borne hantavirus was detected in a common shrew (*Sorex araneus*) from Switzerland, the Seewis virus (SWSV). Further SWSV strains were later found in Hungary, Finland and Russia. Within EDENext, the presence, distribution and genetic variability of SWSV was investigated in various Sorex species captured in Germany, the Czech Republic and Slovakia between 2004 and 2011. The results confirmed the presence of SWSV in Germany, as well as in the Czech Republic and Slovakia (Schlegel et al. 2012). Furthermore, the first comprehensive sequence analyses of SWSV strains from these countries were achieved indicating a broad geographical distribution and high genetic divergence of SWSV in Central Europe. A more detailed analysis of the molecular evolution of SWSV may shed more light on the complex evolution and host adaptation processes of hantaviruses in general. In light of these results, future investigations should aim at assessing the zoonotic potential and pathogenicity of SWSV to evaluate their potential threat for Public Health.

In Finland, Seewis virus was reported in 2009 from archival common shrew (*Sorex araneus*) samples collected in 1982. To elucidate the diversity of hantaviruses in soricomorphs in Finland, 180 individuals were screened by Ling et al. (2014), representing seven species captured from 2001 to 2012: hantavirus RNA was screened using RT-PCR, and hantaviral antigen using immunoblotting with polyclonal antibodies raised against truncated SWSV nucleocapsid protein. The overall hantavirus RNA prevalence was 14% (26/180), and antigen could be demonstrated in 9 of 20 SWSV RT-PCR positive common shrews. Genetic analyses revealed that four soricomorph-borne hantaviruses circulate in Finland, including Boginia virus (BOGV) in water shrew (*Neomys fodiens*) and Asikkala virus (ASIV) in pygmy shrew (*Sorex minutus*). Interestingly, on two study sites, common shrews harboured strains of two very different hantaviruses: Seewis virus and a new distinct,

Ling J, Sironen T, Voutilainen L, Hepojoki S, Niemimaa J, Isoviita, V.- M.,Vaheri, A., Henttonen, H. and Vapalahti O (2014). Hantaviruses in Finnish soricomorphs: evidence for two distinct hantaviruses carried by *Sorex araneus* suggesting ancient host-switch. *Infection, Genetics and Evolution* 27:51-61.

genetically distant (identity 57% at amino acid level) virus (Altai-like virus). This is the first ever evidence of coexistence of two clearly distinct hantavirus species circulating simultaneously in one host species population. The findings suggest an ancient host-switching event from a yet unknown host to *S. araneus*. In addition, phylogenetic analyses of partial S and M segment sequences showed that SWSV in Finland represents a unique genotype in Europe.

Seoul hantavirus in rats

Until recently, very little was known of the globally distributed Seoul hantavirus in brown rats (*Rattus norvegicus*) in Europe. Following the observation of a human case that originated from a pet rat in the UK, screening in Sweden also revealed Seoul hantavirus in pet rats. The virus has also been found in Belgium, The Netherlands (Goeijenbier et al. 2015) and France. Seoul hantavirus causes a more severe form of haemorrhagic fever (HFRS) with renal syndrome than Puumala hantavirus, the agent for the most common form of HFRS in Europe. The potential for HFRS due to Seoul virus is not well understood in Europe, and diagnostic tools are not widely optimised for this hantavirus. Recognising the emerging character of Seoul hantavirus could have significance for Public Health in large areas of Europe. Special attention should be paid to safety with pet rats.

Cowpox virus in voles in Hungary and Finland

As a result of discontinuing vaccination against smallpox in the late 1970s, different orthopoxviruses (OPVs), such as cowpox virus (CPXV), have become a re-emerging healthcare threat among zoonotic pathogens. Two recent EDENext articles have analysed OPVs in Finland and Hungary.

In northern Europe, rodent populations display cyclic density fluctuations that can be correlated with the human incidence of zoonotic diseases they spread. During density peaks, field voles (*Microtus agrestis*) become one of the most abundant rodent species in northern Europe, yet little is known of the viruses they host. Forbes et al. (2014) screened 709 field voles in Finland, trapped from 14 sites over three years, for antibodies against four rodent-borne, potentially

Schlegel M, Radosa L, Rosenfeld UM, Schmidt S, Triebenbacher C, Löhr P-W, Fuchs D, Heroldová M, Jánová E, Stanko M, Mošanský L, Fričová J, Pejčoch M, Suchomel J, Purchart L, Groschup MH, Krüger DH, Klempa B and Ulrich RG (2012). Broad geographical distribution and high genetic diversity of shrew-borne Seewis hantavirus in Central Europe Virus Genes 2012 Aug 45: 48-55. Doi: 10.1007/s11262-012-0736-7. Epub 2012 Mar 31.

Goeijenbier M, Verner-Carlsson J, van Gorp ECM, Rockx B, Koopmans MPG, Lundkvist Å, van der Giessen JWB and Reusken CBEM (2015). Seoul hantavirus in brown rats in the Netherlands: implications for physicians - Epidemiology, clinical aspects, treatment and diagnostics. *The Netherlands Journal of Medicine,* May 2015, Vol. 73, No. 4.

*Field voles (*Microtus agrestis*). © E. Oksanen.*

zoonotic viruses or virus groups - hantaviruses, lymphocytic choriomeningitis virus (LCMV), Ljungan virus (LV), and orthopoxviruses (OPV). Antibodies against all four viruses were detected. However, seroprevalence of hantaviruses, LV and LCMV was low in field voles. On the other hand, OPV antibodies (most likely cowpox, CPWV) were more common but restricted geographically to south-east Finland. This is interesting because in sympatric bank voles, cowpox virus occurs more widely. Within these positive field vole sites, antibody prevalence showed delayed density dependence in spring and direct density dependence in the autumn. Higher seroprevalence was found in spring than autumn. These results substantially increase knowledge of CPXV infection dynamics.

In Hungary, data on OPV prevalence among its rodent host species have been absent. Rodents belonging to four species - striped field mouse (*Apodemus agrarius*), yellow-necked mouse (*A. flavicollis*), wood mouse (*A. sylvaticus*) and bank vole (*Myodes glareolus*) - were live-trapped by Oldal et al. (2015) at 13 sampling plots in the Mecsek Mountains, Hungary, from March to September in 2011 and 2012. Among the 1,587 tested rodents, 286 (18.0%) harboured OPV-specific antibodies. Seroprevalence was highest in bank voles (71.4%) and striped field mice (66.7%). Due to a masting event in the autumn of 2011 across Central Europe, the abundance of bank voles increased dramatically in the 2012 season, raising the overall OPV seroprevalence. These are the first data on OPV occurrence and seroprevalence in rodents in Hungary. The common circulation of OPV in rodents in densely populated areas warrants further studies to elucidate the zoonotic potential of OPVs and the risk for Public Health.

Forbes KM et al. (2014). Serological Survey of Rodent-Borne Viruses in Finnish Field Voles. *Vector-borne Zoon Dis.* 14:278- 283. Doi: 10.1089/vbz.2013.1526.

Oldal M et al. (2015). Serologic Survey of Orthopoxvirus Infection Among Rodents in Hungary. *Vector-borne Zoon. Dis* 15:317-322. Doi: 10.1089/vbz.2014.1731.

2. Development of novel diagnostic tools for recently detected Soricomorpha-borne hantaviruses

Despite numerous recent reports on shrew- and mole-associated hantaviruses from America, Europe, Asia and Africa, since the identification of the first non-rodent borne hantavirus - Thottapalayam virus (TPMV) in 1971 – little is so far known about the antigenic and immunological characteristics of TPMV and the newly isolated Soricomorpha-borne hantaviruses. The differences in antigenicity

compared to that of rodent-borne hantaviruses, including the prototype Hantaan virus (HTNV), has not yet been studied, and the understanding of the pathogenicity and the course of infection, as well as the humoral immune response in shrews is poor. Until now, limits in diagnosis, particularly due to the lack of commercially available species-specific secondary antibodies, have hampered progress in this matter.

Within EDENext, TPMV-specific monoclonal antibodies and anti-shrew immunoglobulin G (IgG) antibodies were generated (Schlegel et al. 2012). Using these antibodies, serological tools for the detection of hantavirus-specific antibodies and hantavirus antigens in shrews were subsequently developed. The novel monoclonal antibodies have proved to represent useful tools for the detection of TPMV, and antigenically related hantaviruses, in cell culture whereby the shrew anti-TPMV-antisera could serve as a positive control. The generation of mouse anti-shrew-IgG allowed the detection of hantavirus-specific antibodies in transudates and sera derived from immunized and experimentally infected shrews. Hence, the established serological tools are assumed to be helpful in discovering novel insectivore-associated hantaviruses and in characterising the humoral immune response and antigen expression in hantavirus-infected insectivores. Moreover, they may be used for diagnosis and surveillance purposes in order to estimate the potential threat which TPMV and antigenically related hantaviruses pose to Public Health.

Human antibodies to shrew-borne hantaviruses?

Puumala virus carried by the bank vole (*Myodes glareolus*) co-circulates with Seewis virus (SWSV, carried by the common shrew (*Sorex araneus*)) in Finland. While PUUV causes 1,000–3,000 nephropathia epidemica cases annually, the pathogenicity of SWSV to man is unknown. To study the prevalence of Seewis antibodies among hantavirus fever-like patients' sera, Ling et al. (2015) used recombinant SWSV nucleocapsid (N) protein as the antigen in enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), and immunoblotting (IB). While characterising the recombinant SWSV N protein, they observed that a polyclonal rabbit antisera against PUUV N cross-reacts with SWSV N, and vice versa. They initially screened in SWSV N

IgG ELISA 486 (450 PUUV seronegative and 36 PUUV seropositive) samples sent to Helsinki University Central Hospital Laboratory for PUUV serodiagnosis during 2002 and 2007. In total 4.2% (19/450) of PUUV seronegative were reactive in SWSV N IgG ELISA, and none of 58 PUUV seronegative samples were reactive in SWSV N IgM ELISA. None of these reactions could be confirmed by IFA or IB. Furthermore, out of 36 PUUV seropositive serum samples, three showed reactivity in SWSV N IgG and 10 in SWSV N IgM ELISA. One of the PUUV-seropositive samples was IgG positive in IFA and four in IB. Finally, researchers used a competitive IgG ELISA to confirm that the observed reactivity toward SWSV N protein is actually a cross-reaction and not a true SWSV response. In conclusion, **no evidence of SWSV infection** was found among a panel of 486 patient serum samples. However, they could demonstrate a cross-reaction anti-PUUV serum with shrew-borne hantavirus N protein.

3. Analysing the effects of environmental change on rodent populations

Anthropogenic disturbance of ecosystems may, inter alia, result in a changed distribution and abundance of vector species harbouring zoonotic pathogens. The bank vole, *Myodes glareolus,* is the rodent reservoir of Puumala virus (PUUV), the pathogen that causes nephropathia epidemica (NE), a mild form of haemorrhagic fever with renal syndrome (HFRS), the most common disease caused by hantavirus infections in Europe. Its incidence is highest in the boreal zone in northern and north-eastern Europe. Here, human NE epidemic peaks correlate with high bank vole abundance, reflecting the seasonal and cyclic multiannual dynamics of the host. Apart from factors causing fluctuations of rodent populations as such, an important reason for this epidemiological pattern regarding human NE cases has been hypothesized to result from differences in landscape structures between temperate and boreal Europe. In boreal Finland, forests, the preferred habitat of bank voles, cover more than 77% of land, whereas in temperate Belgium it is only 23%. In consequence, the NE incidence rate is more than ten times higher in Finland than in Belgium.

An EDENext study investigated the connection between forest age and the abundance of PUUV-in-

fected bank voles, and what influence the intensive management of boreal forests has on the dynamics of PUUV in bank voles (Voutilainen et al. 2012). Furthermore, the correlation between the age of forests, bank vole abundance and the abundance of non-host species with the likelihood of PUUV-infections in bank voles was analysed. Results suggest that even though habitat alterations through intensive forest management can potentially reduce the hantavirus load in the environment, PUUV is commonly found in both old forests and young plantations. In conclusion, despite intensive management measures in forests, the majority of the Fennoscandian boreal forest landscape constitutes a source for hantavirus infection of humans.

Voutilainen L, Savola S, Kallio ER, Laakkonen J, Vaheri A, Vapalahti O and Henttonen H (2012). Environmental Change and Disease Dynamics: Effects of Intensive Forest Management on Puumala Hantavirus Infection in Boreal Bank Vole Populations. *PLoS One* 2012; 7: e39452. Doi: 10.1371/journal.pone.0039452. Epub 2012 Jun 20.

4. How long can wild rodents excrete hantaviruses?

The knowledge of viral shedding patterns and viraemia in the reservoir host species is a key factor in assessing the human risk of zoonotic viruses. The shedding of hantaviruses (Bunyaviridae) by their host rodents has widely been studied experimentally, but rarely in natural settings. Voutilainen et al. (2015) studied the dynamics of Puumala hantavirus (PUUV) shedding and viraemia in naturally infected wild bank voles (*Myodes glareolus*) in the field. In a monthly capture-mark-recapture study they collected shedding data from more than a hundred voles. They analysed those 18 bank voles with the longest follow-up history for the presence and relative quantity of PUUV RNA in the excreta and blood from two months before up to eight months after seroconversion. The proportion of animals shedding PUUV RNA in saliva, urine and faeces peaked during the first month after seroconversion, but continued throughout the study period with only a slight decline. The quantity of shed PUUV in reverse transcription quantitative PCR (RT-qPCR) positive excreta was constant over time. In blood, PUUV RNA was present for up to seven months but both the probability of viraemia and the virus load declined with time. The findings contradict the current view of a decline in virus shedding after the acute phase and a short viraemic period in hantavirus infection – an assumption widely adopted in current epidemiological models. The authors suggest life-long shedding as a means for hantaviruses to survive over host population bottlenecks, and to disperse

in fragmented habitats where local host and/or virus populations face temporary extinctions. The results indicate that the kinetics of pathogens in wild hosts may differ considerably from those observed in optimal conditions in laboratory settings. Go to the field!

Voutilainen et al. (2015). Life-long shedding of Puumala hantavirus in wild bank voles (*Myodes glareolus*). *J. Gen. Virology* (in press). Doi: 10.1099/vir.0.000076

5. Analysing the effects of climate changes on rodent populations

Rodents play a key role in many ecosystems, for example as prey animals for a variety of predators and by affecting the structure of plant communities through their grazing. They also affect human societies, not only by causing economic losses in forestry and agriculture, but also because they pose a serious concern for Public Health as reservoir hosts for a number of zoonotic diseases. The hypothesis that warmer winters cause the disappearance of multiannual vole cycles was tested in an EDENext study (Korpela et al. 2013). A reliable analysis of population dynamics and of effects of climate thereon requires extensive spatial and temporal data. Therefore, all data available on vole abundances and climate collected at 33 locations throughout Finland during the period 1970 to 2011 were combined. The results of this study allow the assumption that vole population dynamics exhibit geographic and temporal variations that are associated with a variation in climate. Reduced cyclicity should be observed when winter temperatures are mild. The spatiotemporal variation in dynamics correlated with seasonal temperatures: cyclicity was weakened by increasing temperatures in the north, but strengthened in the south. Further, the study results do not support the hypothesis that warm winters are uniformly leading to irregular or more stable dynamics in boreal vole populations. Long and cold winters were not a prerequisite for high amplitude multiannual cycles, nor were warm winters with a reduced duration of snow cover associated with reduced winter growth rates.

Small mammalian specialist predators, i.e. small mustelids such as least weasels and stoats, have commonly been attributed as the important cause of pronounced cyclicity of voles in the boreal zone. Korpela et al. (2014) analysed the impact of climate on the long-term trends in cyclicity

in Finland. In several parts of Europe reports of collapsing rodent cycles have attributed the changes to warmer winters, which weaken the interaction between voles and their specialist subnivean (under-snow) predators. Using national long-term population data on voles, predators, birds of prey and climate, collected throughout Finland from 1986 to 2011, they analysed the spatio-temporal variation in the interactions between populations of voles and specialist, generalist and avian predators, and investigated by simulations the roles of the different predators in the vole cycle. They tested the hypothesis that vole cyclicity is dependent on predator–prey interactions during winter. The results strongly support the importance of small mustelids for the vole cycle. However, in the models weakening specialist predation during winters, or an increase in generalist predation, was not associated with the loss of cyclicity. Strengthening of delayed density dependence coincided with strengthening small mustelid influence on the summer population growth rates of voles. In conclusion, a strong impact of small mustelids during summer appears highly influential to vole population dynamics, though this importance between winter and summer may vary geographically, and deteriorating winter conditions are not a viable explanation for collapsing small mammal population cycles. Changing climate may affect this interaction between voles and specialist predators.

Korpela K, Delgado M, Henttonen H, Korpimaki E, Koskela E, Ovaskainen O, Pietiainen H, Sundell J, Yoccoz NG and Huitu O (2012). Non-linear effects of climate on boreal rodent dynamics: warm winters do not negate high-amplitude cycles. *Global Change Biology* 2013 Mar 19: 697–710.

Korpela K, Helle P, Henttonen H, Korpimäki E, Koskela E, Ovaskainen O, Pietiäinen H, Sundell J, Valkama J and Huitu O (2014). Predator-vole interactions in boreal Europe: the role of small mustelids revised. *Proc. R. Soc. B* 281. 20142119.

6. Analyses of the evolution and molecular epidemiology of hantaviruses

A fatal case of nephropathia epidemica (NE)

Despite the usually mild course of NE (case fatality rate is less than 0.1%), fatal cases have been reported, and 5% of patients need dialysis. In EDENext, the first complete PUUV gene sequence was directly recovered from a fatal case from Finland. In order to find out whether the fatal outcome of the disease was due to a particular genetic variant of PUUV, wild-type PUUV isolates originating from bank voles trapped in and near the house of the patient were searched for identical or closely related genetic variants (Plyusnina et al. 2012). Indeed, one completely sequenced rodent-originated PUUV genome was identical to the fatal human case PUUV genome.

Subsequent phylogenetic analysis confirmed that this virus was closely related to genetic variants identified earlier, approximately 60km north-west from where the infection occurred. These findings demonstrate that the fatal human NE case and local wild-type PUUV strains are genetically linked. The results of the sequence analyses also revealed that no mutations had been acquired in the genome of PUUV during the transmission of the virus to the patient resulting in a fatal outcome. In conclusion, the fatal outcome was not due to a unique or even rare genetic hantavirus variant causing the infection but may rather be a consequence of a possible defect in the antiviral defence mechanisms in the patient's immune system. The patient showed a HLA (human leukocyte antigen)-B8-DR3 haplotype which is associated with a more severe outcome of NE, including a C4A null allele, i.e. a major antivirus defence system complement was impaired. Furthermore, the patient had been a heavy smoker, which has been shown to be a real risk factor in contracting NE. In regard to Public Health, identifying the risk factors that may lead to a fatal outcome of a hantavirus infection is of great importance in reducing the disease burden.

Proposal of a new classification of Dobrava-Belgrade virus

More than 20 years ago, a human pathogenic hantavirus was isolated from a patient with clinically severe haemorrhagic fever with renal syndrome (HFRS) and, at the same time, from a yellow-necked mouse (*Apodemus flavicollis*) captured in Slovenia. After it had become evident that both isolates were identical, it was named Dobrava-Belgrade virus (DOBV). Subsequently, DOBV and DOBV-like viruses were found in various rodent species of the *Apodemus* genus in several European countries and Russia. Phylogenetic analyses of the isolates from *A. flavicollis* and *A. agrarius*, occurring largely sympatric in Slovenia and Slovakia, clearly showed that DOBV isolates cluster in distinct evolutionary lineages according to their reservoir hosts. This was confirmed when virus sequences from a third host, *A. ponticus*, were analysed. As a result of EDENext research, a novel intra-species classification of DOBV into four related genotypes, i.e. Dobrava, Kurkino, Saaremaa, and Sochi, has been proposed based on phylogenetic analyses of the S segment (Klempa et al. 2013). The four genotypes show characteristic

Plyusnina A, Razzauti M, Sironen T, Niemimaa J, Vapalahti O, Vaheri A, Henttonen H and Plyusnin A (2012). Analysis of complete Puumala hantavirus genome, Finland. E*merg Infect Dis* 2012 Dec 18: 2070-2. Doi: 10.3201/eid1812.120747.

*Yellow-necked mouse (*Apodemus flavicollis*).*
© Gianfranco Scieghi.

differences in their phylogeny, host reservoirs, geographical distribution and in their pathogenicity in humans. According to the current stage of knowledge, the order of virulence in humans of the DOBV genotypes is assumed to be as follows: Dobrava > Sochi > Kurkino > Saaremaa. In terms of Public Health, it will be important to determine which genetic differences between the four virus genotypes are responsible for the different virulence, and whether differences in the susceptibilities of resident populations influence the severity of disease.

Klempa B, Avsic-Zupanc T, Clement J, Dzagurova TK, Henttonen H, Heyman P, Jakab F, Kruger DH, Lundkvist Å, Maes P, Nemirov K, Papa A, Tkachenko EA, Ulrich RG, Vapalahti O and Vaheri A (2012). Complex evolution and epidemiology of Dobrava-Belgrade hantavirus: implications for taxonomy. *Arch Vir.* Doi 10.1007/s00705-012-1514-5.

7. Reviews on hantavirus and other rodent-borne pathogens causing haemorrhagic fever virus infections

Hantavirus infections in Europe and their impact on Public Health

A review by Vaheri et al. (2013) highlights the most important Public Health issues of hantavirus infections in Europe. There are several hantavirus species in Europe causing haemorrhagic fever with renal syndrome (HFRS). The most common is Puumala virus that causes a mild form of HFRS called nephropathia epidemica (NE). Dobrava-Belgrade virus (DOBV) consists of four genotypes that differ in pathogenicity: Dobrava causes severe symptoms and a high case fatality rate (up to 12%), Sochi causes HFRS of medium severity, Kurkino causes a rather mild disease and Saaremaa a possibly mild or no disease. Seoul virus, carried by rats, causes HFRS with medium severity but is rare. In addition to renal changes, cardiac, pulmonary, ocular and hormonal disorders are quite common during the acute stage of a PUUV infection, the major cause of HFRS in Europe. Although PUUV-associated HFRS has a low (0.1-0.4 %) case fatality rate, complications and long-term consequences regularly occur. About 5% of hospitalised patients require dialysis and some need prolonged intensive care treatment. In this context, prospective studies are needed to define the role of hantavirus infections in the development of chronic kidney diseases, hyper-tension, hormonal disorders and other possible chronic diseases. Research is also needed to understand the mechanism of shock and vascular leakage in order to properly treat the severe forms of HFRS.

Vaheri A, Henttonen H, Voutilainen L, Mustonen J and Vapalahti O (2012). Hantavirus infections in Europe and their impact on public health. *Rev Med Virol.* 2013 Jan 23: 35-49. Doi: 10.1002/rmv.1722. Epub 2012 Jul 3.

Despite the availability of diagnostic tools in many endemic areas in Europe, there are still several countries where HFRS has not yet been recognised by the medical community and may therefore be worryingly underdiagnosed. Field investigations are therefore needed to evaluate the occurrence and distribution of hantaviruses within rodent populations in addition to risk-based sero-surveys of patients to detect new endemic areas. In Europe, thousands of HFRS cases are diagnosed annually and the numbers are increasing. As no vaccine or specific antiviral therapy is in general use in Europe, the development of vaccines containing components of a vole-derived virus (PUUV) and of a mouse-derived virus (DOBV) must be recommended.

Epidemiology, diagnostics and treatment of rodent-borne haemorrhagic fever virus infections

A review by Goeijenbier et al. (2013) gives a practical overview of the epidemiology, diagnosis and treatment of the most important rodent-borne haemorrhagic fever pathogens that are transmitted directly from rodents to humans: Leptospira, hantaviruses, and New and Old World arenaviruses. These belong to an important group of zoonotic pathogens causing severe disease all over the world. Having become a serious challenge for clinicians and laboratory researchers they are of particular importance for Public Health due to increases in international travel and adventure tourism in regions where the pathogens occur. Clinical symptoms, transmission routes and other epidemiological features of the pathogens are often similar and they often have an emerging pattern. High case fatality rates are characteristic for a fulminant manifestation of the diseases. The rather non-specific clinical picture of rodent-borne haemorrhagic fevers, particularly in the early phase, may lead to misdiagnosis. Besides, treatment strategies are usually based on the success in treatment of individual patients or on case reports rather than on randomised controlled trials. Consequently, the compilation of treatment protocols is more anecdotal than evidence based. Moreover, vaccines are often not available or are not approved by the authorities. Because of the rather unspecific clinical symptoms and similar laboratory parameters, along with the partial overlapping geographical distribution,

the accurate diagnosis of rodent-borne haemor-
rhagic fevers depends mainly on the availability
of sensitive and specific tests and high-level
clinical practice. Preventive Public Health mea-
sures include rodent control, the development of
vaccines and education of the public directed at
avoiding contact with rodents or rodent excreta.
For applying these measures, a fundamental un-
derstanding of the pathogens and their specific
rodent hosts is necessary. Compilation of the
present data on rodent-borne haemorrhagic fever
pathogens will provide valuable information for
clinicians.

Goeijenbier M, Wagenaar JFP,
Goris MGA, Martina BEE, Hent-
tonen H, Vaheri A, Reusken
CBEM, Hartskeerl R, Osterhaus
ADME and van Gorp ECM (2013).
Rodent-borne haemorrhagic fe-
vers: under-recognised, widely
spread and preventable - epide-
miology, diagnostics and treat-
ment. *Crit Rev Microbiol* 2013
Feb; 39: 26-42.

Uncovering the mysteries of hantavirus infections — a review of hantavirus disease pathology and immunology

Hantaviruses are negative-sense single-stranded
RNA viruses that infect many species of rodents,
shrews, moles and bats. Infection in these re-
servoir hosts is almost asymptomatic, but some
hantaviruses can be transmitted via aerosols of
rodent excreta to humans, in which case they can
cause two diseases: haemorrhagic fever with re-
nal syndrome (HFRS), which is primarily caused by
Hantaan virus (HTNV) and related viruses in Asia,
Puumala virus (PUUV) and Dobrava virus (DOBV) in
Europe, and Seoul virus (SEOV) worldwide; or han-
tavirus cardiopulmonary syndrome (HCPS), which
is caused by Sin Nombre virus (SNV) and related
viruses in North America, and Andes virus (ANDV)
and related viruses in Latin America. Vaheri et
al. (2013) discuss the basic molecular properties
and cell biology of hantaviruses and offer an
overview of virus-induced pathology, in particu-
lar vascular leakage and immunopathology. These
diseases are characterised by increased capilla-
ry permeability (causing vascular leakage) and
thrombocytopenia. These pathologies are thought
to be caused by viral infection of endothelial
cells, which does not disrupt the endothelium but
nonetheless leads to dramatic changes in both the
barrier function of the endothelium as a whole
and the function of infected endothelial cells.
It has also been suggested that cytotoxic CD8+ T
cells (CTLs) trigger capillary leakage and that
cytokines contribute to the increased capilla-
ry permeability. The terminal soluble complement
complex can also increase vascular permeability,
and complement activation is linked to severity
of hantavirus infection.

Hantavirus infections and the diseases that they cause are a growing Public Health problem, and there is currently no therapy or vaccine in global use. Moreover, by the time the symptoms of HFRS or HCPS appear, viraemia has already started fading, so the administration of antiviral drugs might not be beneficial. Thus, a better understanding of the viral infection and of the involvement of factors such as vascular leakage, cytokine storm and CTL proliferation in disease pathology will be essential to the development of new therapies. Detailed knowledge about the exact contribution of these different host response factors to hantavirus pathology is currently limited, and it is unclear whether one factor acts as the primary cause of pathogenesis and others are secondary causes. It is possible that insights into the pathogenesis of hantavirus infections will also be gained by studying the pathological mechanisms which lead to vascular leakage in infections with other viruses causing haemorrhagic (flaviviruses, filoviruses and arenaviruses).

Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Mäkelä S and Mustonen J (2013). Uncovering the mysteries of hantavirus infections. *Nature Reviews in Microbiology* 11:539-550. Doi:10.1038/nrmicro3066.

8. The importance of immunogenetics for hantavirus infection in rodents and humans

It is often not well understood that both in humans and animals there is great genetic and immunogenetic variation in disease susceptibility. Charbonnel et al. (2014) reviewed the associations of immunity-related genes with susceptibility of humans and rodents to hantaviruses, and with severity of diseases in humans. Several class I and class II human leucocyte antigens (HLA) haplotypes are linked with severe or benign human hantavirus infections, and these haplotypes varied among localities and hantaviruses. The polymorphism of other immunity-related genes including the C4A gene and a high-producing genotype of tumour necrosis factor (TNF) gene associated with severe PUUV infection. Additional genes that may contribute to disease or to PUUV infection severity include non-carriage of the interleukin-1 receptor antagonist (IL-1RA) allele 2 and IL-1β (-511) allele 2, polymorphisms of plasminogen activator inhibitor (PAI-1) and platelet GP1a.

In addition, immunogenetic studies have been conducted to identify mechanisms that could be linked with the persistence/clearance of hanta-

viruses in reservoir hosts. Persistence was associated during experimental infections with an upregulation of anti-inflammatory responses. Using natural rodent population samples, polymorphisms and/or expression levels of several genes have been analysed. These genes were selected based on the literature of rodent or human/hantavirus interactions. The comparison of genetic differentiation estimated between bank vole populations sampled over Europe, at neutral and candidate genes, has allowed to evidence signatures of selection for *Tnf, Mx2* and the *Drb Mhc* class II genes. Altogether, these results corroborated the hypothesis of an evolution of tolerance strategies in rodents. In other words, the bank vole has quite different immunogenetic responses to Puumala virus in different parts of Europe, which could partly explain the variable epidemiology of Puumala virus in Europe.

Charbonnel N, Pagès M, Sironen T, Henttonen H, Vapalahti O, Mustonen J and Vaheri A (2014). Immunogenetic Factors Affecting Susceptibility of Humans and Rodents to Hantaviruses and the Clinical Course of Hantaviral Disease in Humans. *Viruses* 2014 (6) 2214-2241; 2014. Doi:10.3390/v6052214.

9. From single infection studies to understanding of multiple infections and interactions among parasites/pathogens

Until quite recently most epidemiological studies on wild pathogens and reservoirs were done using single pathogen models. During the past decade it has been increasingly understood that parasites/pathogens interact and these interactions affect the immunology and susceptibility of hosts. In this EDENext article, the authors described spatial heterogeneity in immunity in bank vole populations with respect to landscape features and parasite coinfections, the focus being on Puumala hantavirus and the bank vole's common parasite, the nematode *Heligmosomum mixtum*. They assessed the consequences of this heterogeneity for the risk of Puumala hantavirus (PUUV) infection. The authors had earlier found a positive association between infection with *H. mixtum* and PUUV. They found that the tumor-necrosis factor a (Tnf-a) was more strongly expressed in voles infected with PUUV than in uninfected voles or in voles co-infected with the nematode *H. mixtum* and PUUV. Indeed, *H. mixtum* may limit the capacity of the vole to develop pro-inflammatory responses. This effect may increase the risk of PUUV infection and replication in host cells. Overall, the results suggest that close interactions between landscape features, co-infections and immune gene expression may shape PUUV epidemiology.

Guivier E, Galan M, Henttonen H, Cosson JF and Charbonnel N (2014). Landscape features and helminth co-infection shape bank vole immunoheterogeneity, with consequences for Puumala virus epidemiology. *Heredity* 112: 274-281. Doi: 10.1038/hdy.2013.103

10. Modelling using the example of Puumala hantavirus

Even though the data are similar in nature, modelling zoonotic diseases in humans differs from modelling the distribution of individual species because the distribution of pathogens with zoonotic potential may depend on the presence of more than one species. An EDENext study used data from Sweden on Puumala hantavirus (PUUV) infections in humans, reported between 1991 and 1998, as a case study in order to compare existing approaches when modelling spatial distributions recorded as points. In Sweden, PUUV, the pathogen causing nephropathia epidemica (NE), is the only hantavirus and is hosted by bank voles (*Myodes glareolus*). The majority of NE cases (90%) are notified from the four northernmost counties.

Previous studies have shown that the prevalence of NE is linked to host abundance and human activities with an increased risk of exposure to rodents, such as forestry, farming, wood cutting, construction work, camping, cleaning and/or redecorating buildings where rodents live. Virus prevalence and transmission depend on the local environmental, anthropogenic, genetic, behavioural and/or physiological factors. This study focused on three different groups; environmental factors influencing the distribution of NE in relation to bank vole habitat, ex vivo virus survival, human presence and exposure. Logistic binomial regression and boosted regression trees were used to model presence and absence data. Occurrence and potential sites where the disease may occur were modelled using cross-validated logistic regression. Finally, the ecological niche model Maxent, based on presence-only data, was used. The results of this study showed that zoonotic diseases can be modelled with diverse methods (Zeimes et al. 2012). The final purpose of the model, if explicative or predictive, and the availability of data, as well as the specificity of the system at hand, determines the choice.

Zeimes et al. (2015) also investigated the European spatial distribution of NE with a rich set of environmental variables. The influence of variables at the landscape and regional level was studied through multilevel logistic regression, and further information on their effects across the different European ecoregions was obtained by comparing an overall niche model (boosted

regression trees) with regressions by ecoregion. The presence of NE is likely in populated regions with well-connected forests, more intense vegetation activity, low soil water content, mild summers and cold winters. In these regions, landscapes with a higher proportion of built-up areas in forest ecotones and lower minimum temperature in winter are expected to be more at risk. Climate and forest connectivity have a stronger effect at the regional level. If variables stay at their current values, the models predict that NE may intensify but should not spread (although southern Sweden, the Norwegian coast and The Netherlands should be kept under watch). Models indicate that large-scale modelling can lead to a very high predictive power. At a large scale, the effect of one variable on disease may follow three response scenarios: the effect may be the same across the entire study area, the effect can change according to the variable value, and the effect can change depending on local specificities. Each of these scenarios impacts large-scale modelling differently.

Zeimes CB, Olsson GE, Ahlm C and Vanwambeke SO (2012). Modelling zoonotic diseases in humans: comparison of methods for hantavirus in Sweden. *Int J Health Geogr* 2012 Sep 11: 39. Doi: 10.1186/1476-072X-11-39.

Zeimes CB, Quoilin S, Henttonen H, Lyytikainen O, Vapalahti O, Reynes JM, Reusken C, Swart A, Vainio K, Hjertqvist M and Vanwambeke S (2015). Landscape and regional environmental analysis of the spatial distribution of hantavirus human cases in Europe. *Front. Public Health,* March 2015, 3, 54:1-10. doi:10.3389/fpubh.2015.00054

Interview: Heikki Henttonen

Heikki Henttonen, a professor of forest zoology at the Finnish Forest Research Institute (METLA), part of the Natural Resources Institute Finland (Luke), has more than 200 scientific publications to his name. A Fulbright Scholar in the 1980s, Heikki continues to have strong links with the United States of America and is an invited research associate at the University of Alaska Museum and the University of New Mexico's Museum of Southwestern Biology. Heikki is on the editorial board of the journals *Mammalia and Zoonotic and Vector-borne Diseases.* Heikki was a member of both the EDEN and EDENext steering committees (and was president of the latter) and coordinator for rodent-borne diseases.

In your opinion, what have been the notable achievements of EDEN and EDENext?

Heikki Henttonen (Natural Resources Institute Finland): *"I think European collaboration is one important point because EDEN and EDENext have made possible so much European collaboration, which would not have happened without them, without that financing. Another point is working in this network of collaboration opens up the training of a young generation, who from the beginning have learned to work on a European or global-scale network and they know each other. For any kind of research in the future they have a network ready. When they make applications, they can combine themselves in a reasonable way. This kind of European-scale collaboration would not be possible in the USA, for example, they are too competitive, they cannot collaborate as we do. In EDEN, at least in rodent-borne systems, everyone has understood the value of collaboration from the very beginning. In many cases, teams collected material and then one team would data analyse it, but all are co-authors, so that makes it efficient and high quality. Starting from the first contacts, maybe 13 years ago, it has been extremely interesting and it has really given a lot to me...It is one of the happier things to be old yourself but see the young people developing and then you see some of the really smart ones appearing. It's so nice to help these young students."*

What have you liked best?

Heikki: *"Maybe it is this European-scale collaboration. We have been able to look, say, at Puumala hantavirus and the host, bank vole, in Europe, but now we have been able to look at the disease pattern in different climatic biomes and we have been able to compare. Many people have said, 'one host, one virus, there is one model' but that is exactly what it is not! We need comparative models from different parts of Europe and this is what we have been trying to do in the EDEN and EDENext rodent programmes."*

Outstanding research moments?

Heikki: *"We have had many interesting findings, we have found a lot of new things. One of the most interesting things was the life-long shedding of Puumala hantavirus by voles, because we had no idea about how long it was shedded. We knew it was a chronic infection in bank vole and some lab experiments suggested it was two months and then it was over. We followed individual animals for almost a year and we had blood samples, then urine, faeces, saliva samples, every month and we could establish that it is life-long shedding, there wasn't even a decline in shedding. It is also interesting that it wasn't continuous through one route. You could have virus in the saliva, then a break, then again in the saliva or it could be in the urine, every time they were shedding through one route at least."*

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The future of research on tick-borne pathogens:

shifting from the 'one pathogen-one disease' vision to the pathobiome paradigm

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INTRODUCTION

Since the discovery of the Lyme disease agent in 1982 by Willy Burgdorfer (see Burgdorfer 1984), the importance of *Ixodes* ticks as vectors of zoonotic pathogens has continued to rise. The fact that *Borrelia* species responsible for Lyme borreliosis (LB) have been discovered so recently may not be because they did not exist before but because, at that time, no accurate tools were available to discover them. Indeed, the genus *Borrelia* has been recently found in the Tyrolean Iceman, demonstrating that it was already associated with man 5,300 years ago (Keller et al. 2012).

Although the most frequent tick-borne disease reported in European citizens is LB, with more than 65,000 new cases each year (Rizzoli et al. 2011), patients bitten by ticks can also be exposed to many other micro-organisms. These include viruses, bacteria, and parasites, some of which (for example, *Borrelia miyamotoi*) were identified in ticks decades prior to their association with human disease, whereas others were discovered only very recently and their Public Health importance remains so far unknown (for example, *Rickettsia helvetica*, *Candidatus* Neoehrlichia *mikurensis*, *Babesia venatorum* (formerly referred to as sp. EU1)).

Ticks represent an important and increasing threat for Public Health in Europe for three main reasons. Firstly, ticks are haematophagous during their three life stages and ingest a huge volume of blood (compared to other vectors) on many species, which increases the likelihood of acquiring and therefore transmitting pathogens. Indeed, ticks are hosts to a great variety of micro-organisms. Secondly, spending most of their life off-host in the environment, ticks are very sensitive to any changes which impact the environment. Furthermore, socio-economic changes may lead people to be more frequently present in tick habitats, increasing their exposure to tick-borne pathogens which in turn may lead (or have already led) to an important burden of tick-borne diseases. Thirdly, because of the large variety of pathogens that ticks are able to harbour and transmit, clinical and laboratory diagnosis of tick-borne diseases (TBD) is challenging.

Recently the use of next-generation sequencing (NGS) technology revealed that ticks harbour, in addition to pathogens, many other micro-organisms which co-exist and might interact with pathogens. Our vision of tick-borne pathogens therefore needs

to evolve to a more integrated view which considers the 'pathobiome', representing the pathogenic agent integrated within its biotic environments including other pathogens, commensals or symbionts. Shifting the paradigm from pathogens to pathobiome has many consequences in terms of research. The most demanding challenges of the coming years will be:

1. To investigate whether all new micro-organisms revealed by NGS cause human and/or animal disease following tick bites, or whether they are non-pathogenic tick-endogenous micro-organisms.

2. To identify whether interactions exist between pathogens or between pathogens and other tick-endogenous micro-organisms, as well as the consequences in terms of pathogen transmission, persistence, virulence and evolution.

3. Based on the new knowledge, the development of adequate strategies to better diagnose and combat tick-borne diseases.

In this review, we analyse how this new vision will revolutionise our understanding of tick-borne diseases, whose number has grown considerably since the discovery of the Lyme disease agent, and we discuss the implications in terms of research plans for the future in order to efficiently prevent and control the threat posed by ticks in Europe.

STATE OF THE ART OF THE 'OLD VISION' OF TICK-BORNE PATHOGENS: AN EVER-INCREASING LIST

In Europe, the most prevalent tick-borne disease in humans is LB, caused by a group of bacteria belonging to the *Borrelia burgdorferi* sensu lato group with at least five different species infecting humans in Europe (Rizzoli et al. 2011). Recently, *B. miyamotoi*, belonging to the relapsing fever group, has been detected in patients in USA, Japan, Russia and The Netherlands (Platonov et al. 2011, Chowdri et al. 2013, Krause et al. 2013, Hovious et al. 2013) and is transmitted by the tick species involved in LB. Ticks, especially *Ixodes ricinus*, can also be infected with other pathogens that might be transmitted to humans (Heyman et al. 2010). Amongst them, *Anaplasma phagocytophilum* is responsible for granulocytic anaplasmosis, *Candidatus* Neoerhlichia *mikurensis* may cause severe febrile illness in immunocompromised patients (Welinder-Olsson et al. 2010, von Loewenich et al. 2010), and Rickettsiae of the spotted fever group are known (*R. monacenis, R. conorii*) or suspected (*R. helvetica*) to cause rickettsioses (Parola and Raoult 2001). Another bacterial pathogens, *Francisella tularensis*, causes tularaemia. The Q fever agent *Coxiella burnetii* has also been detected in *I. ricinus*, but the role of this tick species in the epidemiology of these diseases is probably not significant (Duron et al. 2014, Angelakis and Raoult 2010).

Protozoans of the genus *Babesia*, mainly *B. divergens*, cause babesiosis in immunocompromised humans, and *B. venatorum* pathogenicity to humans has been confirmed (Hildebrandt and Hunfield 2014). *B. microti*, a well-known human tick-borne pathogen in the USA, has also been identified in ticks with, until now, one single human case in Europe (Hildebrandt et al. 2007). *Ixodes* species also transmit arboviruses, tick-borne encephalitis virus being the most important in terms of Public Health in Europe (Charrel et al. 2004). An increasing number of new species, strains or genetic variants of other micro-organisms are being detected in ticks, resulting in an ever-increasing list of (potential) pathogens capable of infecting livestock, companion animals and humans. However, we need to take into account that a significant portion of these 'new' species/genotypes are not truly emerging, but are only newly detected. This increasing biodiversity of pathogens is not generating answers but rather complex questions regarding the ecological cycles of pathogens, their interspecific interactions, cross-influence on infection mechanisms and differential diagnosis and synergic clinical importance.

Identification of micro-organisms in ticks has been largely dominated by the use of conventional molecular approaches mostly based on the use of specific primers combined with (real-time) PCR, and less frequently by culture-dependent methods. However, pathogen detection in an arthropod is not sufficient to validate its vector competence.

Following new or unexpected pathogen detections in ticks, it is therefore essential to conduct vector competence studies, and to evaluate the risk of exposure for both humans and animals. These types of studies require living ticks raised under controlled conditions, though because of their complex biological cycle and feeding biology, maintenance of tick colonies and their infection with micro-organisms is not easy. However, several methods have been successfully developed and used to infect hard ticks with pathogens, for example, feeding ticks on infected animals, injecting pathogens through the cuticle, by using thin capillary tubes and feeding ticks on infected blood through artificial or animal-derived membranes (Liu and Bonnet 2014). These me-

thods have been successfully employed to validate vector competence for a number of tick-borne pathogens, including Lyme spirochetes (Burgdorfer 1984), *A. phagocytophilum* (Massung et al. 2003), *Babesia* sp. EU1 (or *B. venatorum*) (Bonnet et al. 2009) and *Bartonella* sp. (Cotte et al. 2008, Reis et al. 2011). However, for some established tick-borne pathogens such as *Ca. N. mikurensis* (for which no cultivable strain has so far been obtained) and *R. helvetica*, the vector competence of ticks has not yet been proven. They became *de facto* tick-borne pathogens under more or less strong 'epidemiological evidence'.

The problem posed by the detection/diagnosis of TBDs

When a clinical history of tick bites is mentioned, Lyme disease is the primary diagnosis, but in some cases the borreliosis diagnosis remains unconfirmed by conventional serological tests (Perronne 2014). People bitten by ticks can also be infected by tick-borne encephalitis virus (TBEV), causing severe encephalitis, which is well diagnosed by serological tests. No specific treatment is currently available but TBE can be successfully prevented by active immunisation (Kaiser 2012).

As already mentioned, among vectors ticks are capable of transmitting the largest variety of pathogens, and pathogens other than Lyme or TBE agents might be involved in TBDs. Interestingly, the majority of those pathogens have been discovered during the past 20 years. The symptoms induced by those pathogens are often mild (high fever, fatigue, body aches, chills etc.) and can be confused with symptoms caused by infection with other agents. This is probably the reason why medical practitioners have a poor knowledge of them and they are almost never detected in humans while being frequently identified in ticks and/or reservoir animals. A striking example is *B. miyamotoi*. This *Borrelia* species was first isolated from Japanese *Ixodes* ticks in 1995 and was considered a non-pathogenic endogenous tick bacteria until the first human cases of *B. miyamotoi* infection were reported in Russia in 2011 (Platonov et al. 2011). Since then, human infections have been described in the USA and most recently in The Netherlands (Chowdri et al. 2013, Krause et al. 2013, Hovius et al. 2013, Gugliotta et al. 2013). In European countries such as France, Estonia, Poland and Switzerland, circulation of *B. miyamotoi* in *I. ricinus* and in wild animals has been confirmed (Vayssier-Taussat et al. 2013), with the French genotype identical to an isolate from a Dutch patient (Cosson et al. in press). No human cases of *B. miyamotoi* infections have been reported in these countries, but the absence of serological or molecular tests for *B. miyamotoi* combined with the lack of knowledge on these bacteria among medical practitioners makes diagnosis particularly difficult. Thus it is likely that the absence of human infections is rather due to missed diagnoses than to an actual absence of infection.

Patients bitten by ticks can also be co-infected by several pathogens. For example, Horowitz et al. (2013) described co-infection rates ranging from 2 to 5% for *Borrelia* species and *A. phagocytophilum* in patients with erythema migrans who were diagnosed with Lyme disease. Co-infections between *B. afzelii* and *R. monacensis* were also identified in skin biopsies of erythema migrans patients in The Netherlands (Tijsse-Klasen et al. 2013). However, co-infections are rarely diagnosed in routine practice, raising the problem of

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co-infection in humans as a relevant, albeit poorly studied issue, with strong implications for Public Health.

As a result, after a tick bite, if people are infected by pathogens other than Lyme disease spirochetes or TBEV, the cause of their disease is rarely identified. In recent years unexplained syndromes occurring after tick bites have become an increasingly important issue leading to considerable discord between scientists, patients and infectious disease institutes.

THE TECHNOLOGY-DRIVEN REVOLUTION OF TICK-BORNE PATHOGENS' VISION: FROM PATHOGEN TO PATHOBIOME

Until now, most studies aiming to identify tick-borne pathogens in ticks have been able to assess simultaneously only a limited number (Halos et al. 2010, Reis et al. 2011). This is partly due to technological limitations. Indeed, complete screens of micro-organisms in natural host populations were out of reach using standard laboratory procedures. Within the past few years, the rapid development of Next-Generation Sequencing (NGS) methods has revolutionised epidemiology and the diagnosis of infectious diseases, facilitating the complete screening of pathogens in their hosts, the discovery of new pathogens or the detection of unexpected ones. NGS has recently been successfully used to identify the bacterial communities associated with *I. Ricinus* (Carpi et al. 2011, Nakao et al. 2013, Hawlena et al. 2013, Williams-Newkirk et al. 2014) based on the amplification and sequencing of hyper-variable regions of the 16SrRNA encoding genes (metagenomic profile), illustrating a highly diverse microbial community (108 genera representing all bacterial phyla). As expected, these approaches have made it possible to find without a priori the best known tick-borne pathogens such as the *Borrelia*, *Anaplasma, Coxiella, Francisella* and *Rickettsia* genus. Among these genera, mostly known as pathogenic for vertebrates, some species are considered as endosymbionts (for example, the *Rickettsia*-endosymbiont of *I. Scapularis*) (Gillespie et al. 2012) and the distinction between pathogens and endosymbionts is often tricky. For instance, some authors consider *Rickettsia* species as endosymbionts that are transmitted vertically in arthropods, and only secondarily as pathogens of vertebrates (Perlman et al. 2006).

For the *Coxiella* genus, the species *C. burnetii* is mostly considered as a vertebrate pathogen while numerous other *Coxiella* species have been found to be associated with ticks. Phylogenetic analyses combined with experimental approaches suggested that all of them might also be considered as endosymbionts of ticks (Duron et al. 2014, Almeida et al. 2012). Thus the pathogenic nature of *C. burnetii* could be an exception within the genus (Machado-Ferreira et al. 2011). Besides the well-known vertebrate pathogenic species *F. tularensis* (occasionally found in ticks), *Francisella*-like-endosymbionts associated with *Dermacentor* spp. have been described but their pathogenic nature remains to be investigated (Kreizinger et al. 2013). The *Wolbachia* and *Arsenophonus* genera are also bacteria associated with arthropods (mostly insects) and affect the reproduction and/or immunity of their hosts (Rudolf et al. 2009, Werren et al. 2008). They have also been found associated with ticks (Ahantarig et al. 2013). However, a recent experimental approach has revealed that at least in *I. ricinus*, the finding of *Wolbachia* is due to parasitism by a parasitoid wasp (*Ixodiphagus hookeri*) (Plantard et al. 2012). The role of *Arsenophonus* as tick endosymbionts has still to be demonstrated. Finally, the *I. ricinus* endosymbiont *Midichloria mitochondrii* constitutes a particular example. This bacterium was initially observed within tick cells, especially in ovarian cells of *I. ricinus* (Lewis 1979). The use of molecular probes specific for this alphaproteobacteria have made it possible to detect them in almost 100% of *I. ricinus* females derived from natural populations (Sassera et al. 2006), but also in other tick species (Epis et al. 2008). Recently *M. mitochondrii* is increasingly considered as a potential vertebrate pathogen (Bazzocchi et al. 2013).

The use of NGS technology will undoubtedly shed new light on the intriguing bacterial communities associated with ticks (Hawlena et al. 2013). The clear-cut frontiers between the so-called 'vertebrate pathogens', 'arthropod pathogens' or 'arthropod symbionts' may thus vanish into a more subtle and complex vision of bacterial-vector-vertebrate communities. Additional investigations, including experimental approaches, should be conducted to define their role in ticks. A better knowledge of these bacteria could even constitute useful resources for developing anti-vectorial control methods.

Besides the known micro-organisms (belonging either to pathogens, endosymbionts or both), NGS has also revealed that the majority of RNA/DNA sequences carried by ticks belonged to still unknown micro-organisms. For example, 80% of the viral nucleic sequences obtained from tick extracts may belong to as-yet unidentified micro-organisms (Vayssier-Taussat et al. unpublished data). Among these new viral sequences, we identified genera transmissible to humans and/or animals via arthropods, including Bunyaviridae (Nairovirus and Phlebovirus), Rhabdoviridae (Vesiculovirus) and Reoviridae (Coltivirus) (Vayssier-Taussat

et al. unpublished data). A similar study has been undertaken by Lipkin et al. in the USA to characterise the virome of different tick species. The presence of Powassan virus, a well-known human pathogenic tick-borne virus, and eight novel viruses belonging to Nairovirus, Phlebovirus and Mononegavirus genera were identified (Tokarz et al. 2014).

By having a picture of the entire tick microbial community, we can identify that pathogens are intimately associated with the vast community of micro-organisms (including other pathogens) that may influence tick biology, but also pathogen persistence, transmission and virulence, thereby justifying the need for a shift from pathogens to pathobiome.

HOW CAN WE REVEAL MICRO-ORGANISM INTERACTIONS IN THIS VERY COMPLEX ECOSYSTEM?

Micro-organism interactions have mostly been considered as a one-to-one interaction, where the infection by one pathogen influences the acquisition of and/or dynamics of infection by a second pathogen. However, interactions between a set of pathogens are conceivable where different pathogens interact within a network or through 'cascade consequence' (Rigaud et al. 2010, Bordes and Morand 2011). In experimental studies, we can investigate how the presence of one pathogen may interfere with the infection by another. However, this is not possible in a pathobiome perspective where many pathogens and other micro-organisms are present, including poorly known ones.

In such cases, we can conduct population studies and try to seek whether the probability of finding these pathogens together is not random. The first step is therefore to look at the co-occurrence of micro-organisms, even though this can also result from confounding factors which create statistical associations between pathogens without true biological interactions. In population studies, longitudinal or time series data are useful for identifying pathogen associations, identifying whether the presence of one pathogen modifies subsequent infection by another (Telfer et al. 2010). However, such studies are resource-intensive. One-off cross-sectional studies are widely used to screen for the presence of several pathogens because they consume less time and money than longitudinal studies. This is especially the case when emerging or poorly known pathogens or host species are studied. Numerous approaches are available to detect pathogen associations in such contexts. For instance, multivariate analyses (principal component analysis, correspondence analysis, discriminant analysis, co-inertia analysis) (Gauch 1982) help evaluate which pathogens tend to group together. The drawback is that there is usually no statistical test associated with these analyses (but see, for example, permutation methods, Tollenaere et al. 2008, Salvador et al. 2011).

Recently, we developed a new association screening approach to detect overall and detailed multi-pathogen associations (Vaumourin et al. 2014). This method was shown to be very powerful, but still its use for micro-

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organisms >10 requires more than 1,000 samples. Strong methodological developments have been made in network analyses (Bascompte 2007) over the past few years in many fields (for example, metabolic pathways in medicine (Ravasz et al. 2002, Qin et al. 2012), peer-to-peer networks in computer science (Fox 2001) and scientific collaboration in social science (Newman 2004)). They also offer an attractive representation of multiple pathogen relationships. They provide indices of association such as connectedness (Yodzis 1980), nestedness (Bascompte et al. 2003) and betweenness (Freeman 1977). However, to date, statistical tests regarding network parameters have rarely been used, though developments in this field are promising.

SURVEILLANCE AND DIAGNOSIS FOR TBD

Considering the large number of potential tick-borne pathogens that can be involved in diseases, either alone or in association, there is a need for the urgent development of adapted methods that take into account this variety but also the biology of tick-borne pathogens. For instance, many tick-borne pathogens colonise blood (infecting blood cells or not) of vertebrate hosts. Thus it makes sense to detect the presence of their DNA in the blood of infected human patients or animals. However, blood infection does not occur for all tick-borne pathogens. For example, Lyme spirochete species do not stably infect the blood of humans and therefore detecting their DNA in the blood of a patient bitten by ticks is tricky and requires either bacterial DNA detection in more specific samples (such as skin biopsies) or serological tests, even though their specificity and sensitivity are not always optimal. Molecular identification of tick-borne pathogens has mostly been based on the use of specific primers combined with real-time PCR, which can only detect a selected and limited number of species. To overcome these problems, new tools allowing high-throughput monitoring of tick-borne pathogens have to be urgently set up.

Based on data on circulation and the presence of tick-borne pathogens in ticks in different geographical regions in Europe obtained by NGS, we developed a tool using a so-called microfluidigm system allowing multiple parallel real-time PCRs for TBD surveillance that might be adapted to diagnosis (Michelet et al. 2014). This new tool has the unique ability to simultaneously analyse multiple pathogens (up to 48 different species of pathogens) in the same samples. It presents a major advantage and can be easily adapted to new situations as it is entirely possible to remove primers/probes sets in order to modify the panel of targeted pathogens. If developed by private companies, these kinds of tools will represent an important improvement in the diagnosis of TBD.

VACCINATION

Considering the number of pathogens which could be transmitted by the same tick species, establishing tick vaccines could be a smart and environmentally friendly alternative to protect human and animal populations against tick-borne diseases through the control of vector infestations and reducing pathogen infection and transmission. For this purpose, research on molecular interactions between ticks and pathogens as well as the identification of suitable targets for vaccine development are major challenges for the implementation of new TBD control strategies (Liu and Bonnet 2014). Among these, target molecules playing key roles in tick infestation and vector capacity are particularly promising (de la Fuente and Merino 2013). To date, the only commercially available anti-tick vaccine is based on the *R. microplus* midgut protein BM86, interfering with tick feeding and subsequent egg production (de la Fuente et al. 2007). However, thanks to the combination of the techniques of tick infection in the laboratory and new methods of molecular investigation, promising candidates have been recently identified. These include tick proteins derived from *I. ricinus* (Decrem et al. 2008, Liu et al. 2014), *I. scapularis* (Dai et al. 2009), *Rhipicephalus microplus* (Merino et al. 2013, Labuda et al. 2006), as well as candidates common to several hard tick species (de la Fuente et al. 2006). Improving our understanding of molecular interactions between ticks and tick-borne pathogens is a pre-requisite for the conception of future generations of vaccines and for vector and disease control.

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PUBLICise Health Public Health Telegram: Tick-borne diseases

EDENext's Public Health Telegrams (PUBLICise Health) were established to inform interested individuals and institutions about research results on vector-borne diseases with direct or indirect impact on Public Health issues. Edited and produced by EDENext's Public Health team, each issue concentrates on the work of one of the project's five specialist groups. Here we present an updated version of the issue dedicated to the work of the team examining tick-borne diseases, first published in December 2013.

1. Tick-borne viruses - an overview

Ticks are recognised as vectors for several pathogens of Public Health relevance. Hubálek and Rudolf (2012) offer an extensive overview of the currently known European tick-borne viruses with a special focus on their taxonomy, host and tick vector range, pathogenicity for vertebrates including humans, and their significance for Public Health. Besides the known highly pathogenic Crimean-Congo haemorrhagic fever virus (CCHFV) and tick-borne encephalitis virus (TBEV), the authors also report other tick-borne virus pathogens such as louping-ill and African swine fever virus.

Hubálek Z and Rudolf I (2012). Tick-borne viruses in Europe. *Parasitol.* Res. 2012, 111: 9-36. Doi: 10.1007/s00436-012-2910-1.

2. Effect of deer density on tick infestation of rodents and TBE hazard, Part I and II

The occurrence and population dynamics of wild ungulates, in particular deer (different *Cervidae* species, for example roe deer *Capreolus capreolus*), represent some of the most important risk factors for the risk assessment of diseases transmitted by the pan-European tick species *Ixodes ricinus*. The spatial and temporal variation in the abundance of deer does not only affect the number of ticks in the environment, but also the rate of ticks infected with various pathogens. In the case of tick borne encephalitis (TBE), the probability of TBEV circulation is enhanced above a certain

threshold deer density as shown in a joint study carried out in Italy and Slovakia and published within two EDENext publications (Cagnacci et al. 2012, Bolzoni et al. 2012). The effect of variable deer population density also has consequences on the pattern of tick infestation of the yellow-necked mouse (*Apodemus flavicollis*), one of the most important small mammal hosts supporting high TBEV circulation through co-feeding ticks in the natural habitat. By comparing sites in Italy and Slovakia with moderate and low deer population density to the presence and/or absence of TBEV occurrence in these areas, researchers from the Fondazione Edmund Mach and the Slovak Academy of Sciences have obtained quantitative estimates of the variation of the number of co-feeding ticks on rodents (Cagnacci et al. 2012) and included these estimates within a mathematical model (Bolzoni et al. 2012) that allows for the computation of the basic reproductive number of the infection and various host densities.

Cagnacci F, Bolzoni L, Rosà R, Carpi G, Hauffe HC, Valent M, Tagliapietre V, Kazimirova M, Koci J, Stanko M, Lukan M, Henttonen H and Rizzoli A (2012). Effect of deer density on tick infestation of rodents and the hazard of tick-borne encephalitis. Part I: empirical assessment. *International Journal for Parasitology* 42(2012) 365-372.

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3. THE IMPACT OF CRIMEAN-CONGO HAEMORRHAGIC FEVER (CCHF) ON PUBLIC HEALTH

Crimean-Congo haemorrhagic fever (CCHF) is a haemorrhagic fever in humans, caused by infection with the Crimean-Congo haemorrhagic fever virus (CCHFV). It is the only biosafety level 4 agent currently present in Europe. Every year, more than 1,000 human clinical cases and up to 50 fatalities are reported from more than 30 countries in Asia, the Middle East, south-eastern Europe and Africa. CCHF can be considered a major emerging disease threat to the European Union due to its high infectiousness, potential of human-to-human transmission and present lack of suitable prophylaxis and therapeutic interventions. Additionally, the ongoing range expansion of its tick vectors within Europe underlines the imminent threat this disease poses to Public Health in Europe. The EDENext study by Mertens et al. (2013) summarises the current state of knowledge about CCHF and provides guidance for Public Health analysts and managers. Special emphasis is given to the importance of vector and virus distribution, the

impact of climate and the different modes of virus transmission, as well as to the current limitations in diagnostics, treatment and vaccination. Based on this knowledge, recommendations for protection measures for individuals and the population at large, including case management options, and Public Health management and communication strategies are proposed. CCHFV is primarily transmitted by ticks of the genus *Hyalomma*, which can be found in many regions of southern and south-eastern Europe. Up to 20% of these ticks have been found positive for CCHFV in endemic areas of Turkey. Mammals such as hedgehogs, hares, sheep and cattle are infested by these ticks and thereby may become infected and amplify the virus, yet without suffering from any clinical disease. The virus circulates in a tick-vertebrate-tick cycle, but can also be transmitted horizontally and vertically within the tick population. Most human infections occur by tick bites and by crushing infected ticks, but infections are also possible through contact with blood and other body fluids of viraemic animals. Moreover, there are several reports about person-to-person transmissions following unprotected handling of infected blood samples from patients, by accidental needle stick injuries, and by accidents during surgery. Currently, there is no CCHFV vaccine available and the therapy is restricted to symptomatic treatment.

Mertens M, Schmidt K, Ozkul A and Groschup MH (2013). The impact of Crimean-Congo haemorrhagic fever virus on public health. *Antiviral Res.* 2013 May; 98(2):248-60. Doi: 10.1016/j.antiviral.2013.02.007. Epub 2013 Feb 28.

4. DISCOVERY OF CCHF TICK VECTOR IN HUNGARY

Crimean-Congo haemorrhagic fever (CCHF) is a zoonotic viral disease transmitted primarily by tick species of the genus *Hyalomma*. These ticks are endemic to regions with (sub)tropical or Mediterranean climates, such as Albania, Turkey and Uzbekistan. Migratory birds may occasionally carry *Hyalomma* larvae and nymphs from their original habitat to European countries north of the Mediterranean basin. However, it was previously assumed that the cool climate in these areas of Europe would prevent the ticks from completing their life cycle successfully and therefore both prohibit the development of

adult ticks possible of transmitting CCHF and the establishment of *Hyalomma* populations. In the EDENext study by Hornok and Horváth (2012) ticks were collected from cattle and wild ruminants in a region of southern Hungary, where, according to prediction models, *Hyalomma* ticks are most likely to occur and establish themselves in the future. Adult *Hyalomma marginatum rufipes* males were found on two occasions. This tick species was therefore detected for the first time in Hungary, marking the (so far) northern-most occurrence of this CCHF vector in Central Europe. A human case of CCHF as well as CCHF virus seropositive animals have already been reported in Hungary. The northern spread of *Hyalomma* ticks and thus potentially of the CCHF virus is of uttermost importance to European Public Health. To monitor and possibly prevent the further expansion of this zoonotic disease beyond its original range, further investigations of the current distribution of CCHF tick vectors in Europe have to be undertaken.

Hyalomma rufipes *from cattle.*
© Sándor Hornok, Szent István University, Hungary

Hornok S and Horváth G (2012). First report of adult *Hyalomma marginatum rufipes* (vector of Crimean-Congo haemorrhagic fever virus) on cattle under a continental climate in Hungary. *Parasit.* Vectors 2012; 5:170. Doi: 10.1186/1756-3305-5-170

5. HIGH TBEV INFECTION RATE IN AUSTRIAN HORSES

To evaluate the status of West Nile virus (WNV) within the domestic horse population in Austria, the sera of 257 horses of the same breed, distributed among three federal states, were screened via a commercial ELISA for flaviviruses antibodies. ELISA-positive sera were further tested by virus-specific neutralisation assays for the three flaviviruses circulating in Austria: WNV, Usutu virus (USUV) and tick-borne encephalitis virus (TBEV). While no specific WNV antibodies could be determined, a comparatively high seropositivity rate of 40.4% for TBEV-specific antibodies was detected, with a significantly higher antibody prevalence in younger horses and in stallions. This result is remarkable as comparable studies in Austria and Germany show significantly lower TBEV prevalence in horses and other domestic animals. The age and gender aspect is equally surprising, and is contradictory to results from comparable studies. TBEV is a virus associated

with tick-borne encephalitis (TBE), a viral infectious disease causing inflammations of the central nervous system in various vertebrate species, including humans, but very little is known about the disease in horses. Overall, the results of the study by Rushton et al. (2013) indicate the necessity of further investigations of TBE prevalence in equines and other domestic animals in Central Europe for the sake of evaluating the Public Health risk posed by TBEV.

6. TICK-BORNE DISEASES IN ROMANIA

The geopolitical position of Romania as a bridge connecting southern and central Europe has to be respected in regard to its implication for the Public Health system of the European Union, of which Romania has been a member since 2007. In this country, cases of Lyme disease and TBE have been identified. However, little is known about the Public Health impact of these diseases, and none of the other tick-borne pathogens present in Europe have been reported as causes of infection in Romania. Therefore, the EDENext study by Paduraru et al. (2012) in which various zoonotic tick-borne pathogens (including *Rickettsia monacensis*, *R. helvetica*, *Anaplasma phagocytophilum, Ehrlichia muris, Francisella tularensis*, and *Babesia* sp. EU1) are described for the first time in Romania can be considered an important step towards future evaluations of the prevalence of these diseases in the country and the implications of these results for national and international (European Union) Public Health surveillance.

7. DRIVING FORCES FOR TICK RANGE EXPANSION

The distribution of the tick *Ixodes ricinus*, which serves as a vector of a large variety of pathogens of medical and veterinary importance (among others, TBE, babesiosis, Lyme borreliosis and tularaemia), has increased

significantly all over Europe over the past decades. *I. ricinus* specimens are now detected in more northern areas and habitats at higher altitudes than their prior range. Medlock et al. (2013) assume ongoing climatic change to be a driving factor of the tick range expansion among several other anthropogenic factors. Milder climatic conditions may allow one of the tick's major host species, the European roe deer (*Capreolus capreolus*), whose abundance has increased in several regions as a consequence of current wildlife management, to spread to and inhabit previously inhospitable areas at higher altitude, thereby serving as a means of transport for the ticks. Furthermore, increasing temperatures reduce the length of snow cover, impacting positively the survival and spread of the ticks. Among other driving factors, land use changes due to agriculture and forestry management in various European countries over the past decades, and thereby suitable habitat expansion for *I. ricinus*, including urbanized areas, are discussed in regard to their effects on the tick range expansion. In conclusion, the authors note that multiple factors drive the range expansion of *I. ricinus* in Europe and that enhanced tick surveillance with harmonised approaches for international data comparison would allow better follow-up of tick population trends at the European Union level, thereby improving the prediction of health risks related to tick-borne diseases.

Medlock J, Hansford K, Bormane A, Derdakov M, Estrada-Peña A, George JC, Golovljova I, Jaenson T, Jensen JK, Jensen P, Kazimirova M, Oteo J, Papa A, Pfister K, Plantard O, Randolph S, Rizzoli A, Santos-Silva M, Sprong H, Vial L, Hendrickx G, Zeller H and Bortel W (2013). Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. *Parasit Vectors*. 2013; 6: 1. Doi: 10.1186/1756-3305-6-1

8. WOODLAND FRAGMENTATION INCREASES LYME DISEASE RISK

Over the past decades, the increase in the number of reported cases of Lyme disease throughout Europe has led to growing Public Health concern regarding the pathogenicity of this disease. Lyme disease, or Lyme borreliosis, is the most frequent vector-borne disease of humans in the Northern Hemisphere. The causative agent is the spirochaete *Borrelia burgdorferi* sensu lato, which is transmitted in Europe mainly by the tick species *Ixodes ricin*us. Lyme disease is strongly connected to

various environmental factors, particularly in forest and agricultural landscapes, where the activity ranges of ticks and their hosts (including humans) overlap. However, these factors are not homogeneously distributed among the different landscapes. An understanding of the underlying processes in the transmission system is crucially needed to explain the spatial distribution risk of Lyme disease.

In this EDENext study, Li et al. (2012) demonstrate a cellular automata model for Lyme disease. The study includes a heterogeneous landscape model with three interactive components (hosts, tick population and a disease transmission function). Scenarios of various landscape configurations are simulated and compared. Based on the simulation results, the authors conclude that Lyme disease risk correlates to the density, shape and aggregation level of woodland areas, among others due to the latter's impact on the local host population and thus spread of the disease-carrying ticks. This correlation is useful for understanding and predicting the occurrence of tick-borne diseases in dynamic forest landscapes.

9. DETECTION OF *BORRELIA MIYAMOTOI* IN VECTORS AND RESERVOIR OF LYME DISEASE SPIROCHETE IN FRANCE

In France, as elsewhere in Europe, the most prevalent TBD in humans is Lyme borreliosis, caused by different bacterial species belonging to the *Borrelia burgdorferi* sensu lato complex and transmitted by the widespread tick species, *Ixodes ricinus*. However, the diagnosis of Lyme disease is not always confirmed and unexplained syndromes occurring after tick bites have become an important issue. Recently, *Borrelia miyamotoi* belonging to the relapsing fever group and transmitted by the same *Ixodes* species has been involved in human disease in Russia, the USA and the Netherlands. In this study, researchers identified B. *miyamotoi* in ticks and bank voles in France and demonstrated that its genotype is identical to those already described in ticks from Western Europe, as well as to the one identified from a

sick person in The Netherlands (Cosson et al. 2014). These results suggest that even though no human cases have been reported in France, surveillance has to be improved. Moreover, researchers show that ticks could simultaneously carry B. *miyamotoi* and Lyme disease spirochetes, increasing the problem of co-infection in humans.

Cosson J-F, Michelet L, Chotte J, Le Naour E, Cote M, Devillers E, Poulle M-L, Huet D, Galan M, Geller J, Moutailler S and Vayssier-Taussat M (2014). Genetic characterization of the human relapsing fever spirochete *Borrelia miyamotoi* in vectors and animal reservoirs of Lyme disease spirochetes in France. *Parasites & Vectors* 2014, 7:233. Doi:10.1186/1756-3305-7-233.

10. *IXODES RICINUS* AND ITS TRANSMITTED PATHOGENS IN URBAN AND PERI-URBAN AREAS IN EUROPE: NEW HAZARDS AND RELEVANCE FOR PUBLIC HEALTH

Tick-borne diseases represent major public and animal health issues worldwide. *Ixodes ricinus*, primarily associated with deciduous and mixed forests, is the principal vector of causative agents of viral, bacterial, and protozoan zoonotic diseases in Europe. Recently, abundant tick populations have been observed in European urban green areas, which are of Public Health relevance due to the exposure of humans and domesticated animals to potentially infected ticks. In urban habitats, small and medium-sized mammals, birds, companion animals (dogs and cats) and larger mammals (roe deer and wild boar) play a role in the maintenance of tick populations and as reservoirs of tick-borne pathogens. The presence of ticks infected with TBEV and high prevalence of ticks infected with *Borrelia burgdorferi* s.l., causing Lyme borreliosis, have been reported from urbanized areas in Europe. Emerging pathogens, including bacteria of the order Rickettsiales (*Anaplasma phagocytophilum, Candidatus* Neoehrlichia *mikurensis, Rickettsia helvetica* and *R. monacensis*), *Borrelia miyamotoi*, and protozoans (*Babesia divergens*, *B. venatorum*, and *B. microti*) have also been detected in urban tick populations. Understanding the ecology of ticks and their associations with hosts in a European urbanized environment is crucial to quantify the parameters necessary for risk pre-assessment and identification of Public Health strategies for control and prevention of tick-borne diseases (Rizzoli et al. 2014).

Rizzoli A, Silaghi C, Obiegala A, et al. (2014). *Ixodes ricinus* and Its Transmitted Pathogens in Urban and Peri-Urban Areas in Europe: New Hazards and Relevance for Public Health. *Frontiers in Public Health*. 2014;2:251. Doi:10.3389/fpubh.2014.00251.

11. RICKETTSIAE IN QUESTING *IXODES RICINUS* TICKS IN THE CZECH REPUBLIC.

In a study implemented by Venclikova et al. (2014), a total of 1,473 *Ixodes ricinus* ticks (1294 nymphs, 99 males, and 80 females) were collected and screened for the presence of human pathogenic rickettsiae (*Rickettsia helvetica*, *R. monacensis, Candidatus* Neoehrlichia mikurensis, and *Anaplasma phagocytophilum*) in natural and urban ecosystems using molecular techniques. The minimum infection rate (MIR) for *Rickettsia* spp. was 2.9% in an urban park and 3.4% in a natural forest ecosystem; for *Candidatus* Neoehrlichia mikurensis, MIRs of 0.4% in the city park and 4.4% in natural habitat were observed, while for *A. phagocytophilum* the MIR was 9.4% and 1.9%, respectively. The study provides the first data on the occurrence of human pathogenic rickettsiae in questing *I. ricinus* ticks in the Czech Republic.

12. BIRDS AS POTENTIAL RESERVOIRS OF TICK-BORNE PATHOGENS: FIRST EVIDENCE OF BACTERAEMIA WITH *RICKETTSIA HELVETICA*

Birds have long been known as carriers of ticks, but data from the literature are lacking on their role as a reservoir in the epidemiology of certain tick-borne pathogens. Therefore, the aim of a study carried out by Hornok et al. (2014) was to evaluate the presence of three emerging, zoonotic tick-borne pathogens in blood samples and ticks of birds and to assess the impact of feeding location preference and migration distance of bird species on their tick infestation. Blood samples and ticks of birds were analysed with TaqMan real-time PCRs and conventional PCR followed by sequencing. During the spring and autumn bird migrations, 128 blood samples and 140 ticks (*Ixodes ricinus*, *Haemaphysalis concinna* and a *Hyalomma* specimen) were collected from birds belonging to 16 species. The prevalence of tick infestation and the presence of tick species were related to the feeding and migration habits of avian hosts. Birds were shown to be bacteraemic

with *Rickettsia helvetica* and *Anaplasma phago-cytophilum,* but not with *Candidatus* Neoehrli-chia mikurensis. The prevalence of rickettsiae was high (51.4%) in ticks, suggesting that some of them may have acquired their infection from their avian host. Based on the present results birds are potential reservoirs of both *I. Ricinus*-transmitted zoonotic pathogens, *R. helvetica* and *A. phagocytophilum*, but their epidemiological role appears to be less impor-tant concerning the latter, at least in Cen-tral Europe.

13. ISOLATION AND PROPAGATION OF A *SPI-ROPLASMA* SPECIES FROM SLOVAKIAN *IXODES RICINUS* TICKS IN *IXODES* SPP. CELL LINES

Ixodes spp. ticks may occasionally harbour spiroplasmas - helical mycoplasmas in the class Mollicutes; a previous study in Slo-vakia reported an overall prevalence of *Spi-roplasma ixodetis* of 3% in *I. ricinus*. In the present study, extracts of unfed adult *I. ri-cinus* collected from vegetation in south-wes-tern Slovakia were added to a panel of cell lines derived from *I. ricinus* and I. *scapu-laris* embryos (Bell-Sakyi et al. 2015). The cultures were monitored at intervals over the subsequent 16-18 months. *Spiroplasma*-like mi-croorganisms were detected in cultures of both tick species after 2-3 months and subcultured onto fresh, uninfected cells of the appro-priate cell line up to seven times. Molecular analysis using PCR assays targeting fragments of the 16S rRNA, ITS and rpoB genes confirmed the identity of the microorganisms as a Spi-roplasma species, with between 98.9% and 99.5% similarity to *S. ixodetis*. The sequences of the Spiroplasmas isolated from three diffe-rent pools of ticks collected on two different occasions were identical for all three genes tested.

Molecular taxonomy of sand flies:

which species are out there and how can we find out?

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INTRODUCTION: A TAXONOMIC PUZZLE

Phlebotomine sand flies (Diptera: Psychodidae, Phlebotominae) are the only proven vectors of leishmaniases, a group of important but neglected human and animal diseases. These diseases are endemic in 88 countries, putting an estimated 350 million people at risk and afflicting 10 million people (World Health Organization 2010). In addition, sand flies also transmit other human and animal pathogens such as bacteria (*Bartonella*) and viruses (Bunyaviridae, Reoviridae and Rhabdoviridae families), further increasing their medical and veterinary significance. While the occurrence of bartonellosis is restricted to certain regions in the New World, current studies reveal an unexpectedly high diversity of sand fly-borne viruses and show that the transmission of different arboviruses occurs more often than previously thought across a wide range of the sand fly distribution area.

Despite this obvious and indisputable importance of sand flies in human and veterinary medicine, inadequate and inconsistent attention has been paid to their taxonomy and morphological identification. Several taxonomic reviews and keys for the identification of sand fly species have been serving as 'gold standard' references for several decades; however, these fundamental resources have become gradually outdated and nowadays do not reflect the current views on particular groups of sand fly species. Moreover, with a detailed morphological assessment of some vector species' populations, the actual discriminative power of some morphological characters that these keys rely on, and their ability to conclusively identify species, has been subject to critical debate. Nevertheless, proper identification of sand fly species is necessary not only for the purposes of faunistic entomological surveys. Its importance is considerably greater in epidemiological studies conducted in endemic areas of leishmaniasis, where the presence of morphologically similar species with different vectorial capacities and roles in the transmission of the pathogen can obscure vector-parasite relationships and their consequences for adequate control measures.

The conventional approach to the identification of sand fly species is based on morphological features and therefore requires the dissection of freshly collected or properly stored specimens and the mounting of their head and abdomen which bear the decisive characters (genitalia, cibarium and pharyngeal armature). Both the preparation of the slides and the identification are laborious and time-consuming, and demand a certain degree of proficiency and expertise. Moreover, dried or incomplete specimens are often impossible to identify. Determination is further obscured when specimens bear intermediate morphological characters. All these aspects of morphological analysis contribute to the conclusion that traditional identification of sand flies should be supported by complementary molecular methods.

The Mediterranean basin is an important cultural and historical region, which also abounds in many prime leisure destinations for summer holidaymakers. However, it is also an area well-known for transmission of *Leishmania infantum* and several phlebo-viruses (Maroli et al. 2013). It is inhabited by rich sand fly fauna which comprises many taxonomically problematic and difficult species groups and complexes. While the identification of sand fly species may be a difficult task in areas where their presence has been long established, it is even more challenging in those regions where they have recently appeared.

There is growing evidence of changes in the distribution of a number of sand fly species within Europe. While these changes can be partially attributed to growing attention to sand flies in regions where they had been overlooked in the past, a northward spread to areas where they had not been found in previous surveys has been well documented for a number of species: *Phlebotomus ariasi, P. perniciosus, P. neglectus*, all belonging to the subgenus *Larroussius*, and *P. mascittii* from the subgenus *Transphlebotomus* (reviewed by Medlock et al. 2014). Different aspects, namely changes in climate, human activities and environmental changes, are believed to cause these shifts in distribution. While many may argue about the actual contribution of these aspects, it remains apparent that the expansion of sand flies into new areas introduces them to environments where a lack of expertise in species identification can be a pressing problem.

Of the leishmaniases, the most debilitating to both humans and animals (mainly dogs) are visceral forms of the disease caused typically by *Leishmania infantum* and *L. donovani*. The impact of human and canine leishmaniasis in the Mediterranean area emerges with a high prevalence of asymptomatic *L. infantum* carriers. Domestic dogs are the main reservoirs of the disease with several million dogs infected in south-western Europe (Moreno and Alvar 2002). This is mainly aggravated by the natural abundance of proven and potential vector species and constitutes a serious concern for Public Health authorities. Various sand fly species of the subgenera *Larroussius* and *Adlerius* serve as vectors of leishmaniasis (Killick-Kendrick 1990, Ready 2013). So far, five species of the subgenus *Larroussius* have been proven to transmit human visceral leishmaniasis in this region: *Phlebotomus ariasi, P. perniciosus, P. tobbi, P. neglectus* and *P. perfiliewi* (Ready 2013). Nevertheless, several other species of this subgenus are considered as probable vectors based on circumstantial evidence, namely their distribution, biting habits and taxonomic position.

Of the subgenus *Adlerius, Phlebotomus balcanicus* has been proven to serve as a vector of *L. infantum* in Georgia, probably as a secondary vector along with *P. kandelakii* from the subgenus Larroussius (Giorgobiani et al. 2012). Several more species are suspected to be incriminated in the transmission of visceral leishmaniasis, for example *P. halepensis* in Turkey and Azerbaijan. However, in many foci, species involved in the transmission have not yet been identified due to difficulties with the morphological identification of females, which bear the pathogen but whose identification is dubious and requires expertise. In addition, subge-nus *Transphlebotomus,* comprising only three described species (*P. mascittii, P. economidesi* and *P. canaaniticus*), should be a focus of further investigations concerning their possible roles in *Leishmania* transmission. While these species are again difficult to distinguish morphologically, it has been proposed that *P. mascittii* be incriminated in the transmission of canine leishmaniasis in Germany (Naucke et al. 2008). Hence the taxonomical as well as the epidemiological significance of this subgenus should be thoroughly re-evaluated.

DNA BARCODING: A WAY TO RECOGNISE BOTH KNOWN AND UNKNOWN SAND FLY SPECIES

DNA barcoding is a method based on the identification of a given portion of DNA, typically the first half of a mitochondrial gene cytochrome oxidase I (COI), approximately 650 base pairs in length (Hebert et al. 2003). It is not a taxonomic system itself and does not aim to replace traditional taxonomy, rather it offers a complementary method which combines the classical morpho-logical approach with identification of specimens on the basis of sequence similarity (Vogler and Monaghan 2006). Therefore, a database of type sequences named BOLD (Barcode of Life Database) has been established for the acquisition, storage, analysis and publication of DNA barcode records. The identification system within BOLD, which is based on a linear search of a global alignment of all reference sequences, makes it possible to identify unknown query sequences.

DNA barcoding represents a sufficiently reliable, rapid and cost-effective solution for species identification, capable of identifying life stages other than adults. This feature is potentially very useful for studies on sand fly breeding sites, which are generally poorly characterised despite their potential attraction as possible targets for control measures. Nevertheless, in the case of

accidental findings of immature sand fly life stages, there is no comprehensive morphological approach available and DNA barcoding would be much appreciated. Recently, the question of whether DNA barcoding is a suitable methodological approach for the discovery of new species has been much debated (Moritz and Cicero 2004). While the delimitation of a new species exclusively on the grounds of a divergence of mtDNA sequence is not considered reasonable for numerous reasons (Sites and Marschall 2003), divergent or discordant mtDNA sequences can stimulate taxonomic reassessment based on nuclear genes and morphology, in other words 'reverse taxonomy'. Thus DNA barcoding proves beneficial to research on groups like sand flies where complexities such as cryptic species are often encountered.

We investigated barcode sequences of specimens belonging to the subgenus *Transphlebotomus* originating from localities in Turkey and Crete. This subgenus comprises only three so far described species (*Phlebotomus mascittii, P. economidesi, P. canaaniticus*) which have a markedly restricted distribution in the Mediterranean basin. While *P. mascittii* is recorded from a broad area ranging from the western Mediterranean (Spain, Italy and France) and constitutes the northern limit of sand fly distribution in Europe (Belgium, Germany and Austria), the other two species are more restricted. *P. canaaniticus* is present in eastern Mediterranean countries (Syria, Lebanon, Israel and Palestine) and *P. economidesi* has been recorded only from its type locality in Cyprus. Species of this subgenus are never abundant in sand fly catches throughout their range of distribution, which is often attributed to their presumably cavernous habitats and low biting rates on humans. Therefore, their possible incrimination in transmission of leishmaniases has never been studied, although it has been speculated that *P. mascittii* may be a vector in several small canine leishmaniasis foci in southern Germany, an assumption based on the fact that *P. mascittii* was the only species that was found in this region (Naucke et al. 2008).

Characterisation of COI barcode sequences of all three known *Transphlebotomus* species and their comparison with sequences from our investigated specimens from Crete and Turkey revealed important genetic differences which were corroborated by analyses of several other mitochondrial and nuclear genetic markers. They clearly demonstrated that specimens from several localities on the southern coast of Crete, as well as southern Anatolia in Turkey, in fact represent two putative new species. Their status was further confirmed by detailed morphological analysis of traditional features (pharynx, antennae and genitalia), which revealed minor, yet species-distinctive differences, that had not been noticed in the past (Erisoz Kasap et al. 2015). The description of two new sand fly species from a Mediterranean region, the first for several decades, represents a prime example of how molecular techniques, namely DNA barcoding, can help to reveal overlooked biodiversity and provide direction for taxonomists' investigations.

The key factor that affects (and often limits) the applicability and resolution power of DNA barcoding is the availability of a robust database of barcodes encompassing all major (and possibly also most of the minor and potentially present) sand fly species in a given region. Unfortunately, publicly available barcodes in the BOLD database and other resources, such as GenBank, represent only a fraction of species and do not constitute a collection that would be relevant for any particular endemic area of leishmaniasis transmission (Mediterranean basin, East Africa and the Indian subcontinent etc.). A research project dedicated to creating such a database for an epidemiologically important region for leishmaniasis transmission would create a strong tool enabling the routine and conclusive identification of sand fly species surveyed in that area.

MOLECULAR MARKERS: HANDY TOOLS FOR SPECIES IDENTIFICATION

Besides DNA barcoding, molecular taxonomy methods can offer practical solutions to resolve particular problems in species identification within some difficult species complexes or closely related species without the necessity of analysing sequences of the studied specimens. Several methods of DNA fingerprinting can help to design tools for such tasks.

To characterise species-specific markers for their easy and rapid identification, we studied *Phlebotomus neglectus* and *P. syriacus*, closely related species which both belong to the *P. major s.l.* species complex. Established by Lewis (1982), this complex comprises up to six species or sub-species of similar morphology and a wide distribution area ranging from the Mediterranean basin to Russia, China and the Indian subcontinent. Until now there is no exact delineation of their actual distribution but the general concept assumes that only *Phlebotomus neglectus* and *P. syriacus* are present in the Mediterranean region, contributing to the transmission of *Leishmania infantum. P. neglectus* is a

Type locality of newly described species Ph. killicki *in Crete, Greece.*

proven vector in several countries while *P. syriacus* is a probable vector based on circumstantial evidence (distribution, biting habits and taxonomic position). Nevertheless, it is very difficult to distinguish between these two species based on their morphology. The decisive characters in female genitalia are very difficult to assess in mounted specimens, while the length characters of male terminalia probably exhibit a continuum rather than the two distinctive and species-specific ranges that are given by traditional keys. Thus, these two closely related yet genetically distinct vector species are hot candidates for the development of molecular markers which would make it possible to conclusively identify the species.

When considering methods of DNA fingerprinting, one option is to resort to Random Amplified Polymorphic DNA (RAPD) markers. Comparing reproducible RAPD patterns of *P. neglectus* and *P. syriacus* acquired by a number of random decamer primers, we identified several candidate differential bands, sequenced them and designed specific primers for their amplification. We also exploited Restriction Fragment Length Polymorphism (RFLP) markers. By preparing an alignment of several gene sequences (namely cytB and EF alpha which are routinely used in phylogenetic studies) and predicting restriction sites within these sequences, we identified two restriction enzymes that find a single restriction site in the sequence of one species but lack any site in the sequence of the second species. Therefore, after application of a restriction enzyme, a decisive pattern distinguishes the two species

By combining these two approaches, we have developed a panel of molecular markers that conclusively distinguish these two species. The main advantages of these markers are that they are rapid and cost-effective as they do not require sequencing analysis but only routine PCR amplification and, in the case of RFLP-based markers, quick enzyme restriction. However, the key element in developing such molecular markers is to test them on a large collection of different populations within each species. The general applicability of markers based on specimens stemming from a limited number of species populations can be severely hampered by intraspecific variability among different populations. Moreover, it is also important to apply the markers on other sand fly species that are present in the region to avoid possible cross-positivity. We successfully tested our markers on a large number of specimens from different populations (*P. neglectus*: Italy, Croatia, Hungary, Montenegro and Albania; *P. syriacus*: Turkey, Syria, Lebanon and Israel) and also several species of the subgenus *Larroussius* as well as other subgenera present in the Mediterranean region. We therefore believe they are applicable on the wide range of distribution of our desired species. While there are numerous examples of such species pairs or complexes in the Mediterranean region in the subgenus *Larroussius*, as well as other subgenera, such ad hoc molecular markers could prove to be valuable tools for routine species differentiation.

MALDI-TOF PROTEIN PROFILING: A NOVEL METHOD FOR SPECIES IDENTIFICATION

Protein profiling by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has been established as a routine technique for the identification and successful discrimination of clinically important micro-organisms. Compared to classical phenotyping and molecular methods, this approach is simple, accurate and inexpensive. It requires minimal sample preparation and is especially useful for routine assessment of many field samples. All these qualities make this approach particularly attractive for applications on medically important insects of complicated taxonomy, including sand flies.

The procedure typically starts with acetonitrile/acid-based extraction of peptides and small proteins followed by mixing the resulting sample with a suitable aromatic acid serving as a MALDI matrix. After co-crystallization, the crystals are irradiated, desorbed

and ionized by a short laser pulse to generate peptide/protein ions in the gas phase, which are then measured by mass spectrometer. The obtained mass spectrum represents a characteristic and unique protein fingerprint which can either be compared with a reference spectra database for rapid and effective species identification, or can be evaluated using statistical methods for species discrimination and typing

Over the past decade, the use of MALDI-TOF MS protein profiling for species classification and identification has been expanded to many eukaryotic organisms including medically important haematophagous arthropods. Very recently, it was successfully applied on biting midges, tsetse flies, mosquitoes, fleas and ticks. We have demonstrated, for the first time, the applicability of MALDI-TOF MS to identify and distinguish Phlebotomine sand fly species, of both sexes, under different conditions of storage and homogenisation and to test its discriminatory power regarding subgenera, species and populations (Dvorak et al. 2014). The discriminatory power of the MS-based approach was initially tested on five Mediterranean species of four different subgenera, all proven vectors of important *Leishmania* parasites, namely *Phlebotomus papatasi* (specific vector of *L. major*), *P. sergenti* and *P. arabicus* (specific vectors of *L. tropica*), *P. perniciosus* and *P. tobbi* (vectors of *L. infantum*).

Specimens of all five sand fly species analysed produced informative, reproducible and species-specific protein spectra that enabled conclusive species identification. The method also distinguished between two *P. sergenti* colonies originating from different geographical localities. Protein profiles within a species were similar for specimens of both sexes. We thoroughly tested the conditions of specimen storage and sample preparation in order to establish a standardised protocol that would be generally applicable and reflect the situations encountered in sand fly field surveys. We had in mind the utilisation of a single specimen for several approaches (i.e. MALDI-TOF protein profiling, DNA-based methods and traditional morphological analysis).

After the first successful application of the method on sand flies, we focused on processing more species from the Mediterranean basin and elsewhere, and our protein profile collection currently comprises 20 species of several genera and subgenera. We also tested the sensitivity of the method for distinguishing between different and geographically distant or close populations of the same species by analysing populations of *P. neglectus* from Croatia, Hungary and Romania and *P. perniciosus* from several catch sites in Spain. Preliminary results indicate that protein profiling is able to differentiate between distant populations while closer populations, with presumable gene flow, appear to produce almost identical protein spectra. Finally, we addressed the possibility of cross-analysing data produced by a machine made by one manufacturer (Bruker Daltonics) using software from another major manufacturer (Shimatzu) as it would much enhance the applicability and spread of the method. It was demonstrated that this is indeed possible (Mathis et al. 2015).

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Dvorak V, Halada P, Hlaváčkova K, Dokianakis E, Antoniou M, Volf P (2014). Identification of phlebotomine sand flies (Diptera: Psychodidae) by matrix-assisted laser desorption/ionization time of flight mass spectrometry. *Parasites & Vectors* 7:21.

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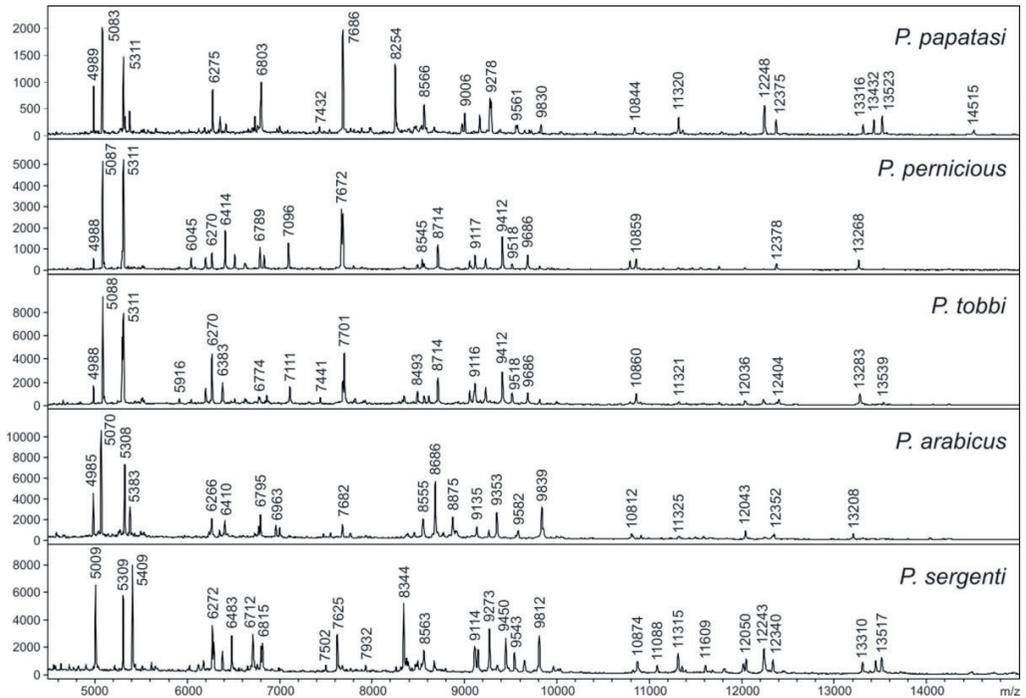
MALDI-TOF MS protein profiles of five different species of the genus Phlebotomus *showing species-unique peaks for conclusive species identification. The peaks in the mass spectra represent peptides or small proteins obtained from the* sand fly *bodies using acidic extraction.*

Dvorak et al. Parasites & Vectors *2014 7:21 doi:10.1186/1756-3305-7-21*

Species identification of sand flies using MALDI-TOF MS appears to be a feasible approach and will soon become a novel promising tool to improve biological and epidemiological studies on these medically important insects, being a rapid, simple, reproducible and cost-effective alternative, even to DNA-based methods. The key goal of ongoing research in this field is the construction of a robust reference database of species-specific protein spectra that would be publicly available and comprise an adequate amount of protein spectra to encompass intraspecific variability together with species biodiversity.

CONCLUSION

Leishmaniases are important yet neglected human and animal diseases that deserve adequate attention from both research and Public Health bodies. It has been clearly demonstrated that while they constitute a serious health problem in many regions of the world, they cannot be underestimated even in well-developed regions such as Europe. They are dynamic diseases and the circumstances of transmission are continually changing in relation to environmental, demographic and human behavioural factors. Recent studies show that the epidemiology of leishmaniases in the Mediterranean basin is changing, old historical foci re-emerge and new foci are established. Introduction of new strains from other regions is a possibility that should be seriously considered (Antoniou et al. 2013).

Phlebotomine sand flies are the only proven vectors of *Leishmania* parasites and as such they should be under the spotlight of consistent and intensive research. Many sand fly species contribute to the transmission of leishmaniases in natural foci, others

show the ability to transmit the causative agents, at least under laboratory conditions, and therefore represent possible vectors when the parasites are introduced into their natural environment (Volf and Myšková 2007). For the purposes of epidemiological studies, as well as appropriate control measures, it appears crucial to thoroughly study sand fly biology and understand their taxonomy as well as developing methods for accurate, conclusive and rapid species identification. Advances in understanding sand fly taxonomy as well as some practical tools for species identification have been possible due to the recent deployment of new molecular techniques. It must be emphasised that these novel methods do not replace but rather support and complement the traditional morphological approach as there is always a need for a baseline reference and 'gold standard'. The new methods for species identification described earlier must be based on broad and sufficient representations of sand fly species and populations from different areas.

Large collaborative projects such as EDEN and EDENext, which bring together researchers from many endemic countries, constitute the ideal platform for data collection, exchange of expertise and research coordination. We believe that the many scientific achievements of the phlebotomine-borne disease group within EDENext have contributed to the introduction and promotion of novel techniques and established a network of institutions which will continue to collaborate in the future.

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PUBLICise Health Public Health Telegram: Phlebotomine-borne diseases

EDENext's Public Health Telegrams (PUBLICise Health) were established to inform interested individuals and institutions about research results on vector-borne diseases with direct or indirect impact on Public Health issues. Edited and produced by EDENext's Public Health team, each issue concentrates on the work of one of the project's five specialist groups. Here we present an updated version of the issue dedicated to the work of the team examining Phlebotomine-borne diseases (Diptera, Psychodidae, subfamily Phlebotominae), first published in September 2014.

1. The role of phlebotomine sand flies in spreading diseases which are of Public Health concern

The EDENext review by Maroli et al. (2013) describes the role of phlebotomine sand flies as vectors in spreading different diseases, i.e. leishmaniasis, sand fly fever, summer meningitis, vesicular stomatitis, Chandipura virus encephalitis and *Bartonella* (Carrión's disease), and outlines their relevance for Public Health. The emergence of new diseases and pathogens or their re-emergence in areas previously considered to be free can be associated with ecological factors and changes in land use, such as deforestation, urbanisation or irrigation measures, that result in changes in the vector populations, and with human factors, for example an increase in global travel and trade that favours the spread of vector species to previously unaffected areas. Climate changes, for their part, may allow not only the northward expansion of vector species but also virus persistence during winter.

Among sand fly-borne diseases, leishmaniasis is the most widespread infection worldwide. Like other tropical infectious diseases, such as Chagas' disease and sleeping sickness, it is generally regarded as a neglected disease, because of the lack of effective control and treatment measures. With few exceptions, phle-

botomine sand flies are the only haematophagous insects that transmit leishmaniasis. Among the more than 800 estimated phlebotomine sand flies species, around 100 species have been recorded as potential vectors of human leishmaniasis. The review provides a list of these species by endemic country, the *Leishmania* species agent transmitted (if known) and the clinical forms of leishmaniasis in humans. In addition, other human diseases and their growing impact on human health are outlined.

Phlebotomine sandflies are involved in the transmission of several viral agents, among which the most important are grouped into the *Phlebovirus* genus (Bunyaviridae), which includes the sandfly fever Sicilian and Toscana viruses, and the *Vesiculovirus* genus (Rhabdoviridae), which includes vesicular stomatitis, and the Chandipura and Isfahan viruses. *Vesiculovirus*, which is well-known to affect livestock animals in southeastern USA and Latin America, is increasingly reported to cause infection in humans in India. In addition Carrión's disease caused by *Bartonella bacilliformis*, a motile, aerobic and Gram-negative bacterium, formerly restricted to elevated altitudes of Peru, Ecuador and Colombia, is spreading to non-endemic areas of the Amazon basin.

Finally, Maroli and co-authors discuss the spread of leishmaniasis as well as the other mentioned diseases and the lack of suitable treatment due to the rather poor awareness of national Public Health authorities, especially in poor countries. Though advances in phlebotomine research with regard to vector control have been made, in particular studies on the social and environmental variables relevant for *Leishmania* ecology and transmission, the development of geographical-spatial and analytical models are needed.

2. Leishmaniasis in Greece

Leishmaniasis is a protozoan disease transmitted by phlebotomine sand flies in a zoonotic or an anthroponotic cycle, depending on the parasite species and the geographical location. Though leishmaniasis is regarded as a tropical or subtropical disease, it has become endemic in other geographical regions such as the Medi-

terranean Basin and has recently been reported to have spread to parts of Central Europe, for example *Leishmania infantum* in northern Italy, Hungary and southern Germany. Therefore, Leishmania constitutes a Public Health problem in Europe, not only in endemic areas but also in geographic regions that are at risk of introduction.

The first case of visceral leishmaniasis in the Mediterranean region was reported from the Greek island of Spetses in 1835. Though the frequency of mosquito and sand fly transmitted diseases decreased due to the use of DDT against malaria in the years 1946-1952, the disease has re-emerged and spread across Greece in the past 35 years with an increasing number of human and dog cases per year. The aim of the EDENext review by Ntais et al. (2013) was to investigate the current situation in Greece of leishmaniases: the geographical distribution of human and canine leishmaniases, *Leishmania* species as well as sand fly vector species and the distribution of the diseases in relation to epidemiological and environmental factors using geospatial tools.

Leishmania seropositive dogs were found in 43 of 54 prefectures with an average of 22% indicating that the disease is widely spread across Greece. Favourable factors were found to be altitude, presence of water bodies, land use, wind speed, mean land surface temperature, mean relative humidity and mean annual rainfall. Dogs kept outside (strays, guard or hunting dogs) are at higher risk of getting infected and, in turn, to infect sand flies. *L. infantum zymodeme* (Z) MON-1 found predominantly in the Mediterranean Basin was present in all parts of the country, while the rare ZMON-98 was less frequent and the dermatotropic *L. tropica* was found only in Crete. Thirteen different sand fly species are known to occur in Greece and are widely distributed across the country, 10 of which belong to the genus *Phlebotomus* and three to the genus *Sergentomyia*.

The importance of infected dogs lies in their role in transmitting the parasite *L. infantum* to sand fly vectors, which in turn can infect humans. Controlling the disease in the dog population is the best way to reduce the risk of infection in the human population. Chan-

ging human habits and living conditions, the movement and travel of humans and dogs, and changes in climatic and environmental conditions and in animal husbandry practices that increase vector abundance enhance leishmaniasis cases in humans and dogs and the geographic spread.

In another EDENext publication Ntais et al. (2014) reported the introduction of *Leishmania tropica* ZMON-58 to the island of Crete and the infection of a local dog. *L. tropica* ZMON-58 was isolated from a young Afghan refugee with cutaneous lesions who came to Crete a few months earlier. In the same area the same zymodeme was isolated from a local dog with symptoms of visceral leishmaniasis. Since *L. tropica* ZMON-58 has so far only been described in six human cases in Afghanistan, this is the first record of *L. tropica* in a dog and presents another example of the introduction of a vector-borne pathogen to an unaffected area that supplies a suitable vector population allowing new transmission cycles. In a closed ecosystem such as Crete, hosting nine different *Phlebotomus* species, there always is the risk of exchanging genetic material between *L. infantum* and *L. tropica* and the risk of zymodeme variants resulting in new hybrids with a potentially changed epidemiology, pathogenicity and drug resistance. Globalisation favours changes in the epidemiology of many pathogens and the introduction to new areas. Pathogens and vectors become established in new foci if they encounter the right conditions and hosts for spread. Therefore, monitoring vectors and reservoirs in areas at risk for introduction and providing information to Public Health authorities is of great importance to prevent the spread and establishment of a novel disease in new, so far unaffected areas.

Sifaki-Pistolla et al. (2014) evaluated the use of a veterinary questionnaire as a cost effective tool for locating, recording and predicting the spread of canine leishmaniasis in an area, comparing the collected questionnaire data with the results of two epidemiological surveys accomplished in Greece (Ntais et al. 2013) and Cyprus. In order to assess the risk of human *Leishmania* infections in an area, surveillance studies in the local dog population are necessary. As epidemiological studies

Ntais P, Sifaki-Pistola D, Christodoulou V, Messaritakis I, Pratlong F, Poupalos G and Antoniou M (2013). Leishmaniases in Greece. *Am J Trop Med Hyg.* 2013 Nov;89 (5): 906-15. Doi: 10.4269/ajtmh.13-0070. Epub 2013 Sep 23.
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of dogs are costly and time-consuming, the aim of the study was to evaluate the questionnaire, consisting of 14 brief multiple-choice questions, in order to collect information on the disease which made it possible to reveal spatially-explicit indicators of canine leishmaniasis prevalence for use in predictive modelling. In addition, the questionnaire offers a two-way exchange of information; it provides information not only to scientists and specialists but also to veterinarians as they become aware of what is considered important by the specialists. Although the questionnaire data cannot provide a quantitative measure of canine leishmaniasis in an area, it demonstrates the dynamic of the disease. Overall, the veterinary questionnaire can be used as an early warning system and a screening tool to assess the risk of leishmaniasis in a country or region and therefore promoting Public Health.

Sifaki-Pistola D, Ntais P, Christodoulou V, Mazeris A and Antoniou M (2014). The Use of Spatial Analysis to Estimate the Prevalence of Canine Leishmaniasis in Greece and Cyprus to Predict Its Future Variation and Relate It to Human Disease. *Am J Trop Med Hyg.* 2014 Jun 23. pii: 13-0459.

3. Risk factors for cutaneous leishmaniasis in Turkey

Cutaneous leishmaniasis (in the form of a cutaneous lesion at the infected sand fly bite site), is the most common form of human leishmaniasis, with millions of human cases reported from southern Europe, Asia, Africa, South and Central America every year. Other mammal species, such as dogs and rodents, play a role as reservoirs or hosts for *Leishmania* parasites and contribute to the spread of the disease. The EDENext study by Votýpka et al. (2012) evaluated risk factors for non-typical cutaneous leishmaniasis caused by *L. infantum* in the Cukurova region in South Anatolia, Turkey. By using structured questionnaires in interviews with local people, epidemiological and clinical characteristics, application of personal protection measures and knowledge about *Leishmania* parasites were analysed. Ownership of a dog, raising cattle and sleeping without a bed-net were associated with a significantly increased risk of *Leishmania* infection in statistical-univariate analyses, with dog ownership having the greatest correlation with leishmaniasis. The authors recommend further research on dogs to clarify the transmission cycle and the role of dogs in the epidemiology of cutaneous leishmaniasis in this area.

A medium-scale field trial with insecticide-treated bed-nets was conducted in south Anatolia to measure the protective efficacy of Olyset® Plus, a new long-lasting factory-treated insecticidal net (LLIN) incorporated with 2% permethrin and 1% of the synergist piperonyl butoxide (PBO), against cutaneous leishmaniasis (CL) in a new hyper-endemic focus caused by a *Leishmania infantum/L. donovani* hybrid parasite transmitted by proven vector *Phlebotomus tobbi*, between May 2013 and May 2014. The study area comprised eight villages; two of them were selected as an intervention village with Olyset® Plus net (Kizillar) and a control village without net application (Malihidirli). Six villages with surrounding allopatric barriers were utilized as a buffer zone cluster between intervention and control villages. Monthly entomological surveys were performed in the intervention and control villages and Damyeri, representing the other six villages, to collect adults of *Phlebotomus tobbi*. Results showed a significant reduction in cutaneous leishmaniasis incidence in the intervention village from 4.78% to 0.37%. The protective efficacy rate of LLIN was 92.2%. In contrast, incidence rates increased in the control village from 3.67% to 4.69%. We also evaluated residual insecticide levels of used nets after six and 12 months of usage. It was determined that the nets had retained full insecticidal strength. These results highlight the value of real-world data on bed net effectiveness and longevity to guide decisions regarding sand fly control strategies (Gunay et al. 2014).

4. Updates from phlebotomine sandfly-borne disease research in Portugal

Zoonotic leishmaniasis caused by *L. infantum* is endemic in Portugal. Within the scope of EDENext, surveys concerning sand fly, canine and feline diseases have been conducted in the internationally renowned tourist hot spot of the Algarve region in southern Portugal (Maia et al. 2013, 2014). *L. infantum* prevalence ranged from 10 % in cats and 16 % in dogs. The simultaneous presence of dogs, cats and *Phlebotomus perniciosus* infected with *L. infantum* in the Algarve region shows that the region continues to be an endemic area of this parasitic zoonosis.

Votýpka J, Kasap OE, Volf P, Kodym P and Alten B (2012). Risk factors for cutaneous leishmaniasis in Cukurova region, Turkey. *Trans R Soc Trop Med Hyg.* 2012 Mar; 106(3):186-90. Doi: 10.1016/j.trstmh.2011.12.004. Epub 2012 Jan 26.

Gunay F, Karakus M, Oguz G, Dogan M, Karakaya Y, Ergan G, Kaynas S, Kasap OE, Ozbel Y and Alten B (2014). Evaluation of the efficacy of Olyset® Plus in a village-based cohort study in the Cukurova Plain, Turkey, in an area of hyperendemic cutaneous leishmaniasis. *Journal of Vector Ecology,* 39: 395–405. Doi: 10.1111/jvec.12115

Campino L, Cortes S, Dionísio L, Neto L, Afonso MO and Maia C (2013). The first detection of *Leishmania major* in naturally infected *Sergentomyia minuta* in Portugal. *Mem Inst Oswaldo Cruz.* 2013 Jun; 108(4):481-7. Doi: 10.1590/S0074-0276108042013014.

In addition, DNA of *Leishmania major*, which is associated with cutaneous leishmaniasis, was detected in a *Sergentomyia minuta* specimen collected in the same geographic region (Campino et al. 2013). It is the first documented case of *L. major* in a sand fly of the *Sergentomyia* genus in Europe. Further analysis of blood meals of sand flies from Algarve region using the cyt-b sequence showed that *P. perniciosus* fed on a wide range of domestic animals while human and lizard DNA was detected in engorged *S. minuta*. Detection of *Leishmania* infections by PCR using ITS-1 as the target sequence revealed in two specimens of *Sergentomyia minuta* and one *Phlebotomus perniciosus* (Maia et al. 2015).

Overall, the results reinforce the need for systematic investigations of the spatial distribution of phlebotomine populations and, at the same time, of pathogens in both invertebrate and vertebrate hosts in order to improve the understanding of the transmission, distribution and spread of *Leishmania* species.

5. A retrospective analysis of human leishmaniasis epidemics in Italy

The EDENext publication by Gramiccia et al. (2013) gives a retrospective analysis of the multi-annual human leishmaniasis epidemics by *L. infantum* that occurred between 1989 and 2009 in Italy. Starting from 1989, Italy has experienced an increase of visceral leishmaniasis cases that peaked between 2000 and 2004 with more than 200 cases per year and declined thereafter. The study was conducted to identify possible determinants that explain the recent trend of the disease in the country. The visceral leishmaniasis epidemic in Italy seems to be a complex phenomenon with several components: i) an outbreak involving infants and immune-competent adults in the Campania region that declined, presumably naturally; ii) a second outbreak affecting HIV-infected individuals throughout the country, that declined due to antiviral treatment; iii) a generalised increase of cases due to disease spreading within traditionally endemic areas as well as the appearance of a few autochthonous cases in previously non-endemic territories, starting from the early 1990s.

Maia C, Dionísio L, Afonso MO, Neto L, Cristóvão JM and Campino L (2013). *Leishmania* infection and host-blood feeding preferences of phlebotomine sand flies and canine leishmaniasis in an endemic European area, the Algarve Region in Portugal. *Mem Inst Oswaldo Cruz*. 2013 Jun; 108(4):481-7. Doi: 10.1590/S0074-0276108042013014.

Maia C, Ramos C, Coimbra M, Bastos F, Martins A, Pinto P, Nunes M, Vieira ML, Cardoso L and Campino L (2014). Bacterial and protozoal agents of feline vector-borne diseases in domestic and stray cats from southern Portugal. *Parasit Vectors*. 2014 Mar 24; 7(1):115. Doi: 10.1186/1756-3305-7-115.

Maia C, Parreira R, Cristóvão JM, Freitas FB, Afonso MO and Lenea Campino L (2015). Molecular detection of *Leishmania* DNA and identification of blood meals in wild caught phlebotomine sand flies (Diptera: Psychodidae) from southern Portugal. *Parasites & Vectors* 2015, 8:173. Doi: 10.1186/s13071-015-0787-4.

While the appearance of autochthonous visceral leishmaniasis cases in northern parts of Italy could be explained by the de novo colonisation with phlebotomine vectors along with importing *Leishmania*-infected dogs from the endemic south and the generalised increase of cases in endemic areas due to changes in vector density, the causes for both the onset and the natural decline of the outbreak in the Campania region will remain unexplained. The immunity on a population level acquired during the epidemic may be one reason, but is hard to verify. No aggressive control measures were applied that could justify the general drop in incidence. To date there is no clear evidence for a direct association of canine leishmaniasis prevalence and incidence of human disease in a given territory. Although dogs are efficient sentinel hosts for *L. infantum* transmission, the prevalence rate of canine infections does not appear a useful parameter for explaining determinants of human visceral leishmaniasis trends in endemic areas as observed in the Campania region. While the presence of competent vectors in a given territory can be predictive for the occurrence of human visceral leishmaniasis together with the occurrence of canine leishmaniasis cases, the current entomological data is still insufficient for the analysis of epidemic visceral leishmaniasis trends.

Gramiccia M, Scalone A, Di Muccio T, Orsini S, Fiorentino E and Gradoni L (2013). The burden of visceral leishmaniasis in Italy from 1982 to 2012: a retrospective analysis of the multi-annual epidemic that occurred from 1989 to 2009. *Euro Surveill*. 2013 Jul 18; 18(29):20535.

6. Spanish leporids (hares and rabbits) as potential sylvatic *Leishmania* reservoirs

In Spain, dogs are considered to be the main reservoir of zoonotic leishmaniasis caused by *L. infantum*. In rural, periurban and suburban areas of the Madrid region, *Leishmania* is endemic. However, since 2010 the number of human cases of visceral and cutaneous leishmaniasis in four municipalities to the south-west of Madrid has drastically increased, presumably linked to the creation of a park adjacent to the urban area. These outbreaks have driven the investigation of local wild animals in the search for potential reservoirs for *L. infantum*. The low prevalence of leishmaniasis in dogs and the high population densities of hares and rabbits in the newly constructed periurban park have led to the assumption that leporids could sustain a large sand fly population in the

area, suggesting the existence of a sylvatic transmission cycle linked to the urban periphery. In order to find the sources of infection, several xenodiagnosis studies focusing on wild leporids and *Leishmania* phlebotomine vectors were initiated:

Molina et al. (2012) delivered the first evidence by xenodiagnosis that Spanish hares (*Lepus granatensis*), a species endemic to the Iberian Peninsula, are used as hosts by *P. perniciosus*, and that *L. infantum* is successfully transmitted from hares to *P. perniciosus* sand flies. The study discussed the role of hares as potential sylvatic reservoirs for *L. infantum*. In conclusion, hares should be taken into account in future epidemiological studies when endemic sites of visceral leishmaniasis are investigated.

In line with these findings, Martín-Martín et al. (2014) could show that wild rabbits and hares captured in this area show high anti-sand fly saliva antibody levels which indicates that they are frequently bitten by *P. perniciosus*.

A study conducted by Jiménez et al. (2014) in the same area demonstrated that rabbits can also contribute to the spread of leishmaniasis. Wild rabbits (*Oryctolagus cuniculus*) were found to serve as hosts for *P. perniciosus* and therefore are a source of *L. infantum*. Results of blood meal preference analyses of *P. perniciosus*, caught in the same area, also revealed that the majority of sand flies fed on hares and/or rabbits.

In the framework of entomological studies taking place in this area, Jimenez et al. (2013) conducted a preliminary entomological survey in 2011, at the end of the seasonal transmission period when highest rates of infections in sand flies could be expected and before control measures were implemented, to determine the putative species involved. The data connect *P. perniciosus* as *L. infantum* vector to hares and humans as hosts and support the existence of an unusual sylvatic cycle as an alternative to the classical domestic one with dogs as the main reservoir in this area. Most noticeably, the percentage of *L. infantum* positive *Phebotomus* sand flies was comparably high with approximately 60%. In addition, blood feeding preferences, analysed by cytochrome b analysis,

Jiménez M, González E, Iriso A, Marco E, Alegret A, Fúster F and Molina R (2013). Detection of *Leishmania infantum* and identification of blood meals in *Phlebotomus perniciosus* from a focus of human leishmaniasis in Madrid, Spain. *Parasitol Res.* 2013; 112(7):2453-9. Doi: 10.1007/s00436-013-3406-3. Epub 2013 Mar 28.

Jiménez M, González E, Martín-Martín I, Hernández S and Molina R (2014). Could wild rabbits (*Oryctolagus cuniculus*) be reservoirs for *Leishmania infantum* in the focus of Madrid, Spain? *Vet Parasitol.* 2014. Doi: 10.1016/j.vetpar.2014.03.027.

revealed that hares were preferred as hosts, with 60% of sand flies positive for hare blood, followed by humans with 30% and cats with 10%, whereas no dog blood was identified. This can explain the high transmission frequency of leishmaniasis in the green park where high population densities of hares are present (see above). Since blood feeding behaviour plays a significant role in the transmission and maintenance of vector-borne pathogens in natural systems, analyses of blood feeding preferences will therefore help to improve our understanding of the role of a particular host or reservoir in urban foci, leading to more effective control strategies.

Using an *ex vivo* model of infection in murine bone marrow-derived cells, the ex vivo virulence of the *L. infantum* isolates from *Phlebotomus perniciosus* captured in this area of Madrid was evaluated, showing that *L. infantum* isolates captured from this endemic area exhibited high virulence in terms of infection index, cytokine production and enzymatic activities involved in the pathogenesis of visceral leishmaniosis (Domínguez-Bernal et al. 2014).

In summary, this exceptional scenario is likely to be a cause of the urbanisation of leishmaniasis due to the coexistence of periurban and sylvatic transmission cycles involving different, domestic and sylvatic, reservoir species for sand fly vectors. These findings show the complexity of the eco-epidemiological factors that drive *Leishmania* transmission and, therefore, have important implications for Public Health in terms of taking effective measures to control the situation as soon as possible.

Martín-Martín I, Molina R, Rohoušová I, Drahota J, Volf P and Jiménez M (2014). High levels of anti-*Phlebotomus perniciosus* saliva antibodies in different vertebrate hosts from the re-emerging leishmaniosis focus in Madrid, Spain. *Vet. Parasitol*. 2014. Doi: 10.1016/j.vetpar.2014.02.045.

Molina R, Jiménez MI, Cruz I, Iriso A, Martín-Martín I, Sevillano O, Melero S and Bernal J (2012). The hare (*Lepus granatensis*) as potential sylvatic reservoir of *Leishmania infantum* in Spain. *Vet Parasitol*. 2012; 190:268-271.

Domínguez-Bernal G, Jiménez M, Molina R, Ordóñez-Gutiérrez L, Martínez-Rodrigo A, Mas A, Cutuli MT and Carrión J (2014). Characterisation of the ex vivo virulence of *Leishmania infantum* isolates from *Phlebotomus perniciosus* from an outbreak of human leishmaniosis in Madrid, Spain. *Parasites & Vectors* 2014, 7:499. Doi: 10.1186/s13071-014-0499-1.

7. Autochthonous canine leishmaniasis in Hungary

Tánczos et al. (2012) questioned the status of Hungary as a *Leishmania*-free country where only imported cases of *Leishmania* infections in humans and canines had been described before. When a first case of autochthonous canine leishmaniasis was diagnosed in a pug dog in 2007, an investigation of the other dogs from the kennel was initiated to assess the prevalence of *Leishmania*. During the study another dog developed clinical symptoms of leishmaniasis. In

total six out of 20 dogs were found positive by testing for antibodies or by PCR analysis, but only the two dogs mentioned showed clinical signs. None of the dogs was reported to have been abroad and no sand flies could be collected in and around the kennels so the source of infection is not clear. Possible routes of transmission resulting in autochtonous *Leishmania* infections are discussed by the authors considering (i) the natural occurrence of two *Phlebotomus* species in Hungary that may serve as potential vectors for the pathogen, and (ii) the risk posed by infected asymptomatic dogs. The likely increase in numbers of dogs travelling to or coming from endemic areas together with a generally high percentage of asymptomatic dogs and the lack in knowledge of local veterinarians may have consequences for the epidemiology of *Leishmania* and the distribution of the disease in Hungary.

This study presents an example that, with the spread of this zoonotic disease northwards from southern Europe, *Leishmania* poses a severe threat for human and canine health in central and northern European countries.

Tánczos B, Balogh N, Király L, Biksi I, Szeredi L, Gyurkovsky M, Scalone A, Fiorentino E, Gramiccia M and Farkas R (2012). First record of autochthonous canine leishmaniasis in Hungary. *Vector Borne Zoonotic Dis*. 2012 Jul; 12(7):588-94. Doi: 10.1089/vbz.2011.0906. Epub 2012 May.

8. Isolation of (novel) sand fly-borne viruses

In countries around the Mediterranean basin, phlebotomine sand flies are involved in the transmission of several arthropod-borne viruses that belong to the genus *Phlebovirus* within the *Bunyaviridae* family, i.e. Toscana virus (TOSV; species Sandfly fever Naples virus), Arbia virus (species *Salehebad* virus) and Sandfly fever Sicilian virus. In southern European countries Toscana virus constitutes a threat for Public Health as it is one of the major viral pathogens causing central nervous system infections in humans. Findings of new sand fly-borne phleboviruses from Mediterranean countries indicated that the viral diversity in the genus *Phlebovirus* is higher than initially expected. In Tunisia, the recent isolation of Punique virus (PUNV; species *Sandfly fever Naples* virus) raised the question of its role in human infections and as a potential pathogen. The EDENext study by Sakhria et al. (2013) demonstrated the co-circulation of two related sand fly-borne phleboviruses, TOSV and PUNV, both belonging to the same species,

Sandfly fever Naples virus. Serum samples from humans living in endemic areas for visceral leishmaniasis in northern Tunisia were tested by micro-neutralisation assay for neutralising antibodies which allowed these closely related viruses to be differentiated. With seroprevalence rates of 41% for TOSV and 9% for PUNV found in the samples it was demonstrated that PUNV is capable of infecting humans but at a low rate, and that TOSV seems to be responsible for the majority of human infections by sand fly-borne phleboviruses in northern Tunisia. Therefore it should always be considered by physicians for patients with meningitis or unexplained fever.

TOSV was initially discovered in central Italy but it was not until 15 years later that its role as causative agent in human neuro-invasive disease was noticed. TOSV circulates in several European countries, for example Italy, Portugal, Spain, France, Croatia and Turkey, and is now recognised as the leading cause of aseptic meningitis during the warm season. Bichaud et al. (2014) reported the first detection of TOSV from *P. perniciosus* in Corsica demonstrating the presence of the virus on the island. TOSV poses a risk to the Public Health of locals and tourists and therefore always needs to be considered by physicians when patients present with aseptic meningitis and febrile illness during the warm season.

The EDENext publication by Remoli et al. (2014) described the isolation of a novel *Phlebovirus*, named Fermo virus, placed in the species *Sandfly fever Naples* virus. The importance of this finding lies in the virus' ability to fill an ecological niche and to co-infect the same vector which in consequence may lead to the emergence of virus reassortants (with mixed gene segments) exhibiting different degrees of pathogenicity. Further studies need to focus on the distribution of Fermo virus and on its ability to cause infection and disease in humans.

A new phlebovirus, Adana virus, was isolated from a pool of *Phlebotomus* spp. in the province of Adana, in the Mediterranean region of Turkey. Genetic analysis based on complete coding of genomic sequences indicated that Adana virus belongs to the *Salehabad* virus species of the genus *Phlebovirus* in the family *Bunyaviridae*.

Bichaud L, Izri A, de Lamballerie X, Moreau G and Charrel RN (2014). First detection of Toscana virus in Corsica, France. *Clin Microbiol Infect*. 2014 Feb; 20(2):0101-4. Doi: 10.1111/1469-0691.12347. Epub 2013 Aug 30.

Remoli ME, Marchi A, Bucci P, Argentini C, Bongiorno G, Maroli M, Gradoni L, Gramiccia M and Ciufolini MG (2014). Viral isolates of a novel putative phlebovirus in the Marche Region of Italy. *Am J Trop Med Hyg*. 2014 Apr; 90(4):760-3. Doi: 10.4269/ajtmh.13-0457. Epub 2014 Feb 17.

Sakhria S, Bichaud L, Mensi M, Salez N, Dachraoui K, Thirion L, Cherni S, Chelbi I, De Lamballerie X, Zhioua E, and Charrel RN (2013). Co-circulation of Toscana virus and Punique virus in northern Tunisia: a microneutralisation-based seroprevalence study. *PLoS Negl Trop Dis*. 2013 Sep 12; 7(9):e2429. Doi: 10.1371/journal.pntd.0002429. eCollection.

Adana virus is the third virus of the *Salehabad* virus species for which the complete sequence has been determined. To understand the epidemiology of Adana virus, a seroprevalence study using microneutralization assay was performed to detect the presence of specific antibodies in human and domestic animal sera collected in Adana as well as Mersin province, located 147km west of Adana. The results demonstrate that the virus is present in both provinces. The significance of this Adana virus as a possible public health problem in exposed human populations deserves further studies (Alkan et al. 2015).

Alkan C, Alwassouf S, Piorkowski G, Bichaud L, Tezcan S, Dincer E, Ergunay K, Ozbel Y, Alten B, de Lamballerie X and Charrel RN (2015). Isolation, genetic characterization, and seroprevalence of Adana virus, a novel phlebovirus belonging to the Salehabad virus complex, in Turkey. *J Virol* 89:4080 –4091. Doi:10.1128/JVI.03027-14.

9. Geographic spread of sand fly-borne phleboviruses

Sand fly-borne phleboviruses are widely distributed in the Mediterranean basin, North Africa, the Indian subcontinent, the Middle East and central Asia. Except for Toscana virus, the leading cause of aseptic meningitis in endemic regions, phleboviruses are inadequately considered by physicians and are frequently underestimated. However, climate changes, environmental conditions and anthropogenic factors, such as increased trade and travel of humans and animals, have a considerable impact on the distribution of vectors. This, together with the virus' propensity for mutations, reassortment and recombinations of its three-segmented genome increases the risk for virus introduction and makes it probable that phleboviruses will extend their geographic range. Most studies document the distribution of sand fly-borne phleboviruses in Western Europe, while data for Eastern Europe, the Middle East and Africa are very limited. The EDENext review by Alkan et al. (2013) summarises the geographic spread of sand fly-borne phleboviruses with a focus on understudied regions and discusses possible countermeasures and the need to conduct studies aimed at developing new antiviral drugs and vaccines. In terms of Public Health, their potential as emerging pathogens should raise our awareness and highlight the need to understand not only the complex nature of sand fly-borne viruses and but also the biological significance of possible interactions between *Leishmania* parasites and phleboviruses.

Alkan C, Bichaud L, de Lamballerie X, Alten B, Gould EA and Charrel RN (2013). Sand fly-borne phleboviruses of Eurasia and Africa: epidemiology, genetic diversity, geographic range, control measures. *Antiviral Res*. 2013 Oct; 100(1):54-74. Doi: 10.1016/j.antiviral.2013.07.005. Epub 2013 Jul 19.

Maria Antoniou

What did EDEN and EDENext mean for your institute and your students?

Maria Antoniou (University of Crete, Greece): *"EDEN and EDENext have been very important for us because they gave us a chance, first of all, to have money in order to do research and, secondly, to have the students involved with some salary too. There were about six students who worked with EDEN and EDENext money. The money gave us the opportunity to do the research, to collect samples from all over Greece and Cyprus regarding sand flies and sera from dogs for* Leishmania *production and from humans, from patients. And this material will be used as we go on to do work with other things like drug resistance of* Leishmania *and in collaborations for analysis of the sand flies. And also the students had the chance to go to conferences and to PhD meetings and were able to learn how to participate in big groups and how to tackle collaborations and so on. They will be the future scientists on these subjects for Greece and for Cyprus. Some of the students who finished their PhDs on EDEN money are now working in Cyprus for the government and are still helping the EDENext project because they were so excited and happy about all of this. Without any money they collect samples for us and I go to Cyprus to collaborate. One of the PhDs, through work carried out in Cyprus, was able to detect* Leishmania donovani *in Cyprus, which is the first detection in Europe; this is the introduction of a very virulent strain in Europe and so important work that has only been done because of this collaboration."*

Cyprus-born Maria Antoniou is assistant professor of parasitology at the University of Crete's Medical School and is head of the microbiology laboratory of the Medical Department and of the University Hospital in Heraklion, Crete. Maria spent a decade at London University's Imperial College of Science, Technology and Medicine, completing a BSc (ARCS) in zoology, MSc (DIC) in nematology and a PhD in parasitology-nematology, before a sabbatical studying leishmaniasis at INSERM (France's national institute for health and medical research). Her current research includes the protozoan parasites *Leishmania* and *Toxoplasma* and the epidemiology of zoonoses using GIS technology. This includes the epidemiology of leishmaniasis in Greece and Cyprus, isolating the parasite from patients, dogs and sand flies, the study of the geographical distribution of *Leishmania* strains and their vectors, and studies relating to the biology of the *Leishmania* parasite and its resistance to drugs.

It is a good base for the future?

Maria: *"Yes, good relations for the future are very important, not only for our lab but also for the students who go independent and get a job somewhere else. They know that these people do this and I can talk to them, I can ask them for advice and have collaborations with them for future work and I can provide them with materials through their lab to carry out their analysis on important questions which arise through this work."*

These collaborations are very important?

Maria: *"It was a great experience to collaborate with so many people from so many different fields and I think this is important for the professors as well as for the students. For example, now we have people who are experts in GIS which is something we did not have before, beyond an expert in Athens who collaborated with our lab. Now I have two students who can do very quick work with GIS, not just mapping but also statistical analysis. Also I have had the chance to send students to different labs for collaborations and people came to our lab for collaborations, which is bond-building for science but also for personal bond-building, which is very important…We have very, very good relationships which we are still building and we hope to be able to carry on working together. This networking is important. It was a very important stepping stone in the EDEN project, not just for EDEN people, but for the policies of the European Union, on how to fund projects. You can sit in your lab and just do research but discussing with people brings out ideas!"*

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Understanding the e
virus tran

INTRODUCTION

West Nile fever (WNF) is a zoonotic viral disease principally maintained in a zoonotic cycle between mosquitoes and birds (Reiter 2010). Although humans and horses are not competent hosts, they may become infected by West Nile virus (WNV). Most infections are of short duration and asymptomatic but in a few cases the virus causes neural disease, even leading to death (Rizzoli et al. 2015). Over past decades the incidence of WNF in horses and humans in Europe has increased (Rizzoli et al. 2015). In this paper I summarise some of the research conducted by EDEN and EDENext research teams aimed at understanding its transmission dynamics in Europe.

WNV outbreaks in Europe used to be of short duration and small scale (Rizzoli et al. 2015) and for this reason the traditional view for many years has been that migratory birds introduced the virus each spring from its endemic areas in Africa. One of the main results of our research has been to demonstrate that WNV circulation in some regions in Europe does not depend on introductions from Africa. Indeed, the virus can be considered endemic at least in Austria (Bakonyi et al. 2013), Italy (Engler et al. 2013), Romania (Dinu et al. 2015) and Spain (Figuerola et al. 2007, 2008). In all these areas, virus, seroconversions and/or cases of disease in birds, horses or humans have been recorded in the same areas over successive years. In addition, the analyses of the sequences of WNV lineage 1 detected in the western Mediterranean suggest that all the outbreaks occurring between 1996 and 2010 may be explained by a single introduction from Africa and posterior dispersal through Europe (Sotelo et al. 2011). The two original, independent, introductions of WNV lineage 2 in Europe, one in Austria and the other in Russia, and subsequent expansion through Central and Eastern Europe underline the importance of WNV dispersal within Europe (Rudolf et al. 2013, Kolodziejek et al. 2014, Dinu et al. 2015).

The persistence of WNV in temperate climates during the winter may occur through overwintering mosquitoes or through long-term infection in birds (Reisen 2013). Research in Romania and the Czech Republic has confirmed the presence of WNV in pools of overwintering mosquitoes (Reiter 2010). Studies are now under way to estimate the survival of female mosquitoes overwintering in anthropogenic areas. Furthermore, ticks provide an alternative vector for WNV, with the potential to act as long-term virus vectors and infect a wide spectrum of vertebrate hosts through the various tick developmental stages (Kolodziejek et al. 2013)

MONITORING SEROPREVALENCE IN VERTEBRATE HOSTS

Analyses of the seroprevalence data of WNV neutralizing antibodies in a high number of different avian species has shown that WNV seroprevalence was higher in migratory birds and in larger species (see Figure 1) even after controlling for differences in the age of individuals (Figuerola et al. 2008, Lopez et al. 2008); mosquitoes may find it easier to detect larger birds. A higher WNV-induced mortality in small avian species does not seem a likely explanation for such differences in seroprevalence (Figuerola et al. 2008). Given the large interspecific differences in seroprevalence rates, it seems advisable to focus WNV vigilance programmes on larger avian species, because the detection of WNV circulation would require the analyses of a smaller number of individuals, a smaller sampling effort and lower laboratory costs (Figuerola et al. 2008). The use of sentinel captive birds (chickens or pigeons) may help to detect and characterise the seasons of the year with higher WNV circulation (Fall et al. 2013). Capture-recapture serological analyses also provide an effective approach for demonstrating local circulation of WNV in free-li-

the ecology of West Nile
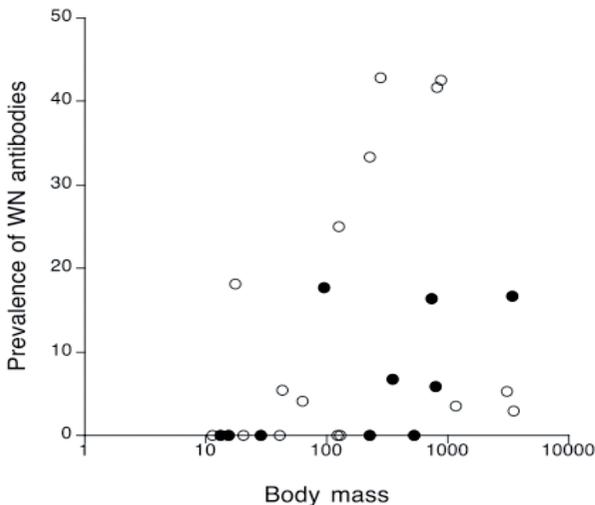# s transmission in Europe

Figure 1. Prevalence of WNV neutralizing antibodies in different avian species. The prevalence of antibodies was higher in migratory species (open circles) than in resident species (filled circles). Antibody prevalence also increased with the body mass of species. (Based on the dataset analysed by Figuerola et al. 2008).

ving wild birds (Figuerola et al. 2007). In this case, the birds provide spatially integrated monitoring of WNV circulation, while information from sentinel birds is restricted to the surroundings of cages. In particular, the common coot (*Fulica atra*) proved a good wild candidate for WNV surveillance in Europe given the high seroprevalence rate of WNV reported by different studies (Figuerola et al. 2007, Hubalek et al. 2008), and its low host competence (Komar et al. 2003).

ENVIRONMENTAL FACTORS RELATED TO WNV INCIDENCE

Analyses of the spatial and temporal distribution of WNV outbreaks or of the seroprevalence rate in different vertebrate species may provide information on the environmental factors associated with higher WNV circulation. The analyses of WNV seroprevalence in horses across Tunisia suggested that

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Engler O et al. (2013). European surveillance for West Nile virus in Mosquito populations. *International Journal of Environmental Research and Public Health* 10: 4869-4895.

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Figuerola J, Soriguer R, Rojo G, Tejedor C, Jimenez-Clavero MA (2007). Seroconversion in wild birds and local circulation of West Nile virus, Spain. *Emerging Infectious Diseases* 13: 1915-1917.

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infection risk was higher closer to wetlands, and was also related to night-time temperature and vegetation cover (Bargaoui et al. 2015). In Europe, WNV outbreaks between 2002 and 2013 were more frequent near wetland areas, in hot and wet summers and positively related to human population size (Tran et al. 2014). WNV amplification may occur in wetlands, where mosquitoes and birds are abundant, but this does not have Public Health significance when people are absent and therefore not exposed to the virus. In the end, it may be that human distribution and human presence in areas of WNV circulation are an important determinant of WNF incidence.

Recent reanalyses of WNV infection cases in humans in Europe have identified landscape and, in particular, the presence of areas characterised by a mixture of forest, human habitation, farmland and transitional habitat as an important factor related to WNF incidence (Marcantonio et al. 2015). Vector population size is also affected by environmental conditions. It has been observed that the abundance of *Culex pipiens* in south-west Spain initially increased with summer temperatures. However, mosquito abundance decreased in hotter summers (Roiz et al. 2014). Climate change may have an effect on the abundance of vectors and pathogens, but the direction of this effect depends on the balance between positive and negative effects. For example, the relationship between temperature and *Cx. pipiens* abundance in south-west Spain was not linear, and winter rainfall was also associated with its abundance. As a result, no net change in *Cx. pipiens* abundance is expected in this area given the projected increases in temperature and decreases in rainfall over the next century. However, forecasting the impact of climate change on WNV transmission risk based only on vector abundance is not straightforward. Firstly, the relationship between WNV transmission risk and environmental conditions may not be linear, nor stationary (Roiz et al. 2014). Secondly, climate may have conflicting effects on different components of WNV transmission, i.e. vector abundance and vector survival (Reiter 2008). Consequently the net effect on transmission risk is difficult to estimate.

ECOLOGY OF WNV TRANSMISSION

WNV surveillance of mosquitoes and birds has demonstrated that WNV is silently circulating in many areas in Europe without resulting in clinical cases in horses and humans. Different processes are involved in the genesis of WNV transmission to humans or horses. After WNV introduction into an area by infected birds, the virus must be amplified through an enzootic cycle involving competent species of vectors and vertebrate hosts. Not all species of vertebrates are competent hosts, and host competence differs widely between avian species (Komar et al. 2003). Consequently, amplification of WNV will be affected by the abundance and vectorial competence of the different mosquito species present, but also by the feeding behaviour of these mosquitoes. We can expect a larger circulation of WNV when and where competent mosquito species are abundant and mainly feeding on competent vertebrate hosts (Kilpatrick et al. 2006, Muñoz et al. 2012). Finally, the spillover of WNV from these usually unnoticed avian-mosquito cycles into humans and horses may involve a different vector species acting as an epidemiological bridge between infected birds and human/horse hosts. The environmental factors affecting the amplification and spillover phase may differ spatially and temporally (Kilpatrick et al. 2006, Muñoz et al. 2012) thereby affecting WNV transmission risk.

The development of universal primers for the sequencing of the COI gene in vertebrates has provided a powerful tool for the study of trophic interactions in haematophagous invertebrates (Alcaide et al. 2009). The recovered sequence can be compared with those stored in GenBank or in the Barcode of Life system to identify the mosquito's host. Several genera of mosquitoes are competent vectors for WNV, though species of the genera *Culex* are the most important vectors present in Europe (Engler et al. 2013). Based on their feeding behaviour in south-west Spain, *Cx. perexiguus, Cx. modestus* and *Cx. pipiens* were considered the main species involved in the avian-mosquito WNV cycle because these species were abundant, feed mainly on birds and are highly competent WNV vectors (see Figure 2). *Cx. perexiguus* was also important for WNV transmission to horses while WNV transmission risk in the studied area was very low and mainly due to *Cx. pipiens* and *Cx. theireli. Cx. perexiguus* seems a very important species for the amplification of WNV in southern Spain. This is a highly ornithophilic species (78% of blood meals had an avian origin) and high vectorial competence (Muñoz et al. 2012). The detection of WNV and Usutu virus in this species has further confirmed its importance for avian Flavivirus transmission (Vazquez et al. 2011). However, this species is not abundant near areas of human habitat, and does not usually feed on humans (0% of blood meals). Therefore, it probably plays a minor role as a bridge vector for the transmission of WNV to humans in south-west Spain (Muñoz et al. 2012, and unpublished information).

The analyses of blood meal origin in different mosquito species and localities confirmed that different species of mosquitoes had different intrinsic feeding preferences (i.e. mosquitoes can be classified as ornithophilic, mammophilic or herpetophilic).
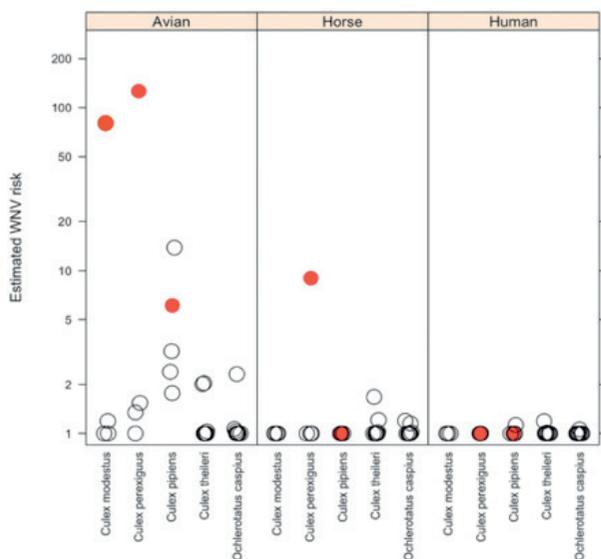
Figure 2. Estimated risk of WNV transmission by different mosquito species in different localities. Transmission risk is calculated as the product of vector abundance, vector competence, fraction of mosquito females feeding on competent hosts (birds) and the fraction of mosquito females feeding in the group of interest (birds, horses or humans). Filled circles indicate the cases where WNV has been detected in the mosquito pools. The figure has been redrawn from Muñoz et al. (2012) original data, updated with new virus detection information and the risk values calculated considering that chickens are not competent hosts for WNV.

However, there are important differences in blood meal origin between localities. Muñoz et al. (2012) have estimated that the probability of finding blood of avian origin in a blood-fed female mosquito is explained by mosquito species (49-65% of explained variation) and locality (30-50%). Given that not all vertebrates are competent hosts for WNV, this results in important differences between localities in the potential for 1) WNV amplification through mosquito-mediated bird to bird transmission and 2) risk of transmission to humans through mosquito-mediated bird to human transmission (Kilpatrick et al. 2006, Muñoz et al. 2012). Unfortunately, no information is currently available on the causes of this local variation in blood meal composition. Differences in vertebrate abundance might explain most of these differences, but ornithophilic and mammophilic species may respond differently to changes in vertebrate community composition. However, very little is known on the functional response of mosquitoes to changes in vertebrate availability, although it is important to highlight that human effects on landscape have important effects on biodiversity distribution.

Recently, an important debate has opened up on the potential role that biodiversity may have in reducing the risk of zoonotic diseases (Randolph and

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Dobson 2012). Although some models support the likelihood of the dilution mechanisms (Roche et al. 2011), it is important to note that the conclusions of these models strongly depend on the assumptions made on the interactions between vectors and hosts. Consequently, until more basic information is available on the interactions between vector and vertebrate distribution and abundance it is difficult to determine if the dilution effect is a general effect of biodiversity or a particular case that may only happen for some pathogens under some environmental conditions. Indeed, understanding the effects of this biodiversity on virus transmission and the impact of anthropogenic habitat transformations may greatly enhance our capacity to predict and manage the risk of WNV outbreaks in the future.

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PUBLICise Health Public Health Telegram: Mosquito-borne diseases

EDENext's Public Health Telegrams (PUBLICise Health) were established to inform interested individuals and institutions about research results on vector-borne diseases with direct or indirect impact on Public Health issues. Edited and produced by EDENext's Public Health team, each issue concentrates on the work of one of the project's five specialist groups. Here we present an updated version of the issue dedicated to the work of the team examining mosquito-borne diseases, first published in March 2014

1. New adhesive traps to monitor urban mosquitoes with a case study to assess efficacy of insecticide control strategies

An Italian research team has assessed the potential of three adhesive traps for passive monitoring of urban mosquito adult abundance and seasonal dynamics, and for assessing the efficacy of commonly used control measures. They note that urban mosquitoes in temperate regions may have a high nuisance value and are associated with the risk of arbovirus transmission. In highly infested urban areas in Italy, for example, this leads to calendar-based larvicide treatments of street catch basins – the main non-removable urban breeding site – and/or insecticide ground spraying. The planning of these interventions, as well as the evaluation of their effectiveness, rarely benefits from adequate monitoring of mosquito abundance and dynamics. Therefore the team designed two novel adhesive traps to monitor *Aedes albopictus* and *Culex pipiens* adults visiting and/or emerging from catch basins in order to evaluate the efficacy of insecticide-based control strategies. The mosquito emerging trap (MET) was used to assess the efficacy of larvicide treatments. The catch basin trap (CBT) was used together with the sticky trap (ST, commonly used to collect ovipositing / resting females) to monitor adult abundance on the campus of the University of Rome 'Sapienza', where catch basins had been treated with insect growth regulators (IGR) bi-monthly and low-volume insecticide spraying was carried out before sunset, and in a nearby control area. Their results from MET showed that, although

all monitored diflubenzuron-treated catch basins were repeatedly visited by *Ae. albopictus* and *Cx. pipiens*, adult emergence was inhibited in most basins. Results obtained by ST and CBT showed a significant lower adult abundance in the treated area than in the untreated one after the second adulticide spraying, which was carried out during the major phase of *Ae. albopictus* population expansion in Rome. They conclude that all three adhesive traps have potential for passive monitoring of urban mosquito adult abundance and seasonal dynamics, and in assessing the efficacy of control measures.

Caputo B, Ienco A, Manica M, Petrarca V, Rosà R and della Torre A (2015). New adhesive traps to monitor urban mosquitoes with a case study to assess the efficacy of insecticide control strategies in temperate areas. *Parasites & Vectors* 2015, 8: 134. Doi:10.1186/s13071-015-0734-4

2. West Nile virus in Europe: knowledge gaps and research priorities

A joint initiative from European experts on West Nile virus (WNV) has seen them analyse 118 scientific papers published between 2004 and 2014 to provide the state of the art on knowledge on WNV. Their work highlights the existing knowledge and research gaps that need to be addressed as a high priority in Europe and neighbouring countries. Noting the continual spread of WNV both in Europe and other regions, they point out that the high genetic diversity of the virus, with remarkable phenotypic variation, and its endemic circulation in several countries, requires an intensification of the integrated and multidisciplinary research efforts built under the 7th Framework Programme. It is important, they say, to better clarify several aspects of WNV circulation in Europe, including its ecology, genomic diversity, pathogenicity, transmissibility, diagnosis and control options, under different environmental and socio-economic scenarios. They add that identifying WNV endemic areas, as well as infection-free areas, is becoming necessary for the development of human vaccines and therapeutics, and for the application of blood and organ safety regulations.

Rizzoli A, Jiménez-Clavero MA, Barzon L, Cordioli P, Figuerola J, Koraka P, Martina B, Moreno A, Nowotny N, Pardigon N, Sanders N, Ulbert S and Tenorio A (2015). The challenge of West Nile virus in Europe: knowledge gaps and research priorities. *Euro Surveill.* 2015;20(20):pii=21135

3. The effects of climate change on mosquito abundance in Mediterranean wetlands

Noting that the impact of climate change on vector-borne diseases is highly controversial,

an EDENext team has tackled one of the principal points of debate, investigating whether or not climate influences mosquito abundance, a key factor in disease transmission. Researchers analysed 10 years of data (2003–2012) from bi-weekly surveys to assess inter-annual and seasonal relationships between the abundance of seven mosquito species known to be vectors of various pathogens, and several climatic variables in two wetlands in south-west Spain. They report that within-season abundance patterns were related to climatic variables (i.e. temperature, rainfall, tide heights, relative humidity and photoperiod) that varied according to the mosquito species in question. Rainfall during winter months was positively related to the annual abundance of *Culex pipiens* and *Ochlerotatus detritus*. Annual maximum temperatures were non-linearly related to annual *Cx. pipiens* abundance, while annual mean temperatures were positively related to annual *Ochlerotatus caspius* abundance. The team then modelled shifts in mosquito abundance using temperature and rainfall climate change scenarios for the period 2011–2100. They note that while *Oc. caspius*, an important anthropophilic species, may increase in abundance, no changes are expected for *Cx. pipiens* or the salt-marsh mosquito *Oc. detritus*. Their results highlight that the effects of climate are species-specific, place-specific and non-linear and that linear approaches will therefore overestimate the effect of climate change on mosquito abundances at high temperatures. Climate warming does not necessarily lead to an increase in mosquito abundance in natural Mediterranean wetlands, they say, and will affect, above all, species such as *Oc. caspius* whose numbers are not closely linked to rainfall but are influenced by local tidal patterns and temperatures. They conclude that the final impact of changes in vector abundance on disease frequency will depend on the direct and indirect effects of climate and other parameters related to pathogen amplification and spillover on humans and other vertebrates.

4. The effect of landscape on mosquito populations in Mediterranean wetlands

An EDENext study by Roiz et al. has shown that landscape significantly affects mosquito

distribution and abundance, and as a result may alter disease risk. The team analysed the relationship between environmental variables estimated by remote sensing and spatial distribution (presence, abundance and diversity) of seven mosquito species which are vectors of West Nile and other pathogens (Usutu, avian malaria and dirofilariasis) in the Doñana Natural Park, Spain. They sampled 972,346 female mosquitoes, the most abundant species being *Culex theileri, Ochlerotatus caspius, Culex modestus, Culex perexiguus, Culex pipiens, Anopheles atroparvu*s and *Ochlerotatus detritus*. Their results suggest that: (1) hydroperiod, inundation surface and NDVI are strongly related to the spatial distribution of mosquitoes; (2) the spatial scales used to measure these variables affects the quantification of these relationships, the larger scale being more informative; (3) these relationships are species-specific; (4) hydroperiod is negatively related to mosquito presence and richness; (5) *Culex* abundance is positively related to hydroperiod; (6) NDVI is positively related to mosquito diversity, presence and abundance, except in the case of the two salt marsh species (*Oc. caspius* and *Oc. detritus*); and (7) inundation surfaces positively condition the abundance and richness of most species except the salt marsh mosquitoes. They conclude that while environmental conditions affect the distribution and abundance of mosquitoes, other factors such as human modification of landscapes may give rise to significant changes in mosquito populations and consequently disease risk.

Roiz D, Ruiz S, Soriguer R and Figuerola J (2015). Landscape Effects on the Presence, Abundance and Diversity of Mosquitoes in Mediterranean Wetlands. *PLoS ONE* 10(6): e0128112. Doi: 10.1371/journal.pone.0128112

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Ecology and eco-epidemiology of *Culicoides*-borne diseases:

what have we learnt and where do we go from here?

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INTRODUCTION

Culicoides biting midges are abundant haematophagous flies capable of acting as vectors for arthropod-borne viruses of veterinary and medical importance. The past two decades have witnessed dramatic changes in the epidemiology of *Culicoides*-borne viruses, including the emergence of exotic viruses in northern temperate regions, increases in global disease incidence and enhanced virus diversity in tropical zones. The drivers of these changes may include altered climate, land use, trade and animal husbandry practices. During this time, several new *Culicoides* species and new wild reservoir hosts have been implicated in disease transmission, highlighting the dynamic nature of pathogen-vector-host interactions and the importance of understanding how the wider natural and agricultural environment influences these processes. Throughout EDENext, the *Culicoides* group has sought to enhance the evidence base for effective disease policy and management by engaging in a wide range of empirical and model-based research activities. Here, we summarise the main findings of the *Culicoides* group in relation to vector behaviour, life history traits, population dynamics and phenology. We then discuss how this enhanced understanding has stimulated a suite of methods aimed at understanding the consequence of midge vector biology for *Culicoides*-borne disease spread and control across Europe. Finally, we identify the key knowledge gaps hindering further refinement of models and improved implementation of disease policy.

VECTOR BEHAVIOUR, LIFE HISTORY TRAITS, POPULATION DYNAMICS AND PHENOLOGY

In response to the recent emergence of bluetongue virus (BTV) and Schmallenberg virus (SBV), researchers within EDENext have studied the behaviour, life history, phenology and population dynamics of *Culicoides* species across Europe and North Africa.

The EDENext *Culicoides* group collaborated with international colleagues outside the project to provide a comprehensive review of the bionomics of *Culicoides* in relation to the transmission of *Culicoides*-borne diseases (Purse et al. 2015). This review focused on potential vector species worldwide and key elements of vectorial capacity, and assessed the sensitivity of *Culicoides* life cycles to abiotic and biotic factors. It also considered the implications for designing control measures and understanding

the impacts of environmental change in different ecological contexts. Finally, critical geographical, biological and taxonomic knowledge gaps were prioritised. These knowledge gaps, along with others identified within the EDENext *Culicoides* group, are discussed in the conclusions of this article.

A critical aspect of providing an evidence base for informing disease management and policy involves assessing how aspects of *Culicoides* vector capacity vary across Europe and North Africa. The EDENext *Culicoides* group assessed vector competence for several *Culicoides* vectors and viruses, for instance investigating the competence of *C. imicola* with three BTV serotypes (BTV-8, BTV-4 and BTV-1). The EDENext *Culicoides* group developed standardised methods for comparative estimates of vector susceptibility to infection and onward transmission potential. One aspect of this work involved significant advances in feeding techniques for North-Western Palaearctic species (*C. imicola,* Obsoletus group species) thereby targeting key potential vectors of orbiviruses. Our group developed novel methods using the Hemotek feeder to improve comparison between sites and countries, allowing the first multi-country comparison of feeding rates in *Culicoides*. These methodological advances were transferred to Senegal and applied to *C. oxystoma,* an important Afrotropical vector species. Importantly, these methodological advances in feeding methods allowed crucial vector competence assessments, standardised across countries, which can be used in disease transmission studies, bionomics and genomics experiments, thereby comprising a vital prerequisite for understanding of *Culicoides*-borne arboviruses.

To investigate existing prevalence of key *Culicoides*-borne arboviruses across our study region, the EDENext *Culicoides* group screened field collected individual *Culicoides* from virus epidemic areas. In Denmark, 200 pools of *Culicoides* (comprising 10 to 50 individuals) were screened for BTV and 300 for SBV in 2012, demonstrating high prevalence between July to September across the whole country (Rasmussen et al. 2014). Similarly, in France pools of 50 *Culicoides* were screened for SBV across the national entomological surveillance network in 2011; and in Senegal seroprevalence was assessed for nine serotypes of African Horse Sickness (AHSV), demonstrating seroprevalance of 65% for AHSV-9 and 60% for AHSV-2 based on serosurveys of donkeys in 2008 (Fall et al. 2015).

Key life history parameters were also empirically assessed for *Culicoides* vector species. Parallel studies in France, Spain and the UK investigated how fecundity, oogeneis and survival within vector species varied in relation to environmental parameters. Understanding how these life history traits vary by species and environment is a key component to building population and epidemiological models capable of predicting how orbiviruses may spread across landscapes in diverse geographic regions.

The *Culicoides* group also combined *Culicoides* surveillance data from the UK and Spain to develop two modelling frameworks for predicting the maximum abundance and seasonal abundance of five key *Culicoides* vector taxa (*Culicoides obsoletus* complex*, C. pulicaris, C. imicola, C. impunctatus, C. newsteadii*) for sites across Western Europe. The first method estimated the effect of environmental covariates (wild and domestic host densities, meteorological variables and land cover) on the maximum abundance of *Culicoides* at each site. This modelling framework was used to generate predictions at new sites across Western Europe falling into similar environmental spaces as those used to train the model. The second method estimated the seasonal abundance of *Culicoides* (*C. obsoletus* complex*, C. pulicaris, C. imicola,*

Night trapping in Denmark.
© *Carsten Kirkeby, Danish Technical University*

C. impunctatus, C. newsteadii) using the surveillance datasets from the UK and Spain containing weekly trapping data from the UK and Spain for approximately 400 sites spanning multiple years from 2005 to 2010. This modelling methodology was used to generate predictions of seasonal activity at new locations across Western Europe, properly accounting for uncertainty in model fitting and parameter estimates using Bayesian methods. In western Senegal, the seasonal dynamics of *Culicoides* vector species were empirically assessed and modelled. The temporal dynamics of the four dominant species, including *C. imicola* and

C. oxystoma, were analysed over 12 months (July 2011-July 2012) using horse-baited and light traps. A cross-sectional study was also conducted with the national veterinary services in Senegal to model the spatial distribution of key vector species across 108 sites and 36 districts (Diarra et al. 2014).

In the UK, the EDENext *Culicoides* group used five years of *Culicoides* surveillance data from national surveillance activities to assess how the phenology of individual species varied geographically and seasonally (Searle et al. 2014). This work demonstrated significant inter-specific differences in *Culicoides* adult phenology, the most notable of which related to the seasonal activity of one species, *Culicoides scoticus,* being approximately eight weeks shorter than that of *Culicoides obsoletus*. The work also demonstrated important species-specific differences in the length of the seasonal vector-free period (SVFP), which were related to host density and local variation in landscape habitat. There was evidence that the current treatment of *Avaritia Culicoides* as a single group in surveillance work inhibits understanding of environmentally-driven spatial variation in species phenology and hinders the development of models for predicting the SVFP from environmental factors.

Importantly, the study demonstrated that active surveillance of haematophagous female *Culicoides* vector populations cannot currently be replaced using remote environmental models of abundance. This failure was most likely related to the diverse ecology of species conflated within this taxonomic grouping. It was found that the timing of the end of the season was the most difficult to forecast, and should perhaps be treated with more caution by policy makers than the beginning of the season, because it varies widely between species, years and locations in response to environmental heterogeneity. To rectify this concern, the study recommended that intensive trapping should take place across a range of climatic zones with species-level identification of *Culicoides* females wherever feasible to facilitate more accurate detection and understanding of the start of the vector-free period in temperate zones.

SPATIO-TEMPORAL DYNAMICS OF VBD SPREAD AND CONTROL

Within EDENext, several group members studied the effect of direct control methods on *Culicoides*. Most of the common insecticidal products authorised in the EU for use in domestic animals are based on pyrethroids. The products are used topically as pour-on solutions, which provide efficacy for at least 4-8 weeks. Other insecticides are used for dipping or spraying. To date, few attempts have been conducted to apply chemical control of *Culicoides* spp. in an area-wide approach and there is currently no veterinary product on the European market authorised for the control of *Culicoides*.

Within EDENext, we conducted coordinated research at laboratory and field level for developing standardised control methods for *Culicoides*. The main aim of this work has been to provide an evidence base for the development of control practices that will reduce the rate of biting by *Culicoides* on animals. As a result of this work, our group has provided data on resistance to insecticides, such as permethrin and deltamethrin, for *C. obsoletus* and *C. imicola* from France, Spain, Senegal and South Africa (Venail et al. 2011, 2015a, b, c). Field evaluation of the efficacy of the ZeroFly® mosquito net (a commercial deltamethrine-impregnated net) was conducted in Senegal. This experiment demonstrated that the number of *Culicoides* captured (all species combined) was not significantly different between control and treatment nets showing that the impregnated net had no significant effect on the capture, instantaneous and delayed mortality rates of *Culicoides*. However, results did vary by *Culicoides* species, with larger bodied species suffering a greater negative impact than smaller species.

The group also assessed the impact of vector control using a UK data-driven *Culicoides* mechanistic population model to explore the impact of vector control on seasonal vector abundance. This mechanistic simulation population model for *Culicoides obsoletus* explicitly links environmental variation with life-history processes, such as development time, fecundity and mortality, and predicts the seasonal abundance of adults. Mechanistic vector dynamics are important as they affect disease seasonality and are affected by environmental variability. Building upon this model framework, the group investigated the efficiency of alternative timing and intensity of vector control; focusing on a control strategy that targets host-seeking adults that come into contact with insecticide, akin to the insecticide-treated nets used for the protection of sheep and cattle and evaluated by the CBD group in Del Rio et al. (2014).

This work demonstrated that for significantly high control efficiencies (a combination of mortality rate of insects and the proportion of the population that contact the treated hosts), we may eradicate the *Culicoides* population. However, for low control efficiencies, there is only a small reduction of the average annual *Culicoides* abundance. Importantly, for intermediate control efficiencies the average annual *Culicoides* abundance was markedly reduced but the peak autumn abundance was greatly increased. This was because pre-adults were released from density-dependent competition due to the decrease in population in the spring peak. As a consequence, although the control strategy had a desirable effect on the whole, the increase in peak autumn abundance may have dramatic consequences for the reproductive value for a disease (R_0, the number of new disease cases generated by one case) in this warmer period and the prevalence and seasonal persistence of midge-borne diseases.

This work also considered the seasonal timing of vector control. Here, the insecticide was present at a constant level for only a limited time in the year (50 days) and the starting day in the year at which the control window started was varied. When the control window was centred over the spring abundance peak (approximately 100th starting day), the spring peak was reduced. However, the knock-on effect was a markedly increased peak in autumn abundance. Furthermore, the mean abundance throughout the year also increased. This was in contrast to windows centred over the autumn peak, which markedly reduced the midge population in the control year but also reduced the subsequent spring population, because fewer individuals entered diapause.

In summary, vector control strategies with differing efficiencies and timing may either successfully reduce the vector population, or deleteriously increase the vector population. Control efforts should concentrate on reducing the autumn peak, as this will reduce the midge population at the peak, as well as on average through the year and in subsequent years. However, the precise timing is crucial for effective control, especially if the strategy is used year upon year, since late autumn control may increase subsequent late summer populations. Key knowledge gaps in predicting the outcome of vector control include the proportion of the adult population visiting the treated hosts in different ecological settings, the rate of decay of impact of nets or treatments on mortality as well as diapause triggers and levels of density dependent competition.

In other work, our group also considered the design of optimal prevention and control strategies for *Culicoides*-borne diseases. The independent or combined impact of vaccination and vector control on the reproductive value for a disease (R_0) will vary seasonally, geographically and between years with different meteorological conditions. EU Member States are presently not able to determine the necessary protective levels of vaccination cover, vector control efforts or movement restrictions based on objective calculations of R_0 or derived indicators for each country or ecosystem. Calculations of R_0 for within- and between-herd spread will allow stakeholders and Member States to adjust the intensity of preventive strategies as well as outbreak control measures to the predicted level of transmission in each country or ecosystem at various meteorological conditions.

Within EDENext, we have developed statistical and mechanistic simulation spread models simulating virus transmission within herds and between herds to facilitate the development of optimal prevention and control strategies. We developed a set of representative outbreak scenarios for various geographical areas, seasons and meteorology including average weather conditions and 90% extremes. This set of outbreak scenarios was used to run different spread models and formed the basis of the model comparison. We then identified spatial and temporal levels of vaccination and vector control that were necessary to keep R_0 below one, thereby preventing spread of the disease, which have been presented as guidelines for control of *Culicoides*-borne diseases in Europe.

Our group also assessed the effectiveness of alternative vaccination strategies on the potential spread of the Schmallenberg virus (SBV) at the landscape scale in Scotland. Here a stochastic mathematical model of SBV spread was used to identify the optimal method for deployment of a vaccine, under different regimes of current and changing temperatures, and in scenarios where *Culicoides* show a marked or no preference for feeding on cattle over sheep. Targeting strategies for vaccination included ad hoc strategies, where farmers could voluntarily take up vaccination, versus tactical strategies such as vaccination of only cattle, only breeding replacement stock, or only in the south of the country to break the transmission chain and reduce overall disease spread (Bessell et al. 2014). This analysis demonstrated that vaccine impact was optimised by targeting high risk areas in the south of Scotland, or by vaccinating only cattle. Importantly, given the recent rise in average temperatures experienced in the UK, the results demonstrated that at higher than average temperatures, and hence increased transmission potential, the relative impact of vaccination was considerably enhanced. Vaccine impact was also enhanced if vectors fed preferentially on cattle. These findings are of considerable importance when planning control strategies for SBV and also have important implications for management of other arboviruses such as bluetongue virus. Critically, environmental determinants and feeding preferences should be researched further to inform development of effective control strategies.
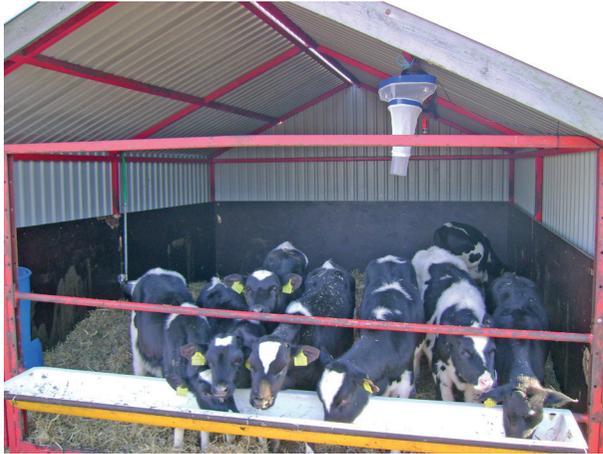
Trapping in Denmark. © René B Bødker, Danish Technical University

In other group work, we developed a spread model (Græsnøll et al. 2014) and used it to compare different preventive vaccination strategies for bluetongue virus (BTV) in Denmark, determining that the most cost-effective vaccination strategy was to vaccinate cattle on pastures (Græsbøll et al. 2014). The group also undertook an intensive study in Denmark to quantify dispersal of vectors between farms (Kirkeby et al. 2013), a key parameter in *Culicoides*-borne disease spread models. In France, Pioz et al. (2014) investigated the effect of vaccination on the propagation of bluetongue virus based on data from the 2008 bluetongue virus serotype 1 of southwest France. The average estimated velocity across the country, despite restrictions on animal movements, was 5.4km/day, which is very similar to the velocity of spread of the bluetongue virus serotype 8 epizootic in France. In comparison to municipalities with no vaccine coverage, the velocity of BTV-1 spread decreased by 1.7km/day in vaccinated municipalities. Our findings emphasise the importance of vaccination in limiting disease spread across natural landscapes. Environmental factors, specifically those related to *Culicoides* abundance and activity, were found to be good predictors of the velocity of BTV-1 spread, indicating that these variables need to be adequately accounted for when evaluating the role of vaccination on bluetongue spread.

The CBD group also collaborated with the EMIDA VICE project to model the monthly mean abundance of *Culicoides* vectors across Western Europe. *Culicoides* surveillance data from Norway, Sweden, Denmark, Germany, Poland, Austria, Switzerland, France and Spain were used to generate the monthly outputs. These predictions were then used to generate monthly Europe-wide R_0 prediction. These outputs showed R_0 values exceeding '1' for most of Europe for several months a year, suggesting that prevention and control of transmission can only be achieved when peak transmission intensity is reduced by more than 90%. As such, reductions in vector abundance or vector contact in the magnitude of 50% will only have limited impact in most areas during the peak transmission period in Europe. In

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conclusion, the reduction in transmission intensity needed for prevention and control of *Culicoides*-borne bluetongue virus and Schmallenberg virus in European ruminants is likely to be so high that for all practical purposes vaccination is currently the only practical solution.

CONCLUSIONS

Research on *Culicoides* lags behind that on most other vector groups, primarily because of their minute size, the lack of colonies of primary vector species and, until recently, the relatively low impact of *Culicoides*-borne animal disease agents in developed countries.

Our work has identified techniques that can reduce *Culicoides* survival and their attack rates on livestock, however these techniques work best for species whose ecology has been well characterised. Moreover, because many regions have multiple potential vectors with widely varying ecologies and diverse habitats, many of these techniques will only be effective locally and are unlikely to control widespread outbreaks.

Long-term *Culicoides* surveillance using trapping is currently restricted to BTV-epidemic regions of Western Europe. This surveillance can track the seasonal abundance and distribution of *Culicoides* vectors aiding in the demarcation of seasonally vector free areas for relaxation of animal trade and movement restrictions. However, the current treatment of the *Avaritia Culicoides* as a single group inhibits understanding of environmentally driven spatial variation in species phenology and hinders the development of models for predicting seasonally vector free periods from environmental factors. As such, we recommend that *Culicoides* surveillance methods should be adapted to focus on concentrated assessments of species-specific abundance during the start and end of seasonal activity in temperate regions to facilitate refinement of ruminant movement restrictions, thereby reducing the impact of *Culicoides*-borne arboviruses.

There remain key knowledge gaps in relation to vectoral capacity parameters for *Culicoides*. These parameters have complex interactions that ultimately determine how diseases are spread. Improved mathematical models must be developed with empirically derived parameters from laboratory and natural environments, and confronted with empirical data from disease outbreaks to create robust and accurate models for predicting virus transmission and spread.

Finally, we know that the landscape context alters disease transmission and spread, particularly through its influence on the abundance and interactions of *Culicoides* vectors with wild and domestic hosts. For instance, short-range upwind flight of *Culicoides* has been shown to be critical in explaining epidemic spread, however an understanding of how short-range midge movements are modulated by landscape and topography is currently lacking. Similarly, recent work has implicated wild ruminant hosts as playing an important role in disease spread, however little is known about how the abundance of wild ruminants affects the behaviour and distribution of vectors.

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PUBLICise Health Public Health Telegram: *Culicoides*-borne diseases

EDENext's Public Health Telegrams (PUBLICise Health) were established to inform interested individuals and institutions about research results on vector-borne diseases with direct or indirect impact on Public Health issues. Edited and produced by EDENext's Public Health team, each issue concentrates on the work of one of the project's five specialist groups. Here we present an updated version of the issue dedicated to the work of the team examining *Culicoides*-borne diseases, first published in August 2014.

1. The impact of biting midges on human Public Health in Europe

The EDENext study by Carpenter et al. (2013) considers the impact of biting midges of the genus *Culicoides* on human health in Europe. Special focus is placed on their potential for transmitting human pathogenic arboviruses. To date, the only arboviruses identified as being primarily transmitted by *Culicoides* between humans are Oropouche virus (OROV) and the Iquitos virus (IQTV), although the latter has not yet been confirmed. These two members of the family *Orthobunyaviridae* cause major epidemics of febrile illness in human populations of South and Central America and the Caribbean. The authors describe factors promoting sustained outbreaks of OROV in Brazil from an entomological perspective and assess areas of the epidemiology of this arbovirus that are currently poorly understood, but may influence the risk of incursion into Europe. Additionally, evidence implicating *Culicoides* in the transmission of other zoonotic infections is examined and placed in context with the presence of other vector groups in Europe.

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2. *Leishmania* vector competence of biting midges

Leishmaniases are diseases caused by various species of the trypanosomatid protozoa genus *Leishmania*. They can cause various symptoms, ranging from a cutaneous form that leads to cutaneous ulcera at the bite site to a highly

pathogenic visceral form. Millions of human cases are reported from Asia, Africa, South and Central America as well southern Europe every year. Female biting sand flies of the genus *Phlebotomus* in the Old World or *Lutzomyia* in the New World are currently the only known vectors of *Leishmania* sp. Recent observations of Australian midges transmitting Leishmania may indicate that biting midges might also play a role as potential vectors. To investigate this theory, Seblova et al. (2012) experimentally infected the colonized biting midge species *Culicoides nubeculosus* with two human pathogenic *Leishmania* species (*L. infantum* and major). An early stage of *Leishmania* development was demonstrated in the midge mid-gut until two days after feeding, but a subsequent loss of parasites occurred indicating that *C. nubeculosus* is unlikely to act as a competent vector.

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3. *Culicoides* biology and ecology in Senegal

A major area of research in EDENext has been in conducting the first systematic surveys of *Culicoides* in Senegal using a wide range of monitoring techniques. In addition to providing detailed phylogenetic analyses (Bakhoum et al. 2013) both spatial and temporal surveys of *Culicoides* on livestock were successfully completed in a region where African horse sickness virus is of high epidemiological importance (Fall et al. 2015a; Fall et al. 2015b; Fall et al. 2015c; Fall 2015d; Diarra et al. 2014; Diarra et al. 2015). These studies are also of Public Health interest in providing baseline data regarding *Culicoides* populations and their overlap with human populations. In some areas of central Africa, certain biting midge species may act as a huge nuisance for human populations and are able to transmit human filarial nematodes. It is highly likely that future studies will examine the impact of *Culicoides* in transmission of human pathogenic arboviruses within Africa following the implementation of virus discovery via next-generation sequencing and these studies provide a strong baseline for these approaches.

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Diarra M, Fall M, Fall AG, Aliou Diop A, Seck MT, Garros C, Balenghien T, Allène X, Rakotoarivony I, Lancelot R, Mall I, Bakhoum MT, Dosum AM, Ndao M, Bouyer J and Guis H (2014). Seasonal dynamics of *Culicoides* (Diptera: Ceratopogonidae) biting midges, potential vectors of African horse sickness and bluetongue viruses in the Niayes area of Senegal. *Parasites & Vectors* 2014, 7:147. Doi: 10.1186/1756-3305-7-147.

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EXAMPLES
OF EDEN AND
EDENEXT IMPACTS

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Four kinds of

INTRODUCTION

In this chapter we provide information regarding the impacts, in other words the consequences, of the implementation of EDEN and EDENext, both on the targeted scientific community and on the more general public, in other words citizens in countries where these projects conducted their activities.

ACADEMIC IMPACT

For research projects, important impact indicators are the number and quality of articles published in international, peer-reviewed journals. In this respect, EDEN and EDENext have been quite successful, with 470 papers released as of mid-June 2015, and 100 more papers already submitted and awaiting publication. Moreover, the quality of these papers fully complies with international standards: indeed, their median impact factor[1] is 3.1 (fig. 1), with an inter-quartile range of [2.4; 3.5], and outliers of up to more than 40 (Bhatt et al. 2013).

Though EDEN ran for a longer time than EDENext, these statistics noticeably improved from the former to the latter. For example, the number of papers rose from 207 to 263, and their median impact factor[1] increased from 2.6 to 3.3: good progress.

The most used journals are shown in Figure 2a. While the two most popular journals (*Parasites & Vectors* and *Vector-Borne and Zoonotic Diseases*, totalling more than 16% of published papers) are specialised in the domain of vectors and vector-borne infections, this is not the case for many of the others. Indeed, journals from the PLoS family, as well as *Eurosurveillance and Emerging Infectious Diseases*, are more general in their scope, with a strong orientation towards Public Health in the case of the latter two. This is a first indication of the special effort made by scientists involved in EDEN and EDENext to have a positive impact on Public Health.

In this respect, the most important findings with an actual or potential effect on Public Health are outlined in the vector group sections of this book. Figure 3 presents a brief summary of the topics covered by EDEN and EDENext papers. These two panels were produced using the word cloud method employed by sociologists to summarise focus group discussions. It was used by the EDENext Public Health group for their work on hantavirus risk perception among high-risk populations (for example, forestry workers) (Dressel and Schülle 2014). Figure 3a emphasises that the *transmission* of vector-borne infections in animal and human populations
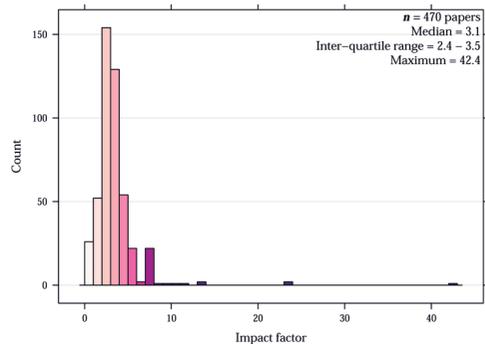
Figure 1. Distribution of impact factors for papers approved by EDEN and EDENext steering committees

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[1] Metric available on Thomson Reuters' Web of Science

was addressed in many papers. Indeed, most vector-borne infections are zoonotic, in other words common to animals and humans. The most cited pathogens belong in this category: hantaviruses, tick-borne encephalitis virus (TBEV), West Nile virus and some *Leishmania* species (for example, *L. infantum*). Moreover, though many publications focused on European ecosystems, Africa is well represented too. For instance, Senegal is involved in 18 papers and Morocco in 12, though the latter country was only represented in EDEN.

Figure 3b shows the words that better differentiate EDEN and EDENext. In EDEN, major attention was paid to hantaviruses and TBEV. For the latter, major advances were made in understanding its epidemiology. For instance, a brilliant demonstration was made by the tick group, led by Sarah Randolph (University of Oxford), showing that climate change could not explain the upsurge of TBE in Baltic countries (Sumilo et al. 2007). Instead, this upsurge was largely a consequence of the socio-economic transition from communism to capitalism and associated environmental changes (Godfrey and Randolph 2011). However, many concerns arose at the same time regarding the emergence of tick-borne pathogens, such as Crimean-Congo haemorrhagic fever virus (Randolph and Rogers 2010), and several *Anaplasma, Babesia, Bartonella, Borrelia,* or *Rickettsia* species (among many others
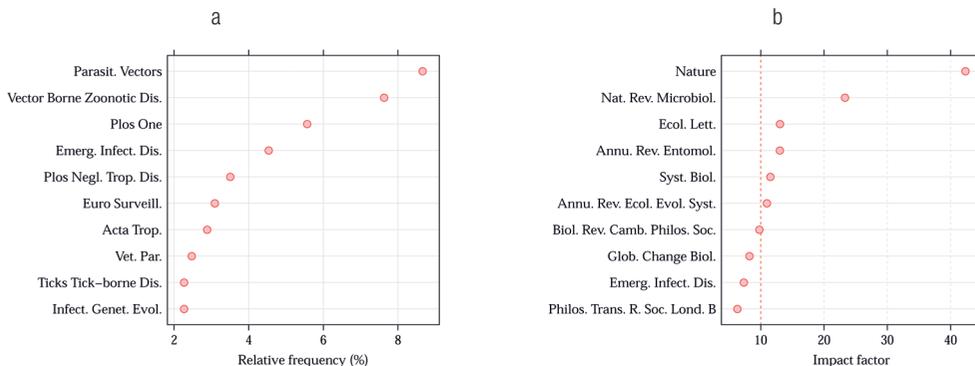
Figure 2. Top 10 journals used by EDEN and EDENext authors

see, for example, Carpi et al. 2011). Therefore, when preparing EDENext, it was decided to place an emphasis on these emerging tick-borne pathogens. The research consortium was then altered to reflect this new orientation.

Other changes in the research scope explain the different word cloud patterns between EDEN and EDENext. Regarding rodent-borne pathogens, the acquired knowledge was capitalised upon and disseminated in high-profile journals (Goeijenbier et al. 2013, Vaheri et al. 2013). In addition, the rodent group wanted to further explore the range of pathogens transmitted by rodents and less emphasis was put on hantaviruses.

Also, at the end of EDEN, we decided to stop research on the risk of malaria re-emergence in the Mediterranean Basin. Indeed, this (rather small) EDEN sub-project was quite productive, with 33 papers. The main conclusion was that though the *Anopheles* vectors are still there (so-called *'anophelism without malaria'*) the risk of malaria re-emergence is low, provided that Public Health efforts are maintained for the quick detection of autochthonous malaria cases and the adoption of appropriate measures to break the cycle in humans (epidemiological surveys and treatment of cases) and vector populations.

Some changes were also implemented in the Phlebotomine sand fly group, reorienting researches on the *Leishmania* transmission mechanisms – particularly the role of sand fly saliva (11 papers on this topic), and extending the scope of pathogens to Phleboviruses. The group was very productive, with (i) 65 publications on sand flies, *Leishmania* transmission and implications for Public Health – see, for example, Antoniou et al. 2013, Gramiccia et al. 2013, Ntais et al. 2013, and (ii) 11 publications on Phleboviruses – see, for example, Alkan et al. 2013 for a review.

Finally, a new vector group was added in EDENext: *Culicoides* biting midges, responsible for the transmission of bluetongue and African horse sickness viruses, as well as the newly emerged Schmallenberg virus. Though this group did not benefit from the 'EDEN-momentum' effect, its scientific productivity was impressive in both quality and quantity, producing 36 publications, including six for work implemented in Senegal. Moreover, many of these papers had a high impact factor, and two of them even fell into the outstanding category (Hartemink et al. 2014, Purse et al. 2015).

NETWORKING AND EXPERTISE

Improved networking is another major impact of EDEN and EDENext. We have clear evidence of this positive impact, at different levels. The first one is provided by examining the network of institutions linked by common scientific publications (Figure 4). The network looks very compact with a highly skewed distribution of degrees (number of links connecting a given institution to others).

(a) EDEN & EDENext in 60 words

(b) Comparison of EDEN & EDENext

Figure 3. Word clouds for EDEN and EDENext publications

This distribution of degrees is characteristic of a scale-free network, which is known to provide an optimal pattern for knowledge transfer (Lin and Li 2010). Therefore, institutions at the heart of the network (coloured dots in Figure 5) are in a very good position to transfer knowledge and skills to their partners in the network.

More evidence is provided by the observed changes in network indices (number of institutions, number of links, degrees, closeness, betweenness) between EDEN and EDENext (Figure 6). All these indices show a sharp increase: networking was more intense after 10 years of projects, with an obvious snowball effect aggregating many more research and Public Health institutions than those which actually received funds from the Commission in the framework of EDEN and EDENext. For instance,

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344 institutions were involved in the network presented in Figure 4, while only 58 institutions were funded in EDEN and EDENext.

Beyond research, this network was formalised and utilised to provide expertise at the European level. A 'European Network for Arthropod Vector Surveillance for Human Public Health' (VBORNET) was set up and funded by the European Centre for Disease Prevention and Control (ECDC, Stockholm), bringing together 400 medical entomologists and Public Health specialists in vector-borne infections. It was coordinated by AVIA-GIS (Belgium) during its existence between 2009 and 2014. The network was used to collect, validate and map data regarding the present distribution of main vector species of Public Health interest. Thus, maps were produced for five invasive mosquito species (Figure 6), seven endemic mosquito species, six tick species, and 10 Phlebotomine sand fly species. They are available on the ECDC website[2].
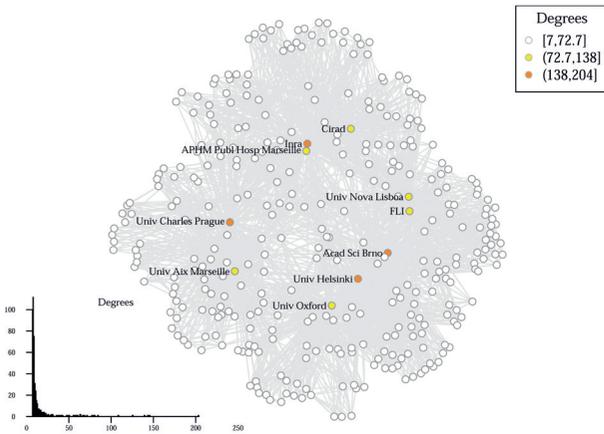

Figure 4. The EDENext network of research institutions according to their affiliations in joint publications (April 2015). Institutions are represented by dots (they are not all EDENext beneficiaries). Line segments show the links between two institutions co-signing an EDENext paper. The degree is the number of links connecting a given institution to others. Institutions with the highest degrees are labelled and represented by coloured dots. The histogram on the bottom left of the network shows the distribution of degrees.
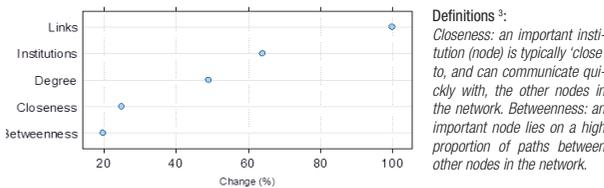

Definitions [3]:
Closeness: an important institution (node) is typically 'close' to, and can communicate quickly with, the other nodes in the network. Betweenness: an important node lies on a high proportion of paths between other nodes in the network.

Figure 5. Changes in institution network indices between EDEN (reference situation) and EDENext

[2] http://www.ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/vector-maps.aspx

[3] Definitions: https://sites.google.com/site/networkanalysisacourse/home

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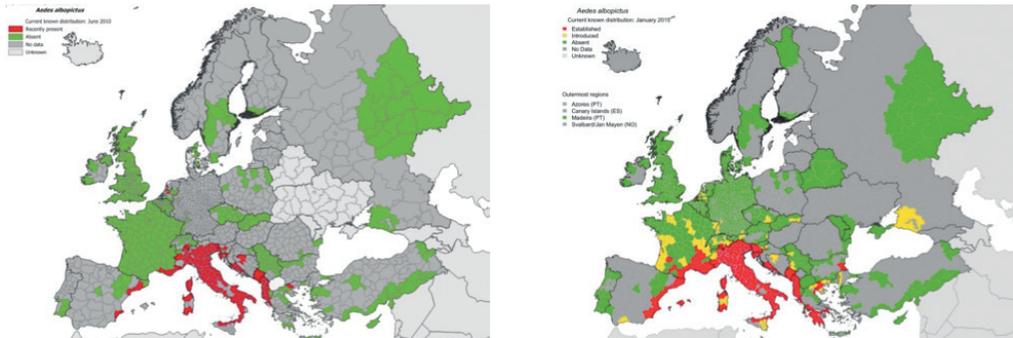
Figure 6. Distribution maps of invasive mosquito species Aedes albopictus *in Europe in June 2010 (left) and January 2015 (right)*

VBORNET has now been superseded by the 'European network for sharing data on the geographic distribution of arthropod vectors, transmitting human and animal disease agents' (VectorNet) – still coordinated by AVIA-GIS. VectorNet has a wider scope than VBORNET, encompassing medical and veterinary Public Health and economics in the same domain. It is being co-funded by ECDC and the European Food Safety Authority (EFSA) over a four-year period (2014-2018). Its geographical scope is wider, now including the whole Mediterranean Basin. It also includes vectors of veterinary importance, such as *Culicoides* biting midges.

Products of these networks are now routinely used by ECDC, EFSA and national Public Health or veterinary services to write review papers (see, for example, Braks et al. 2014, Medlock et al. 2013, Medlock et al. 2012), to feed risk analyses and, more generally, to provide information for better control of vector-borne epidemics and epizootics, with a clear 'One Health' perspective.

CAPACITY BUILDING

During EDEN and EDENext, a PhD network was established and PhD meetings were organised during each annual general meeting. On average, each of these PhD meetings was attended by around 20-30 PhD students and post-docs. These young scientists thus had the opportunity to present and discuss their work with their peers, with the gracious support of senior scientists providing constructive criticism.

Moreover, training courses were organised each year, in particular on the basic and advanced uses of geographical information systems, and spatial epidemiology and species distribution modelling (face-to-face training courses plus distance learning). Also, several workshops were organised on mathematical modelling. Finally, each vector group organised training sessions for field data collections, morphological and genetic identification of vectors, diagnostic methods, etc. Overall, we estimate that around 100 PhD students were fully or partially supported by the project, including 28 who received an EDEN (13) or EDENext (15) number for their PhD dissertation (as of June 2015).

This capacity building aspect of impact is of special importance for developing countries. Thus, we made a special effort for Senegal: four PhDs have already been obtained (Diarra 2015, Diouf 2013, Fall 2013, Fall 2015), and another (Mame T. Bakhoum) will be defended by the end of 2015. Among them, Maryam Diarra obtained the joint best poster award at the GERI 2015 International Conference (among more than one hundred competitors). Among these five Senegalese PhD students, two of them obtained a permanent position at ISRA (Dakar) after their PhD contract: Assane Fall as a medical entomologist, and Maryam Diarra as an epidemiologist. Moreover, another has already been hired by the Directorate of Veterinary Services (Nicolas Diouf). Similar successes were obtained in Morocco and Turkey.

SOCIO-ECONOMIC IMPACT

This kind of impact is the most difficult to assess, because EDEN and EDENext were not designed to have a direct socio-economic impact. However, there were at least two areas where this impact is noticeable and might be evaluated:

• **Employment:** as indicated above, about 100 PhD positions were partially or totally supported by EDEN and EDENext, including 45 in the latter. Moreover, many post-doc positions were at least partially funded by the project (a minimum of 10 for EDENext). Finally, many of these PhD students and post-docs have found a permanent position with a direct link to their work in these projects. We'll give just two examples in cases where we could trace the information back:

- Four PhD students were hired within the framework of the EDEN modelling group: all of them now have a permanent position in universities or Public Health agencies, after a few post-doc positions in excellent teams. For example, Catherine Linard completed a brilliant PhD in Louvain University (Belgium), with several very good papers (Linard et al. 2007a, Linard et al. 2009a, Linard et al. 2009b, Linard et al. 2007b, Tersago et al. 2008, Tran et al. 2008). She is now hired at Namur University (Belgium) after a post-doc at the University of Oxford (Tatem and Linard 2011).

- In Senegal, out of four students who defended their PhD thesis between 2013 and 2015, three now have permanent positions, two at the national agronomic research institute (ISRA) and one at the Directorate of Veterinary Services.

• **Technologies with commercial potential:**

- Diagnostic tests, such as the competition ELISA for CCHFV antibodies, designed and developed by several EDENext beneficiaries with FLI (Germany) as a leader and IdVet (France) as an SME associated with the project (Mertens et al. 2015).

- Integrated information systems such as Vecmap™, a spatial decision support system for targeted vector surveillance and control, designed and developed by several EDEN and EDENext beneficiaries and MEDES (Toulouse, France), led by AVIA-GIS, and co-funded by the European Space Agency. Vecmap is now routinely used in research projects (Ducheyne et al. 2015) and expertise networks such as VectorNet.

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Sharing scientific data:

1+1=3

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INTRODUCTION

"Should publicly funded data be publicly available?" According to the Science Ministers of the OECD, including most developed countries of the world, this should be the case. These Ministers signed a declaration which essentially states that all publicly funded archive data should be made publicly available (oecd.org 2004) and that there is a need for coordinated efforts at national and international level to broaden access to data from publicly funded research to the advancement of scientific research and innovation. EDEN and EDENext are both examples of publicly funded projects which demonstrated that sharing data does indeed result in the advancement of research and technological innovation. Here we discuss why these two projects succeeded in bringing the data and scientists together, we describe the added value of some of the EDEN and EDENext legacy projects, and demonstrate that this does lead to new innovative products.

WAYS TO ENABLE DATA SHARING

Researchers are often wary of data sharing, especially before publication. There are, however, a number of ways in which data can be shared without endangering publication rights. Firstly, data summaries rather than raw results can be provided, for example, for geo-referenced data administrative unit data rather than individual point information. Alternatively, a number of individual datasets can be used to produce a spatial distribution model, but only the model output is shared, rather than the training data that underpin it. An output model based on multiple datasets is likely to be better than one based on a single set of inputs, so all contributors benefit.

Another possibility is to set up a web-based archive, as exemplified by the EDEN and EDENext data sites. The technical justifications for a shared data platform, managed by data management specialists, are primarily standardisation, quality control, improved access and efficiency. One example of standardisation is typified by the diversity of human population datasets available. There are a number of authoritative sources of such data: UNPOP (Feeney 2015), Worldpop (worldpop.org.uk 2015), GRUMP (sedac.ciesin.columbia.edu 2015), Landscan (web.ornl.gov 2015), or even national censuses, all of which are updated on a regular basis. Each dataset will have slightly different numbers, different dates, different spatial and/or temporal resolutions, and consequently provide different denominators for calculation of variables such as disease incidence. If each project member uses a different denominator dataset, then individual results cannot be easily compared. Similar arguments apply to almost every kind of data: land use, satellite imagery and climate, to name but a few.

Two of the justifications for shared data platforms - quality control and improved access - are linked. Specialists are more able to find obscure sources of data and are more likely to be able to translate the sometimes arcane formats in which they are stored into something that non-specialists can deal with. During the processing needed to translate or standardise the acquired files, the specialist can also make sure the data are clean and any errors flagged or corrected. Finally, efficiency is a key factor: centralised preparation and sharing means the processing and acquisition need only be done once as opposed to each researcher doing the same thing themselves. This saves time and resources.

A more indirect way of 'sharing' data is to provide annotated lists of links indicating where data can be found. It should be noted that there are many such lists, but EDENext data is one of the most comprehensive with more than 1,500 such links, and indeed the links are the site's most visited pages.

Other platforms for data sharing are the emerging data journals and data repositories. The principle behind these is that datasets - from as little as numbers underlying graphs or maps in a publication to model outputs and to full-blown data archives - are deposited in an online open access repository, along with descriptive metadata describing the data and the methods used to generate them. This is then assigned a digital object identifier (DOI) and a citable reference with assigned authorship, in the same way as a scientific journal publication. One of the major advantages of this new way to publish is that, though the metadata document is peer reviewed, it is much quicker to produce than a full publication, because only production methods and descriptions are required and so there is no need to analyse the data or present discussions and conclusions. Further, it is perfectly acceptable to publish non-validated or negative data (which is almost impossible to publish in a research paper) provided that the metadata document accurately describes the datasets and any limitations.

The advantages of these data publications are obvious: they are easier to produce than full publications; even incomplete datasets can be given a citable reference as a publication; when the data are used they must be acknowledged in the references (no more 'Smith unpublished' or 'Jones pers comm'); they can be included as publications in a CV; they can be published prior to analyses, and, indeed, even if the analyses fail; they do not preclude publishing full papers using the data; and, of course, they allow access to the data by other researchers. The impact factors of these journals are admittedly low, certainly at the moment as these journals are not yet widely known. However, with the launch of Nature's new Scientific Data (Avants et al. 2014) it seems likely that their profile (and associated impact) will now start to increase quite rapidly.

SHARED DATA: OWNERSHIP, ACCESS AND SECURITY

An important element of data sharing at any level is that the data owners are confident that they know to whom their data is being given, and that they get due credit or acknowledgement. This relies on access to the data being managed or controlled in some way, most often by implementing some sort of user registration with user access permissions set for each dataset. This allows, for example, some datasets to be available to all registered users, whilst others are restricted to particular user groups. This arrangement is another argument for centralised data archives as potential users can be screened for access, or put directly in touch with data owners to request access. The data providers therefore retain ownership and control of their data and can, for example, decide whether direct competitors are to be given their data, and if so can negotiate the conditions under which such access is granted. Demonstrably retained ownership enhances trust in the system and promotes further sharing.

WHY DID IT WORK FOR EDEN AND EDENEXT?

EDEN and EDENext both promoted scientific collaboration at a multi-disciplinary and cross-country level. The framework was ambitious, bringing together a wide variety of scientists from different disciplines and across Europe. What was even more ambitious was to convince them to share the data and to have a commercial company host this platform.

Sharing was not only via personal interaction and capacity building, but also on a more practical level: sharing of scientific data on a common platform now amounting to more than 2,000 datasets. At the onset of EDEN the idea of data sharing seemed frightening to some, idealistic to others, while at the end of EDENext it is accepted as normal.

Seeing other partners open up their historic databases collected for various scientific purposes, ranging from molecular research to spatial modelling, inspired others to do the same thing. Trust was built within the network creating an atmosphere where people were comfortable to discuss, share and publish together. Just looking at the number of papers published (388 submitted EDENext papers up to the end of June 2015, and 269 from EDEN) and the geographical range covered by field activities (see Figure 1), it is undeniable that actively sharing information means a higher output can be achieved.

THaving Euro-AEGIS, a European Economic Interest Grouping composed of two private companies, Avia-GIS (Zoersel, Belgium) and ERGO (Oxford, UK), hosting the platform seemed to benefit all too, although this may have seemed odd in the beginning.
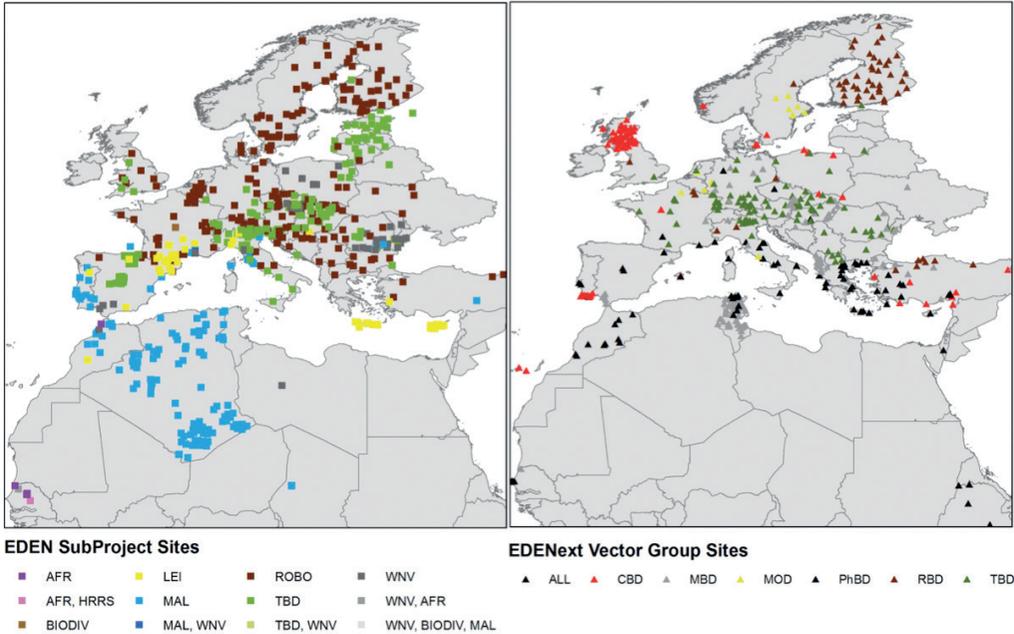
Figure 1: Map showing field sites reported in published (a) EDEN and (b) EDENext publications (status January 2015). Key to Fig 1a: AFR: Africa Platform / BIODIV: Biodiversity group / HRRS: High resolution remote sensing group / LEI: Leishmaniasis group / MAL: Mosquito-borne diseases group - malaria / ROBO: Rodent-borne diseases group / TBD: Tick-borne diseases group / WNV: Mosquito-borne diseases group - West Nile virus. Key to 1b: ALL: All vectors included in study / CBD: Culicoides group / MBD: Mosquito group / MOD: Modelling group / PhBD: Phlebotomine group / RBD: Rodent group / TBD: Tick group

Still as it turned out, the scientists could focus on research and not worry about where which data was stored and if it would remain up-to-date as these technical tasks were taken care of. In addition, Euro-AEGIS was not a competitor in terms of initiating publications and provided training in GIS and spatial modelling to help improve the content of publications. Finally, using this database provided a method to ensure that the data, collected with a lot of effort in the field or in the lab, remain available to all partners involved beyond the end of this or any other multinational and multidisciplinary project, and does not get lost in oblivion, which may even be the most important reason to build and maintain it.

LEGACY OF EDEN AND EDENEXT: CREATING NEW DATA SHARING NETWORKS

In order to be prepared for the challenges posed by vector-borne diseases (VBD), the European Centre for Disease Prevention and Control (ECDC) commissioned in 2008 an assessment of the magnitude and importance of vector-borne diseases in Europe (VBORNE). The priority vector-borne diseases were identified using both qualitative and quantitative methods, indicating that dengue and chikungunya were the highest priority. At the same time, risk analyses (see Figure 2) and fact sheets about a large number of vector-borne diseases were generated. The study concluded that one important aspect of preparedness for vector-borne diseases was the mapping of existing disease vectors and the surveillance of the introduction, establishment and spread of new disease vectors (VBORNE 2008).

Scenarios				Frequency of scenario	Risk assessment related to								
					Vertebrate Host			Invertebrate Vector			Pathogen		
Vertebrate host	Invertebrate vector	Pathogen	Human disease		Intro	Establ	Spread	Intro	Establ	Spread	Intro	Establ	Spread
1	1	1	0	M	0	0	1	0	0	1	0	0	1
1	1	1	1	H	0	0	1	0	0	1	0	0	1
1	1	0	0	L	0	0	1	0	0	1	1	1	1
1	0	1	0	H	0	0	1	0	0	1	0	0	1
1	0	1	1	N	0	0	1	0	0	1	0	0	1
1	0	0	0	H	0	0	1	1	1	1	1	1	1
0	1	1	0	L	1	1	1	0	0	1	0	0	1
0	1	1	1	L	1	1	1	0	0	1	0	0	1
0	1	0	0	L	1	1	1	0	0	1	1	1	1
0	0	1	0	L	1	1	1	1	1	1	0	0	1
0	0	1	1	L	1	1	1	1	1	1	0	0	1
0	0	0	0	H	1	1	1	1	1	1	1	1	1
1 = present					1 = relevant								
0 = absent					0 = not relevant (except for reintroduction and subsequent reestablishment)								

H – High, M – Medium, L – Low, N – Nil, NA – Not applicable

Figure 2: Example of a risk analysis table developed as part of VBORNE.

In order to further gather the available knowledge about the chikungunya/dengue vector, the TigerMaps network was established alongside the VBORNE project, bringing together a large number of specialised European entomologists focusing on the invasive mosquito *Aedes albopictus*. TigerMaps outputs included for the first time a pan-European database (see Figure 3), harmonised at the administrative unit level, not only of confirmed establishment but also of the confirmed absence (or lack of knowledge of both) of the mosquito. All these aspects are important to the study of invasive behaviour (ECDC 2009).

The VBORNE and TigerMaps temporary networks formed a crucial second stepping-stone towards the development of a large pan-European network of VBD specialists: first VBORNET, recently followed by VectorNet. Essential elements of this process were:

• Recognition of the EDEN know-how by ECDC, the largest European Public Health institute, through the successful completion of VBORNE and TigerMaps.

• Opening-up to non-EDEN experts, hence breaking the 'them-and-us' resentment, and at the same time spreading the EDEN spirit.

• Generation of factual data for the common good: both for decision makers and scientists.

• Focusing first on the dynamics of invasive mosquitoes, justifying the need for regular input from a large group of experts spread over many countries.

• Stressing the need to have a precise knowledge of the distribution of resident vectors to assess the risk of introduction of exotic pathogens.

• Solving the administrative issues related to co-funding large networks by two large EU institutes: ECDC and European Food Safety Agency (EFSA).

VBORNET was the first major expansion of the EDEN network, which integrated more than 400 entomologists from all over Europe. They were asked to define where vectors of disease can or cannot be found, while the network coordinators made sure that the information was recorded, validated and archived in a standardised way and, most importantly, made available in a usable format to the scientific and Public Health communities. This resulted in quarterly map outputs (see Figure 4) at various administrative levels for a large number of potential mosquito, tick and Phlebotomine disease vectors prioritised by ECDC.

Data related to Aedes albopictus					
Presence 2007	If yes, since	If no, estimated period of presence (before 2007)	Type of occurrence	Report of the nuisance	Source of information
Yes/no/ no data	year	year/s	Homogenous/ isolated foci	complaints/ no complaints	Publication/newspaper/personal communication

Data related to other mosquitoes				Data related to Aedes albopictus					
Surveillance/ study	Regular surveillance active since	Control	...	Surveillance	Type of surveillance	Surveillance active since	Control	Type of control programme	Organisation of control programme
yes (date)/no/ no data	year	yes/no/ no data		yes/no/ no data	active/ passive	year	yes/no	larval/ adults/ both	public/ private/ both

*Figure 3: Examples of entomological information systematically collected at the administrative unit level as part of the TigerMaps network. Entomological dataset fields related to the (a) occurrences of A*edes albopictus *and (b) surveillance and control for* Aedes albopictus *and other mosquitoes in general*

Sharing entomological data is no longer an issue for network contributors because all can benefit. Indeed, vectors do not stop at country borders and gaining access to the data is beneficial to all parties involved. The enhanced datasets generated by sharing have also provided a more robust input for spatial modelling to underpin a gap analysis to identify areas that should be a priority for extra fieldwork.

In parallel to promoting the establishment of longer-term VBD networks in Europe, ECDC also was inspired by EDEN's environmental data-sharing initiative and ensured the integration of most of its data into its European Environment and Epidemiology Network (E3) geoportal. To initiate this effort, ECDC sponsored the maintenance of the EDEN data website between the EDEN and EDENext projects and launched four dedicated proof of concept studies, showcasing a tick-borne disease (Lyme borreliosis), a mosquito-borne disease (dengue), a water-borne disease (leptospirosis) and a food-borne disease (salmonellosis), confirming the great value of such an archive within the broader concept of spatial epidemiology beyond vector-borne diseases alone (e3geoportal.ecdc.europa.eu 2015). Data from the E3 geoportal is currently being used by ECDC staff and consultants to develop risk analyses.

Finally, a further level of expansion through inter-institutional sharing was achieved when ECDC and EFSA put their institutes behind a further harmonisation of entomological and spatial databases linking Public Health and veterinary VBDs as an important step towards a One Health approach. VectorNet (ecdc.europa.eu 2015) has been set-up to not only expand to veterinary entomologists and include biting midges as an additional vector group, but also to increase the geographical range taking in territories at the fringe of Europe in the east and south. Data gaps that were earlier identified during VBORNET are now subject to targeted surveillance campaigns and will provide high-quality missing data to the current VBORNET and EFSA databases from presence/absence to abundance data to feed risk analysis studies for the introduction, establishment or spread of VBDs. To complete the circle, ECDC uses its E3 Geoportal to feed back the collected and quality assessed data to the entire community.

Vector distribution

Vector Categories: [Ticks ▲▼]
Vector Species: [Ixodes ricinus ▲▼]

NUTS0:
Andorra
Anguila*
Armenia-Hayastan
Aruba*
Azerbaijan
Belarus

NUTS1:
Région de Bruxelles-Capitale / Brussels Hoofdstede
Région Wallonne
Vlaams Gewest

NUTS2:
Prov. Antwerpen
Prov. Limburg (B)
Prov. Oost-Vlaanderen
Prov. Vlaams-Brabant
Prov. West-Vlaanderen

NUTS3:
Arr. Antwerpen
Arr. Mechelen
Arr. Turnhout

D1: Is Ixodes ricinus present: [Indigenous ▲▼]

D2: Environment: ○ Only Present In Sheltered Environment, Specify: [_____]

D3: Date first confirmed record:
○ NA ○ Don't Know ○ Date (ddmmyyyy):

<		July 2015				>
Sun	Mon	Tue	Wed	Thu	Fri	Sat
28	29	30	1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	1
2	3	4	5	6	7	8

[July ▲▼] [2015 ▲▼]

D4: Date last confirmed record:
○ NA ○ Don't Know ○ Date (ddmmyyyy):

<		July 2015				>
Sun	Mon	Tue	Wed	Thu	Fri	Sat
28	29	30	1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	1
2	3	4	5	6	7	8

[July ▲▼] [2015 ▲▼]

D5: Scientist providing information:
5.1.1 Surname (*): Janssen
5.1.2 First Name(s) (*): Peter
5.1.3 Title:
5.1.4 Position:
5.1.5 Phone Office:
5.1.6 Phone Mobile:
5.1.7 E-mail (*):
5.2.1 Organisational legal name (*): Province Antwerpen
5.2.2 Organisational short name:
5.2.3 Department/Faculty/Institute/Laboratory:
5.3.1 PO Box:
5.3.2 Postal Code:
5.3.3 Cedex:
5.3.4 Street name and number:
5.3.5 Town:
5.3.6 Country:
5.3.7 Internet homepage:

D6: Point data: ○ Related GIS Point Data is available

D7: Published source:
Technical report to Institute of Public Health

Figure 4: VBORNET data entry questionnaire

GOING TO OTHER CONTINENTS AND DISEASES: IDAMS, THE 3D CONSORTIUM AND VMERGE

EDEN and EDENext were not limited to Europe. A strong link with West Africa in general, and Senegal in particular, was established and preserved over the entire 10 years. While the previous section focused on Europe only, the spatial data archive is also of great added value in North and West Africa and the rest of the world.

IDAMS (idams.eu 2015) is an FP7 project surveying and mapping dengue and dengue vectors at a global scale. It is associated with two other FP7 projects, Denguetools (denguetools.net 2015), and DenFree (denfree.eu 2015), in a '3D consortium', whose members have access to EDENext spatial data archives, and which also provide resources to add substantial primarily climate and climate change related datasets to the archive. These EDENext-generated environmental data have been used in a number of high-impact publications (for example, Bhatt et al. 2013).

The extensive databases generated by EDEN/EDENext and VBORNET/VectorNet, are also shared with other research groups. Several ECDC studies have requested access to EDENext and IDAMS generated covariate datasets and information from VBORNET has been integrated into broader global vector datasets for the main vectors of dengue: *Aedes albopictus* and *Aedes aegypti*. This worldwide database contains 22,104 records for *Ae. albopictus* and 19,969 records for *Ae. aegypti* and has been submitted to a data journal for publication. The data will be used to produce spatial models for the vectors' current distributions, and EDENext-hosted climate change ensembles will be used to produce projected distributions. Similar initiatives are also in progress, for example, for *Hyalomma* ticks transmitting Crimean-Congo haemorrhagic fever virus.

Finally, VMERGE (vmerge.eu), also funded under FP7, has the overall objective of addressing the risk of introduction, emergence and spread of both known and unknown VBDs associated with mosquitoes and *Culicoides,* with a focus on Rift Valley fever and bluetongue. The aim is to improve the limited understanding of these emerging diseases and their potential for spread throughout northern Africa and Europe and to enhance epidemiological surveillance strategies and tools for better disease detection.

The EDEN and EDENext legacy seems ensured for the future. The data archives are widely used, properly acknowledged and have given rise to scientific and technological innovation. The answer to the prime question of this paper, "Is sharing of public data providing added value for the scientific community?" is a clear and loud "Yes", and more importantly it is possible and it has been achieved. Data sharing is a continuing story for now and the future.

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Vector-borne disease models: R_0 and beyond

INTRODUCTION

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The basic reproduction number, R_0, has a long history, especially in the field of vector-borne diseases. This paper provides a brief overview of the use of this concept in vector-borne disease epidemiology as well as new developments and recent applications, notably within the EDEN and EDENext framework. Furthermore, it describes a novel framework for the inclusion of landscape factors and animal movements in vector-borne disease risk assessments, which can be merged with more quantitative approaches in order to achieve a mechanistic and realistic description of the spatial and temporal variation in vector-borne disease risk.

R_0 FOR VECTOR-BORNE DISEASES

The basic reproduction number, R_0, is a key concept in the epidemiology of infectious diseases. It is defined as the expected number of secondary cases caused by one infectious individual introduced into a naïve population. It can be used as a measure of the potential success of invasion of a pathogen into a population; if the value of R_0 is higher than 1, an outbreak of the infectious agent is possible, whereas if R_0 is less than 1, the infection will die out (Anderson and May 1991, Diekmann and Heesterbeek 2000).

The theory underlying R_0 models for vector-borne diseases (hereafter VBDs) originated in the early 20th century, when Ronald Ross first developed a theoretical framework to describe the transmission of malaria. His work was extended by several others, including George MacDonald in the 1950s and 1960s and, by 1970, the foundations of the Ross-MacDonald theory were established (MacDonald 1957, Smith et al. 2012). The by now well-known Ross-MacDonald formula for the transmission of malaria is usually written as:

$$R_0 = \frac{ma^2bc\,p^n}{(-\ln(p))r}$$

where m is the ratio of mosquitoes to humans, a the mosquito biting rate (on humans), b and c the pathogen transmission efficiencies, p the daily survival rate of mosquitoes, r the recovery rate in humans (i.e. the reciprocal of the infective period of the host) and n the duration of the extrinsic incubation period (EIP). Since the development of this Ross-Macdonald model for malaria, hundreds of models for mosquito-borne diseases have been derived and published, and most of them resemble the original models quite closely (Reiner et al. 2013). The Ross-MacDonald formula applies to situations where one vector species and one host species are involved in the disease transmission.

For those situations where multiple types of infected individuals are involved or where multiple transmission routes are possible, the R_0 value can be derived using the next-generation matrix approach (Diekmann and Heesterbeek 2000). The first step in this approach is to identify the different types-at-birth in the system, in other words to categorise individuals by their state at the moment they become infected. These types-at-birth differ in traits that affect their ability to produce secondary cases, such as infectivity, contacts, life history traits, possible transmission routes etc. For VBDs, there are at least two types-at-birth (host and vector), but often, there are more. For a system with m types-at-birth, the next-generation matrix (hereafter NGM) will be

an $m*m$ matrix, where each element k_{ij} equals the expected number of new cases of type-at-birth i caused by one individual of type-at-birth j.

The basic reproduction number R_0 is calculated as the dominant or largest eigenvalue of the NGM (Diekmann, Heesterbeek and Roberts 2010, Diekmann, Heesterbeek and Britton 2013). For a 2 by 2 matrix, the matrix looks like this:

$$K = \begin{bmatrix} k_{11} & k_{12} \\ k_{21} & k_{22} \end{bmatrix}$$

and the corresponding expression for R_0 is:

$$R_0 = \frac{1}{2} \left[(k_{11} + k_{22}) \pm \sqrt{4k_{12}\,k_{21} + (k_{11} - k_{22})^2} \right]$$

For a VBD system with only one vector, one host and no direct transmission (i.e. k_{11} and k_{22} are equal to zero), this approach gives the same result as the Ross-MacDonald formula. In fact, the Ross-MacDonald R_0 is the square of the R_0 obtained by the NGM method. That is, the Ross-MacDonald formula calculates a person-to-person basic reproduction number (via a mosquito, so there are actually two transmission steps), whereas the NGM method calculates a reproduction number from individual to individual (i.e. the average of the vector-to-host and host-to-vector reproduction), so for one transmission step.

The main advantage of the NGM approach is that it can deal with situations with multiple hosts and/or multiple vectors, as well as with different transmission routes. Direct transmission (for example, via transovarial transmission in mosquitoes, or transplacental or direct contact transmission in hosts) can be easily incorporated, as was shown in a study on the impact of direct transmission between crows on WNV transmission (Hartemink et al. 2007), part of the EDEN project. Another EDEN modelling study illustrated how the NGM method can be applied to a complex disease system, namely tick-borne diseases transmitted by *Ixodes ricinus* ticks, which involves five different types of infected individuals and three different transmission routes (Hartemink et al. 2008). Efficient numerical methods for the calculation of the eigenvalues exist, also for a 3 by 3 (or higher-dimensional) matrix (Stoer and Bulirsch 1983) and most common statistical software packages can routinely perform these calculations.

It is important to note that some modelling studies which calculate R_0 assume exponential rates. It means that authors use a fixed rate for mortality and for becoming infectious. Since the extrinsic incubation period (EIP), that is, the period required for pathogen development in the vector, is more likely to last a fixed number of days (during which no vector becomes infectious), this assumption is often not met. This is particularly so when the EIP is long compared to the life span of the vector, which is often the case in vector-borne disease systems, and it is better to use the assumption used in the Ross-MacDonald formula, which is more realistic from a biological point of view (Hartemink, Cianci and Reiter 2015).

R_0 MAPS

R_0 maps are maps indicating values of R_0 for different locations, calculated for a given infectious agent when taking the local conditions into account with respect to hosts (and possible vectors) and their environment. They can be used to identify areas with a higher probability of a major outbreak after this agent is introduced. This concept has been used to develop risk maps for directly transmitted diseases such as foot-and-mouth (Ferguson, Donnelly and Anderson 2001, Keeling et al. 2001), avian influenza (Boender et al. 2007) and classical swine fever (Boender et al. 2008). Within the EDEN project, R_0 maps were constructed for two vector-borne disease systems: bluetongue (Hartemink et al. 2009) and canine leishmaniasis (Hartemink et al. 2011).

Several R_0 maps have now been developed and published for VBDs, including maps for bluetongue virus (Racloz et al. 2008, Brugger and Rubel 2013), chagas disease (Cordovez et al. 2014) and tick-borne diseases such as Lyme disease (Wu et al. 2013) and Crimean-Congo haemorrhagic fever (CCHF) (Estrada-Peña et al. 2013).
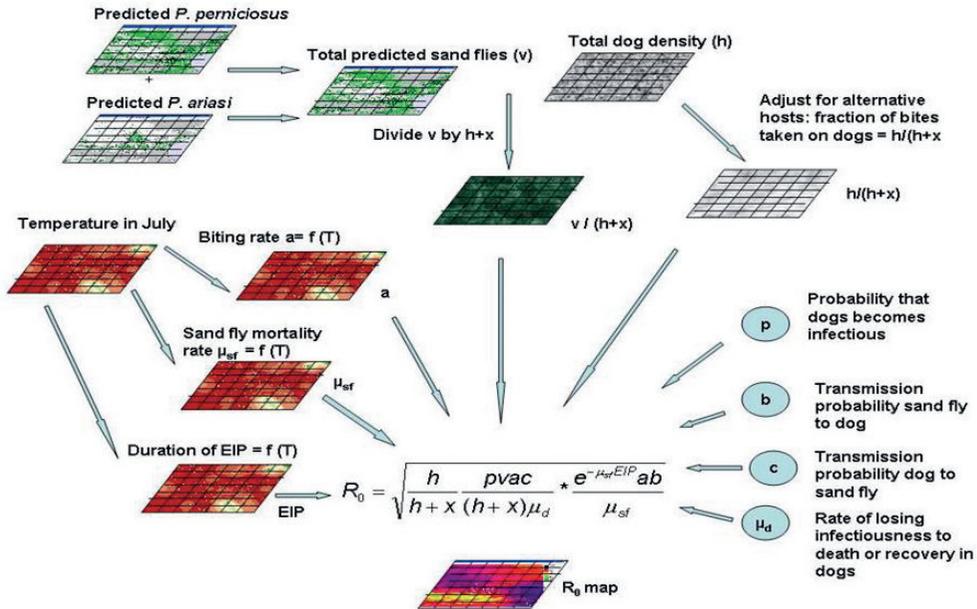

Figure 1. Example of the construction of an R_0 map for canine leishmaniasis (from Hartemink et al. 2011). Parameters of the R_0 formula can be temperature-dependent (in this example, biting rate a, mortality rate and extrinsic incubation period depend on the temperature in July, left side of figure), or constant (parameters in blue circles, right side of figure). Important parameters include the estimated vector abundance and host abundance (top). Calculating the R_0 value for each pixel yields the R_0 map.

IMPACT OF TEMPERATURE

R_0 maps can also reflect the temperature dependency of disease transmission. Several processes influencing transmission dynamics are known to vary with temperature, and this can be taken into account by making the corresponding parameters in the expression for R_0 temperature-dependent. Temperature can have opposing effects on different ingredients of R_0, a phenomenon that has been discussed by, amongst others, Rogers and Randolph (2006). Therefore, caution should be taken when using models to predict the effect of climate change; for instance, models taking only the effect of temperature on the pathogen development rate into account are just too simplistic (Reiter 2001, Reiter et al. 2004). Parameters that are known to be temperature-dependent for many VBD systems are i) the biting rate, ii) the rate of development of the pathogen in the vector (which determines the length of the extrinsic incubation period or EIP) and iii) the mortality of the vector. Even parameters often assumed to remain constant (such as the transmission efficiencies b and c in the expressions for R_0) are affected by temperature in one or more vector species. For example, *Culicoides nubeculosus,* a midge that is normally refractory to the strains of bluetongue virus circulating in Europe at the present time (i.e. b=0), is capable of transmitting bluetongue virus when its larvae or pupae are kept at higher than normal temperatures, as is discussed in Rogers and Randolph (2006).

While R_0 and the NGM approach offer a promising tool to assess the risk of establishment, by means of the per-generation growth rate of a starting epidemic, as well as a way to quantify the contributions of different ingredients or transmission routes to this growth rate, there are also some limitations. One of them is that it is not straightforward to include animal movements and landscape factors into the R_0 calculation. One of the assumptions underlying the R_0 concept is that there is local homogeneous mixing, that is, we consider a population where all individuals can get in contact with each other. An R_0 map thus gives local values of the expected number of infected individuals resulting from one infected individual in a naïve population present at that specific location. An R_0 map at 1km resolution assumes that there is homogeneous mixing within the 1km grid cell or pixel, but no substantial exchange of individuals between cells. Also the configuration of certain landscape factors, and its effect on how animals move though the landscape, cannot easily be incorporated in R_0 formulas, although some studies on metapopulations have looked at the effect of migration rates (for example, Jesse and Heesterbeek 2011).

ROLE OF LANDSCAPE FACTORS, CONNECTIVITY AND FRAGMENTATION

Landscape, and elements characterising it, such as land use, land cover, composition and structure, have been suggested to have a considerable impact on the dynamics and therefore the occurrence of VBDs (Ostfeld, Glass and Keesing 2005, Lambin et al. 2010). Landscape, defined as all the visible features of an area of land (*Oxford Dictionary*), includes the physical elements of landforms (such as mountains, hills, water bodies such as rivers, lakes, ponds and the sea), living elements of land cover (including indigenous vegetation), human elements (including different forms of land use, buildings and structures) and transitory elements such as lighting and weather conditions (*Human Geography Dictionary*).

These landscape elements will affect the presence, spread and persistence of vector-borne and zoonotic diseases as they may influence the presence of vectors, reservoir hosts, susceptible hosts (including humans) and the pathogen, as well as their overlap in time and space. Land use will, among other things, affect the number of people present in an area and the way they use the landscape, which in turn affects their chances of getting in contact with the pathogen, via the vector or, for example, in the case of hantavirus, via contaminated soil. Land cover will, at least partly, determine the availability and quality of habitat of the different species which interact with the pathogen, i.e. the vectors and hosts. Many of these aspects had been covered in EDEN, as summarised in the paper by Lambin et al. (2010). In EDENext, the focus shifted, based on the notion that it is not just the landscape *composition*, but also the landscape *configuration* and *connectivity* that will affect the suitability of an area for a pathogen to establish, spread and/or persist. Habitat connectivity and habitat fragmentation are likely to have an effect on the dispersal of animals and thereby of the pathogens they carry.

Characterising and quantifying the level of connectivity of a habitat or landscape is not straightforward. Notwithstanding the importance of the concept of connectivity, there is no generally accepted and employed formal definition (Crooks and Sanjayan 2006) and many different measures for connectivity have been applied in various research fields. In the field of landscape eco-logy, it has long been recognised that habitat connectivity is determined by two different components (Bennett 1990, 2003). The structural component of connectivity is determined by the spatial arrangement of different types of habitats in the landscape. It refers to the mappable, spatial arrangement of habitats. The behavioural component of connectivity relates to the behavioural response of individuals and species to the physical structure of the landscape.

Consequently, even though living in the same landscape, species with contrasting behavioural responses (to habitat disturbance, for example) will experience different levels of connectivity (Bennett 2003). In the field of vector-borne disease epidemiology, this distinction between the structural and behavioural connectivity is often not made and landscapes are classified as well connected or fragmented mostly based on human perception of the landscape. Given the fact that the effect of landscape configuration on connectivity, population density and contact rates is species- and disease-system specific, and the fact that the way to measure connectivity is not well-defined (and in many cases probably overlooks the way that animals, including humans, actually use the landscape), it is not surprising that different studies on the effect of habitat connectivity and fragmentation on disease risk come up with different answers.

In EDENext, a modelling study looked into the effect of fragmentation on animal movement and tick-borne disease transmission by means of an agent-based model, which takes landscape-specific dispersal rates into account. This study predicts a positive effect of fragmentation level on disease risk (Li et al. 2012). Another study, using a multilevel statistical analysis to find landscape factors associated with the occurrence of hantavirus cases in humans, found a positive association with well-connected forest. It is clear that there is no straightforward relationship between landscape configuration and pathogen transmission; the relationship will be different for each disease system and will depend on the way the vector and host species use the landscape, their dispersal capacity, their habitat requirements and many other factors.

RESOURCE-BASED HABITAT CONCEPT (RBHC)

In order to overcome the problem of not being able to include landscape factors and animal movements in our risk assessments, several members of the EDENext modelling team worked together to explore approaches used in other research fields to see whether these approaches would be applicable in the VBD context. It became clear that the research field of vector-borne disease risk modelling shares many goals and challenges with the field of conservation biology. In both fields, the aims include the mapping of organisms of interest and the study of environmental factors which help the prediction of presence, abundance or dynamics of focal species.

One particular concept, the resource-based habitat concept, has been explored in great detail and seems to be a promising framework to identify systematically the different ecological resources that are necessary for the completion of the transmission cycle and to relate these resources to (combinations of) landscape features and other environmental factors (Hartemink et al. 2014). The framework has been developed in the field of conservational biology, with special application to butterfly conservation, to help identify functional habitats from a mechanistic, biological viewpoint (Dennis, Shreeve and Van Dyck 2003, Vanreusel and Van Dyck 2007). The concept facilitates the study of functional habitats of the species involved in pathogen transmission (i.e. pathogen, vectors and hosts) by regarding the resources required by each of the species and linking them to specific environmental features at the landscape level.

Using the movement capacities of the animals involved, the potential functional habitat range (i.e. the delineated zones that correspond to the combined and integrated information on resources, utilities and scale of movement) can be determined for each species. Once the functional habitat of the vector and host species is determined, the functional habitat of the pathogen can be delineated, by looking at the areas when transmission can occur (i.e. where functional habitats of vector and host overlap and other (thermal) requirements of the pathogen are met).

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In this way, the concept provides new insight into spatial and temporal variation in transmission opportunities and exposure that ultimately determine disease risks. Since application of the concept requires a systematic inventory of the resource required by each of the interacting species, it may also be a tool to identify knowledge gaps. An interesting advantage of the method is that it forces us to think about the spatial configuration of resources and how these influence disease risk, which may lead to novel control options; changing the spatial configuration of key resources across the landscape may decrease the overlap of functional habitat of vector and host, or may decrease the vector population, if essential resources for the vector are not placed within flight range distance.

An interesting example is a study in South America, where transmission of oropouche virus was decreased by removing rotten banana stumps or cacao husks, which are breeding sites for the main vector, *C. paraensis*, in the vicinity of houses (Hoch, Roberts and Pinheiro 1986). Removal of bluetongue-virus vector (*Culicoides* spp.) breeding sites has also been shown to be useful in reducing vector abundance in North America for *C. sonorensis* (Linley, Evans and Evans 1970; Mullens and Rodriguez 1989; Carpenter, Mellor and Torr 2008*a*), even though this has not always translated into reduction of bluetongue infection as
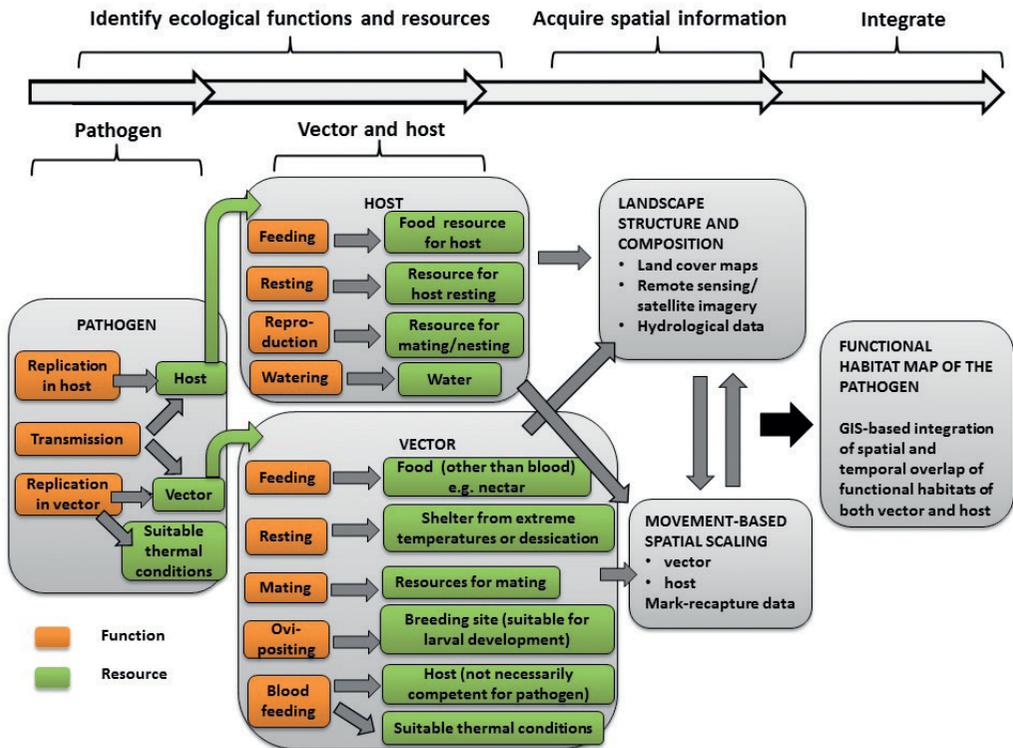

Figure 2: Schematic overview of the application of the resource-based habitat approach to VBDs. In the first phase, ecological functions (orange boxes) and associated resources (green boxes) are identified for the pathogen and the (possibly multiple) vector and host species. Then, information on the movement capacities of the species involved and on the structure and composition of the landscape together, and in mutual dependence, determine the functional habitats of vector and host species. That is, the movement range determines the distance that should be considered when looking at the accessibility of the different resources, whereas the type of terrain may affect the movement range. Transmission opportunities, given the presence or introduction of the pathogen, are determined by the overlap of these functional habitats and by environmental conditions, such as thermal conditions. Figure published earlier in Hartemink, Vanwambeke et al. (2015).

measured by sero-conversion rate (Mayo et al. 2012). So, in summary, RBHC allows for the integration of landscape factors and animal movements in epidemiological spatial risk assessments and hence bridges the gap between existing mechanistic modelling approaches that ignore landscape factors and dispersal and satellite image-based approaches that are based on statistical inference only.

MERGING RBHC AND R_0: INCORPORATING LANDSCAPE FACTORS AND MOVEMENT RANGE IN A QUANTITATIVE WAY

While RBHC may therefore offer promising scope to deal with spatial modelling of VBD risks, it is currently only a conceptual model that gives a qualitative risk assessment, but lacks the advantage of an integrating quantitative approach, such as R_0. The next step would be to take the RBHC framework to the next level, and make it a quantitative approach, by parameterizing it for a case study. This would allow us to take advantage of the properly weighted way in which the R_0 formula combines all the ingredients of the transmission cycle and the spatially explicit and biologically realistic way in which RBHC incorporates the functional use of the landscape.

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Guy Hendrickx

Belgium-born Guy Hendrickx, DVM, PhD, graduated as a doctor in veterinary medicine in 1985 (Gent, Belgium) and has more than 25 years of experience in the field of spatial epidemiology, livestock geography and decision support systems. Between 1987 and 2000, he completed a series of long-term assignments in Africa working on FAO tsetse control projects. Back in Europe he created Avia-GIS in 2001 to help bridge the gap between research and decision making in the field of spatial decision support systems applied to animal and Public Health. He established Euro-AEGIS with ERGO (Oxford, UK) and, as one of his first European assignments, was requested by CI-RAD to build the EDEN consortium and write the EDEN project. In EDEN he was operational manager and secretary of the Steering Committee. He is particularly interested in developing multidisciplinary research networks with a focus on PhD networks. In 2012 his company won the first Janssen Open Kempense Innovation Award for the VECMAP™ software package which is now going to the market.

What role have the EDEN and EDENext PhD networks played?

Guy Hendrickx (Avia-GIS, Belgium): *"When we started talking about EDEN in 2002/2003, there was a huge gap in Europe of people working in the field. Especially because of the pressure on publications, there was more and more emphasis on lab work and having, as quickly as possible, results you could publish in the biggest possible journals. It was all about bioinformatics and anything like lab-type publications, genetics were very much high on the list. So, for a young student looking for a career, it was not very appealing to go the field and, like the presentation we saw this morning [GERI 2015], conducting two years of sampling sand flies and coming up with some graphs and linking this to a few factors was not very appealing.*

"Given this framework, the fact that EDEN was such a large network, covering all of Europe, suddenly made the topic sexy in the sense that it became interesting for PhD students, especially because they were not working alone. When you are doing field work the one major constraint is time - it takes a lot of time - and you can be alone a lot or you may be lucky and be with a very nice team, but if you know that you are part of a group and you can meet every year or at least have a lot of contacts with other people doing similar things in other countries, it makes it very interesting because it gives it a kind of community feeling.

"It very quickly became obvious that we should actually install it [PhD network] in EDEN and in the first year meeting it got organised. Part of the reason it was organised so late in the EDEN project was the very complicated politics and, especially, because people were very reluctant regarding data sharing and working together; there were not many large European projects like it. Before the submission, most of the time was put into how we as teams were positioning ourselves and most of the discussions and attention went into that. So at that time it was like, OK, the work will be done by PhD students but how this would be organised was not defined. In the steering committee meetings of the first year it became more clear and in organising the first annual meeting the idea came to us to organise, before the actual meeting, one or two days as a PhD meeting and that actually sealed it.

"PhD students got their specific time where they could discuss between themselves their results and the idea of having a PhD group became very concrete. Also, there was a PhD prize so each year we could give a prize to the best presentation from a PhD student. It was a significant prize so for the PhD students it was very attractive and at the PhD meetings during the annual meetings there was always plenty of time for presentations, not just 15 minutes, but at least 20 or 25 minutes with questions.

"It took a while before the seniors in EDEN understood the importance of it. I think, on purpose, we actually excluded the seniors from the first PhD meeting, with only PhD students there, to make them feel comfortable. All these studies were just starting so we didn't really need questions to scare them off. It was only at the second PhD meeting in the second year that the seniors were more involved.

It became a structure where it was more like a summer school, only in a fairly cold period of the year! There was a relaxed feeling and everybody knew it was possible to ask very precise or deep questions without anyone feeling personally threatened…It worked out fine.

"In EDENext it was very much embedded in the project, the PhD group was even more like a group than in EDEN. We have had at least 60 PhD students and that's a generation. It is really quite amazing in Europe. It means that over the past 10 years, EDEN and EDENext have put a stamp of quality on a whole generation of PhD students. There are several students from EDEN that have been working in EDENext and that is very nice. It has definitely created a group of people thinking in a certain way about research and, actually, they are still applying that."

SCIENTIFIC ADVANCES FOR BETTER PUBLIC HEALTH

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Public H
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INTRODUCTION

Vector-borne infections comprise a major group of diseases with high impact on human and animal health. Arthropod vectors such as mosquitoes, ticks, midges and sand flies or mammals such as bank voles are responsible for the transmission of pathogens to humans, domestic animals and wildlife hosts. Environmental changes and the dramatic increase in global trade and travel have meant that several diseases are starting to emerge in Europe, causing increasing challenges for Public Health (PH). This is clearly shown by recent events such as dengue emergence in Madeira, Portugal, in 2012, the outbreak of human hantavirus infections with more than 2,800 cases in Germany the same year; and the spread of Crimean-Congo haemorrhagic fever (CCHF) in south-eastern Europe, particularly in Turkey. These events mean that the risks and the consequences triggered by vector-borne infections are just starting to surface in the public awareness.

A prominent role was assigned to Public Health within the EDENext Project, funded by the European FP7 programme. PH in this context can be defined as an approach that focuses on vector-borne diseases as an important risk with an increasing impact on human and animal health. Emphasis is therefore laid on an integrated health approach ('One-Health') as it recognises the interrelated nature of both human and animal health, and recognises the links between health and the environment. The Public Health group was included in EDENext to interface with all five vector-specific groups: ticks, rodents, sand flies, midges and mosquitoes.

The EDENext Public Health work plan was defined and developed as an integrated risk-based approach applied to vector-borne diseases - the first time this has been formally attempted. We have developed a shared definition that has been presented both as an EDENext White Paper (2013) and a risk governance framework for PH activities relating to vector-borne diseases (VBD) (Schmidt et al. 2013). The risk governance framework provides a systematic approach for the analysis, assessment and governance of emerging health risks that adopts the guidelines developed by the International Risk Governance Council (IRGC 2005, 2008). The framework incorporates the five IRGC elements: (i) pre-assessment, (ii) appraisal, (iii) characterisation and evaluation, (iv) management directives and (v) communication, and has adapted them to Public Health.

This paper takes two case studies - on CCHF virus and hantavirus, causing nephropathica epidemica (NE, also referred to as HFRS or FHSR) in humans - to show that it was possible within the large collaborative EDENext project to address all five of these different elements of risk governance. The first chapter defines the relevance and the shared understanding of PH within the project. Chapter 2 shows how this concept has been applied within EDENext. Chapter 3 considers the two disease case studies within this framework.

This paper also reports on our attempts to formally define the additional necessary components to PH activities in the VBD arena. Whereas quantitative risk assessment is always considered to be the primary focal point for decision-making, we will illustrate how concern assessment, particularly the risk perception of vector-borne infections by the general public living in endemic areas, is equally important when identifying appropriate measures to manage the risks to public and animal health. We will also elaborate on the health communication strategies required for PH authorities to raise professional and public awareness of these diseases, especially in endemic regions.

We emphasise that the focus in each of the following sections is on findings that were actually produced within the EDENext project, which shall be used as an illustration of the underlying assumptions and the idea of the framework that we have proposed.

A SHARED UNDERSTANDING OF PUBLIC HEALTH IN THE FIELD OF VECTOR-BORNE DISEASES WITHIN EDENEXT

We have based our EDENext understanding of Public Health on the four main areas identified by Kaplan et al. (2009) and agreed on the following shared definition (see White Paper 2013, p. 5 & p. 11): "The term Public Health used in this EDENext White Paper refers to a holistic, interdisciplinary and multi-level approach. Public Health is concerned with questions of prevention, rather than cure and it is concerned with health at the level of the population rather than the individual. It is, moreover, concerned with the facilitation of health equity in the society through the mobilisation of political, social and economic resources. Hence, Public Health considers various determinants of health, such as the ecological environment, interdependence between animal and human health, political structures, socio-cultural patterns, economic, living and working conditions, social and community networks or individual lifestyle factors."

APPLICATION OF THE PH PERSPECTIVES IN EDENEXT RESEARCH

Research projects on vector-borne diseases are rarely (if ever) designed to concisely answer questions relevant to PH. Three major activities were therefore applied within the EDENext consortium to promote the relevance of PH to research scientists concerned with these diseases and its vectors: EDENext PH reviews, PH guidelines, and PH Telegrams.

APPRAISAL OF THE 'STATE OF THE ART' KNOWLEDGE IN TERMS OF PH (EDENEXT PH REVIEWS)

Before starting new research projects addressing PH topics it was important to screen and critically review available literature on the PH relevance of vector-borne diseases. These meta-analyses were eventually published as PH reviews:

• The impact of Crimean-Congo hemorrhagic fever virus on public health by Mertens et al. (2013) *Antiviral Research* 98: 248-260.

• Hantavirus infections in Europe and their impact on public health by Vahari et al. (2013) *Rev Med Virol.* 23:35-49.

• *Culicoides* biting midges and public health by Carpenter et al. (2013) *Antiviral Research* 100, 102-13.

• Sandfly-borne phleboviruses of Eurasia and Africa: epidemiology, genetic diversity, geographic range, control measures by Alkan et al. *Antiviral Research* (2013) 100:54-74.

• *Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe, new hazards and relevance for public health by Rizzoli et al. (2014) *Frontiers in Public Health* 2:251.

• Arboviruses pathogenic for domestic and wild animals by Hubálek et al. (2014) *Adv. Virus Res.* 89:201-75.

• Hantavirus emergence in rodents, insectivores and bats: What comes next? by Schlegel, et al. (2014) in: *The Role of Animals in Emerging Viral Diseases* (ed. N. Johnson), Elsevier, Academic Press, pp. 235-292

GUIDELINES FOR PH RESEARCH WITHIN EDENEXT (EDENEXT PH GUIDELINES)

The Public Health relevance of particular research activities (such as studies on the distribution and spread of known vector species in new areas, the vector competence of putative local arthropods for arboviruses, the impact of environmental changes on vector distributions, virus prevalence and variety in arthropod vectors etc.) is frequently not appreciated by scientists working on vector-borne diseases. In order to communicate the PH idea and to enhance applicability for users of the PH risk governance framework, specific 'Guidelines for Public Health-oriented Science in EDENext' were produced to specifically help project partners identify and elucidate the PH content of their research. These practically oriented guidelines encouraged researchers to address all PH relevant issues in the design phase of a study, with a range of topics including (i) identifying and characterising risk areas; (ii) understanding transmission of vector-borne infections, (iii) finding options for prevention, control and treatment; and iv) evaluation of the relevance of the results to PH activities.

TRANSLATION OF EDENEXT RESEARCH RESULTS FOR THE LAY AUDIENCE AND STAKEHOLDERS/DECISION MAKERS (EDENEXT PH TELEGRAMS)

EDENext publications were systematically scanned by the PH work group for data relevant to protect human health, then summarised in lay language and accessible formats into so-called Public Health Telegrams on Vector-borne diseases - PUBLICise Health: 'RainboGram' on rodent-borne diseases, 'TiBoGram' on tick-borne diseases, 'MoBoGram' on mosquito-borne diseases, 'CuliBoGram' on Culicoides-borne diseases and, 'PhBD-Gram' on phlebotomine-borne diseases. These can be downloaded directly from the EDENext website (http://www.edenext.eu/publications/publicise-health-edenext-s-public-health-telegram) and updated versions are also presented in this book.

THE EDENEXT PH RISK GOVERNANCE FRAMEWORK: CASE STUDIES ON CCHF AND HANTAVIRUSES

The Public Health risk governance framework developed within EDENext comprises the following five elements of the International Risk Governance Council (IRGC 2005, 2008): (i) pre-assessment, (ii) appraisal, (iii) characterisation and evaluation, (iv) management directives and (v) communication. We selected the IRGC framework rather than other existing risk analysis approaches (see Sedda et al. 2015) because it includes three often neglected components: a focus on pre-assessment, an emphasis on concern assessment (which explicitly takes social, cultural, political, financial and economic considerations into account), and it highlights the central importance of communication within the risk governance process (White Paper 2013, p. 12). The PH risk governance framework was then exemplified in two case studies: Crimean-Congo haemorrhagic fever (CCHF) and nephropathia epidemica (NE). Recently, hanta and CCHF viruses have received considerable scientific and public attention. NE case numbers are rising in several parts of Europe, for example in Germany, and recent outbreaks of CCHF in Turkey and Greece have raised awareness amongst physicians and led to improved detection, though formal risk assessments are still lacking or inadequate.

EXAMPLES FOR THE PRE-ASSESSMENT PHASE IN PUBLIC HEALTH RISK GOVERNANCE: DIAGNOSTIC CAPACITY

According to the IRGC framework, the pre-assessment phase focuses on early warning and monitoring, risk screening and scientific conventions of risk and concern assessment. In our framework these include a number of components (for details see Schmidt et al. 2013): scientific tools and diagnostic (laboratory) capacities for addressing and specifying the dimensions of the emerging health risk, and the identification and evaluation of existing regulatory systems used to justify specific actions.

Two external quality assessment (EQA) studies on CCHF and NE gave a good overview of the capacity and molecular diagnostic performance of European laboratories (Escadafal et al. 2012; 2012a). Since there is no approved vaccine or specific treatment for CCHF, reliable surveillance and diagnosis is essential. Diagnostic techniques include virus culture, serology and, more recently, molecular methods. The international EQA of CCHF molecular diagnostics to assess the efficiency and accuracy of molecular diagnostic methods applied by expert laboratories was organised by the European Network for the Diagnostics of 'Imported' Viral Diseases (ENIVD). Uneven performances using the same method indicates that there is still a need to improve testing conditions and standardise protocols. The purpose of the second EQA study on NE diagnostics organised by ENIVD was to collect updated information on the efficiency and accuracy of NE serological methods applied by expert laboratories. All studies showed a need to improve the diagnostic profile of several laboratories.

The unsatisfactory diagnostic performance in both case studies is related to the rarity of the diseases and the absence of commercial diagnostic assays. The evaluation of the diagnostic performance through an external assessment with inactivated samples offers a unique opportunity to verify diagnostic procedures since most laboratories have not evaluated their assays. Participants can learn how the assay they used performs and whether it needs improvement. Regarding the laboratory capacity, we can presume there is a sufficient number for the diagnosis of these kinds of rare diseases.

APPRAISAL: A FOCUS ON THE PUBLIC'S RISK PERCEPTION OF VECTOR-BORNE DISEASES IN ENDEMIC AREAS

PH agencies need to communicate risk effectively. The complexity of the diseases and the interdependence of international PH systems, involving governmental as well as non-governmental agencies and bodies, mean that only a participatory approach will be effective. This approach must involve key players in the field of PH and take into account public risk perceptions with regard to vector-borne diseases.

Our PH risk governance framework shows that quantitative scientific risk assessment alone is inadequate for effective decision-making and that social science-based concern assessment is as important. Concern assessment is defined as encompassing risk perceptions of the affected population in all groups at risk, as well as their social response to a particular risk. It also encompasses social, economic and trans-boundary effects of the health risk (for more details see White Paper 2013; Schmidt et al. 2013). We have therefore performed a risk perception study on two vector-borne diseases.

This qualitative study was based on in-depth interviews with Public Health authorities and relevant stakeholders, and used focus group interviews with the general public in endemic areas (Dressel 2014). The study was conducted in five European countries supported by the rodent and tick vector groups of EDENext. The study on rodent-borne NE was conducted in France, Finland and Germany; and the work on tick-borne CCHF was completed in the Republic of Macedonia and Turkey.

The focus group analysis covered risk awareness, risk knowledge, risk behaviour and coping strategies, as well as information behaviour and public expectations in times of enhanced risk. Results were categorised by respondents' age, sex, occupation, personal affectedness and region. Fifteen focus groups in total (three per country) with 8-10 attendees each were conducted in endemic areas of each country. One group in each country was composed of professionals such as forest rangers or lumberjacks or veterinarians affected by the particular disease. The setting of the focus group followed certain socio-demographic variables that are known to influence risk perception, information behaviour, coping strategies and knowledge such as sex, age or region. The following summarises the risk perceptions that we found (see also Schüle 2012, Dressel 2012, Dressel 2013, 2014, Dressel, Sekovska and Stefanovska 2014, 2014a, Dressel 2014a, 2014b).

FINLAND (NE)

In Finland, where Simo Tuppurainen assisted in the risk perception and communication study, Puumala virus (PUUV) is widespread throughout most parts the country, but is not generally considered a challenging risk, perhaps because it is a familiar one. We found, however, that rural people, who are most at risk, do accept the risk more than urban residents, who tend to take PUUV more seriously even though they have only occasional contact with voles (particularly through their summer cottages). We also

found that risk perception did not vary with gender but did with age: older people, who are more experienced with the disease, tend to be more concerned than younger ones

FRANCE (NE)

In France, where Gabriela Pfeifle assisted in the risk perception and communication study, there was a striking lack of knowledge with regard to NE/PUUV in all groups (including the occupational risk group) even in the two areas where it is endemic, and so it was considered much less important than other VBDs, in particular leptospirosis. A typical interview quote was: "Funny, or better scary, to see how many things exist I am not aware of," said a man in the 25-45 age range in an urban group. Nevertheless, people felt they had a right to be informed about the risk by public authorities. Occupational risk group attendees were not concerned about NE risk, and saw other risks as more important. Urban residents were more worried about environmental risks than rural people, who considered the risks from VBD generally as a normal part of the natural environment.

GERMANY (NE)

In Germany, where Steffen Schüle assisted in the risk perception and communication study, NE is quite common in several areas. Knowing someone who is/was personally affected by a risk played the most important role in sensitising respondents to the risk at the micro level. In contrast, people who did not know affected people tended to underestimate the risk or were more careless about it. The ecological dimension (such as living close to a forest or having a garden) was mentioned on all levels as a potential risk factor. Older people tended to be more sensitive regarding the perception of their ecological environment and its risk, for example in their awareness of the changing mice population. Overall, focus groups reported that a lack of knowledge led to a feeling of insecurity and not knowing what do to and how to tackle NE /PUUV.

REPUBLIC OF MACEDONIA (CCHF)

In the Republic of Macedonia, where Jovana Stefanovska and Blagica Sekovska assisted in the risk perception and communication study, only generic haemorrhagic fevers are detected and treated, and no specific CCHF diagnostic test is used. There are no specific health campaigns, which would raise CCHF awareness among general practitioners or the public. Participants of both rural and urban focus groups were generally aware that tick bites could cause disease, though CCHF itself was unknown. Risk perception for this particular disease was extraordinarily low, despite the fact that neighbouring countries (Albania, Bulgaria, and Greece) have reported cases.

TURKEY (CCHF)

Contrary to the Republic of Macedonia, in Turkey, where Ayca Bakalacs and Pinar Oktem assisted in the risk perception and communication study, the risk perception of CCHF was high in all groups. Those people who were most at risk were well aware of the fact that they have an increased CCHF exposure risk and they know about necessary protective measures. Women generally stated that they are afraid of being bitten by ticks and getting the disease when going to the fields and barns: "We have panic, we have fear. [...] We are frightened in the barn and in the fields," said a 36-year-old woman in a rural focus group. Men also expressed their awareness of the risk, but did not use emotional words to do so. This was particularly striking among young men, who sometimes did not consider themselves at risk at all. In contrast, older men were even able to define the actual timeframe and specific circumstances of higher CCHF risk situations.

For each focus group, a word cloud was generated about the first association of focus group attendees with regards to the particular risk posed by hantavirus or CCHF. To the authors' knowledge this is the first time this has been attempted in such studies. It was particularly illuminating as the word clouds from all focus groups in any one country accurately reflected their risk

perception and, particularly, their knowledge of the disease. The following three illustrations show the differences between the three case studies on hantavirus.

Figure 1: Word cloud of all Finnish focus groups on hantavirus.

Figure 2: Word cloud of all French focus groups on hantavirus

Figure 3: Word cloud of all German focus groups on hantavirus

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As illustrated by the word clouds, risk perception and knowledge about hantavirus is strongly expressed in Finland, where hantavirus is endemic in most parts of the country. The French word cloud shows the opposite situation: risk perception is low and knowledge is weak in the focus groups conducted in the only two endemic areas of the country. Germany, with its patchy distribution of hantavirus infection, is between Finland and France in regard to the risk perception and knowledge about hantavirus risk found in members of the general public in endemic areas. We have published this new methodological approach for the study of risk perception in a paper on the German hantavirus case study (Dressel & Schüle 2014).

CHARACTERISATION AND EVALUATION

Characterisation and evaluation as defined here requires the results of the health risk assessment to be interpreted in the context of wider social and economic factors, the weighing of pros and cons and trade-offs of different preferences, interests and values, the consideration of ethical issues and the suggestion of possible interventions for risk reduction. For CCHF, we concluded in the White Paper (p. 38): "In summary, the risk of CCHFV to Public Health is regarded as being intolerable, because of its widespread distribution, the agility and the broad host range of the vectors, as well as the effective adaptation of the virus to its vector population enabling efficient virus transmission. This together with its potential to be transmitted from human to human, the risk of nosocomial infections, the potentially high lethality rates and the limitations in therapeutic interventions make CCHFV a significant Public Health threat in South-Eastern and, in future, possibly also Central European countries. The impact of CCHFV on health care workers, on individuals living in a high risk area and on the public should not be ignored. Corresponding educational programmes and control measures should be implemented in these countries."

PUBLIC HEATH RISK MANAGEMENT OF CCHF AND HANTAVIRUS

PH risk management should be based on the analysis of the present health situation, the available (social) scientific knowledge, including risk perception, and it should follow a well defined approach in dealing with vector-borne diseases. Important steps include the identification of those responsible for managing the emerging health risk and promoting awareness of the disease, identification of potential options for handling the health risks at various levels, and the selection of the most suitable management options (Schmidt et al. p. 531).

In their review paper on CCHF, Mertens et al. (2013) suggest several management and control strategies for tackling the PH risk which include: a) the classification of countries or regions into four risk zones (zone 0 = no risk for humans, for example Germany; zone 4 = endemic areas with permanent risk zone, for example, the Tokat region in Turkey); b) the implementation of measures and strategies for risk characterisation and handling the risk (again ranked from level 0 to 5); and c) the implementation of surveillance programmes following international standards and training programmes for those affected by the PH risk.

While there is not even a potential vaccine for CCHF, several inactivated vaccines for NE, based on rodent brains or cultured rodent cells, are already available in South-East Asia, though no targeted vaccine or therapy is in use in Europe (see Vaheri et al. 2012). Better vaccines based on DNA, recombinant proteins or genetically engineered viruses are likely to be developed. In their review Vaheri et al. (2012) emphasise the need for controlled prospective studies as hantavirus infections are underestimated by the medical communities in many countries due to lack of data. The authors conclude: "Very simply, if there are no diagnostics, there is no HFRS" (Vaheri et al. 2012). Monitoring rodent population densities, and preferably also hantavirus infections in rodent populations, can be used to draw risk maps to be used by individuals to minimise their hantavirus exposure risks. This approach also involves continuous education and raising awareness among the public using adequate risk communication strategies.

THE DESIGN OF TARGETED HEALTH COMMUNICATION STRATEGIES

A major PH goal is to establish an interactive communication process, at the governmental level (internal communication between different departments), at the interface between the government and stakeholders, and between the government and/

or stakeholders and the general public. This guarantees that communication is an iterative process that can be adapted and changed as needed (White Paper, p. 21).

Meaningful risk communication (or health communication) strategies can only be established using consolidated knowledge of the risk perception held by the public and stakeholders, considering what they know and what they don't know. This is particularly relevant when health is concerned, as improving the public's risk awareness effectively reduces risk behaviour, the risk of infection and influences the effectiveness of PH interventions.

What people know about a disease usually reflects, at least to some degree, the actual spread of a particular disease in a particular region. Within the EDENext risk perception studies, we have included both countries that are widely affected by a specific VBD (NE in Finland and CCHF in Turkey) as well as countries where the disease risk is only very low (NE in France, CCHF in Macedonia), Germany was intermediate with some endemic regions and some areas with no disease. We found that health risk communication strategies need to be adapted to the specific regional situation. In addition, we found that the area of residence also plays an important role: the more rural the population, the more knowledgeable the population is about a particular disease which predominantly occurs in rural environments; the more urban the population the less familiar it is and the higher the concern is, especially regarding rural health risks. Similarly, age is a strong determining factor for risk awareness and risk knowledge. Gender also played a role in some cases, as we have seen in the case of CCHF in Turkey.

In countries with newly emerging diseases, sensitisation of the population is needed in terms of campaigns that will not provoke panic, but spread a solid knowledge to promote risk awareness of these diseases. This should also include dedicated information campaigns and training for people at occupational risk. In addition, general practitioners, who can be expected to be the first people to correctly recognise and diagnose the disease, should be informed. In countries with current health risks restricted to endemic areas, no widespread information campaign is necessary, but information campaigns should be targeted to the specific needs of the population in endemic areas. Case specific recommendations on targeted health communication strategies that take into account socio-cultural factors of risk perception of VBD were formulated for the five examined countries (Dressel 2014).

In an innovative and fruitful joint approach between EDENext modellers and the Public Health group, focus group participants in the risk perception study reviewed health risk communication material (risk maps) and their opinions were collected. The risk maps were then modified and adapted by the modellers in response to the needs and wishes expressed by people living in endemic areas (Dressel 2014a, 2014b). There is great potential for modellers to support risk communication strategies by providing model outputs that can be used by practitioners as well as for informing the general public (Vanwambeke, Tersago and Sedda 2013; Dressel and Sedda 2013; Niedrig and Dressel 2013; Dressel et al. 2014; Dressel et al. 2015).

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CONCLUSIONS

We have shown in this paper that the great challenge of introducing Public Health perspectives to vector-borne research scientists was not only taken up, but generated several valuable inputs with immediate impact for PH practice. The idea of a common understanding of a "holistic, interdisciplinary and multi-level approach" was taken seriously by the EDENext consortium and the inherent potential of such an approach became apparent in a wealth of publications, models and recommendations to decision makers. The EDENext Public Health risk governance framework has shown its potential and illustrated how each discipline involved, ranging from vector scientists, modellers and social scientists to human and animal epidemiologists, has contributed with its specific expertise to complete and integrate PH relevant knowledge. It is necessary to evaluate, to revise, to adapt and to improve the overall framework after every new or new emerging epidemic in order to pave the way for target-specific risk management and sensible health communication strategies, and to enhance preparedness for future epidemics in the field of One Health.

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Marieta Braks

Marieta Braks studied biology at the University of Utrecht and obtained her PhD in entomology for research on the host-seeking behaviour of mosquitoes in the laboratory and in the field (Tanzania) from Wageningen University in 1999. Subsequently, she accepted a four-year post-doctoral position at the Florida Medical Entomology Laboratory UFL (USA) investigating the invasion ecology of yellow fever and Asian tiger mosquitoes in the USA and Brazil. She then worked at the Entomology Laboratory of the University of California at Riverside and the Department of Vector-borne Diseases of the California State Department of Public Health. In 2007, she returned to the Netherlands to work at the Centre for Zoonoses and Environmental Microbiology and has extensive experience in the surveillance and control of vectors and diseases at RIVM (National Institute for Public Health and the Environment). She joined the EDENext advisory committee in 2012.

For you as a Public Health professional, what have been the outstanding achievements of EDEN and EDENext?

Marieta Braks (National Institute for Public Health and the Environment (RIVM), Netherlands): *"One thing is that in the 10 years EDEN and EDENext have run, people who were not in it were very jealous. I know lots of Americans who are very jealous that you can run such a thing for 10 years with 30 institutions from 15 countries and manage to go through to the end. I think it has often been said that it cannot be done and this is proof that it can, especially [concerning] data sharing. This has been an enormous move because people said that people were not going to do it...I know a lot of people who have worked in this field but didn't find a community, they were very isolated. These projects have made a community of different vectors and different disciplines, from molecular biology and ecology to Public Health and modelling and that is quite an achievement. I know that it has sometimes been a struggle. I have seen a lot of different people who didn't understand each other and if you have 10 years, two times five years, together it proves that it takes that long to get over it, so you need that investment. And I think the next step is for EDENext to be even more applied to Public Health. It's still quite far from my daily usage."*

How could it be closer to your daily professional life?

Marieta: *"If I can give an example. Only two days ago a physician asked me about tularaemia, which is a very complicated disease transmitted by different vectors, sometimes by water, sometimes by hare and maybe some others too. And it's different in every country. When I worked in California it was biting flies, it is ticks in Sweden, mosquitoes in other areas, by moles...it is very complicated. In Holland we have had a few incidents of tularaemia so a physician who is dealing with these patients said, 'You guys don't really know anything about ticks right?' Obviously I was a bit shocked by this but having been in Public Health I didn't go defensive and say we have been investigating for 20 years. Apparently, though, we didn't do anything for him.*

"The problem with the work that we do is that it is not generalised. If you take measles it is the same in Poland, in California or in South Africa, so by now we have figured out how it is transmitted. The problem with tularaemia and all the other diseases is that it is very spatially and temporally variable so you feel that you don't progress because it breaks out somewhere else and the entomologist has to go out in the field again and you may do that for 50 years. So you have to do something...to explain that it is always different and it is not because it is our hobby or anything, but transmission cycles are temperature dependent, host dependent. It's an important message that we have shown across Europe, why we have to do it and that you cannot generalise as you can go hopelessly wrong.

"If you cannot explain this, for the physician it feels as though you are just asking for more money again. And that is what occurred to me last week when I talked to this physician. I thought, we know lots about ticks, we know about Lyme, but you cannot investigate tularaemia

when you do not have it in the country. You can investigate all you want, but there is nothing to investigate. So with these new viruses you have to look. Physicians look things up in the encyclopaedia and they think, 'Come on, why have you not done this?' And we have to find out how to try and translate that. It would help.

"Also, if they ask us something, we have to really share our knowledge instead of stressing the things we don't know. Scientists are always very keen to discuss the things which are variable such as the tick population in a forest, but physicians don't care if it is 50 or 55 ticks, it is not zero!...We are developing a lot of knowledge now, but we only talk about the scientific, the variable things, and we should learn to lean back and be more informative...This is anecdotal but on one occasion a woman had a sand fly bite and was working on the sixth floor. The question was, do sand flies fly that high? Somebody gave the sand fly distribution in the country but that is not what was asked. They asked was she bitten there, should we do something? If you can answer maybe, but it is 99.9% sure she was not bitten there, then say it is probably not. Just give an answer instead of giving data. We know so much and you have to be able to make statements."

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Interview: **Hervé Zeller**

Hervé Zeller is currently senior expert, head of the Emerging and Vector-borne Disease programme, Office of the Chief Scientist, at the European Centre for Disease prevention and Control, Stockholm, Sweden, with special focus on surveillance, risk assessment and prevention of these diseases in the European Union and neighbouring countries. A pharmacist virologist from Lyon University, France (1976), he worked for more than 20 years within the International Pasteur Institute network as a virologist in several countries in Africa and the Americas, mostly on the diagnostics of viral emerging viral diseases: influenza, hepatitis, HIV and emerging and vector-borne viruses. He was involved in field work with multi-disciplinary teams on the eco-epidemiology of arboviral diseases (yellow fever, dengue, chikungunya, Crimean Congo haemorrhagic fever, Rift valley fever, West Nile) and other zoonosis (hantavirus) mainly in Africa. He was director of the French National Reference Centre and WHO Collaborative Centre on arboviruses and viral haemorrhagic fever in the Pasteur Institute Paris/Lyon in France for 10 years (1998-2008) and has participated in several outbreak responses through the WHO Global Outbreak response network (GOARN) in Africa and Europe.

What are your impressions of the EDEN and EDENext projects and the transfer of this knowledge for Public Health?

Hervé Zeller (European Centre for Disease Prevention and Control): *"Out of these two projects funded by DG Research, EDENext was more focused on the biology of vectors and reservoirs and was a continuation of EDEN with a clear link to Public Health and Animal Health…The Public Health component was well integrated from the start in the team work on the field and we see that from the data presented at the end of the project. There were really good results which can be used for Public Health and Animal Health…What we have seen today [GERI 2015] is, I would say, a good example of integration of the results from research for assessing the risks, for models for prediction and so on, which is very important for the future."*

Do EDENext and EDEN provide useful expertise for ECDC and other agencies?

Hervé Zeller (European Centre for Disease Prevention and Control): *"We are using, through EDENext, a large community of contacts; we have many experts involved in different projects under the ECDC, such as the VectorNet project with its network of medical and veterinary entomologists, and Public Health specialists. We have also the possibility to have access to the data which have been produced, and need to be sustained as discussed this morning [GERI 2015, see Sharing scientific data: 1+1=3]. Regarding data sharing I would mention that some of the databases would be of main interest for animal or human health and should be shared with these agencies. We need to find the best ways to keep these data available for further use. On one hand a lot of work has been achieved, and there could be a potential risk of under-use of these results which should be avoided. On another hand we have in ECDC the E3 project, a European Environmental Epidemiology portal which links epidemiology and the environment. It hosts already the data from the project EDEN, and discussions should take place in the next months to establish which databases could be provided by the project and integrated in this portal in the future for use by the wider Research Human and Animal Health community. I think that the talks this morning [GERI 2015, see Sharing scientific data: 1+1=3] illustrated the need to sustain a network through an external project/portal to extend the network to a wider range of people for decision-making and scientific purposes."*

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1 EDEN project
2 EDENext project