Welcome to the fifth issue of the EDENext Public Health Telegram, the newsletter from the EDENext project focusing on our vector-borne disease research results with direct or indirect impacts on Public Health issues.

EDENext is investigating the biological, ecological and epidemiological components of vector-borne disease introduction, emergence and spread, and creating new tools to control them.

EDENext comprises five specialised vector groups. In this issue we concentrate on the work of the group on sand fly or Phlebotomine-borne diseases (Diptera, Psychodidae, subfamily Phlebotominae).

EDENext is seeking to make research more visible, comprehensible and applicable to the public and policy makers, embedding the research results from each vector group in the context of Public Health and vector-borne disease control in Europe. The EDENext Public Health Telegram was established to inform interested individuals and institutions about research results on vector-borne diseases with direct or indirect impact on Public Health issues.

For more details about the project, visit www.edenext.eu

In this issue, research results from the EDENext Phlebotomine-borne disease (PhBD) group include:

1. The role of sand flies (Phlebotominae) in spreading diseases of Public Health concern
2. Leishmaniasis in Greece
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4. Updates from phlebotomine sand fly-borne disease research in Portugal
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6. Spanish leporids (hares and rabbits) as potential sylvatic Leishmania infantum reservoirs
7. Autochthonous canine leishmaniasis in Hungary
8. Isolation of (novel) sand fly-borne viruses
9. Geographic spread of sand fly-borne phleboviruses
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, phlebotomine sand flies (Diptera, Psychodidae, subfamily Phlebotominae) 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 (family Bunyaviridae), which includes the sandfly fever Sicilian and Toscana viruses, and the Vesiculovirus genus (family 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.

Cutaneous leishmaniasis; photograph by CDC/Dr. D.S. Martin


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 Mediterranean 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
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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. Changing 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
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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 (Ntaias 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 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.


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 in this area with long-lasting insecticide-treated bed-nets is in progress.

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.

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 *Leishmania major* in a sand fly of the *Sergentomyia* genus in Europe. 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.

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.

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, 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 behavior 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.

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.

Granada hare (*Lepus granatensis*); photograph by Luis Egido
Landscape of the area of the outbreak; photograph by the Medical Entomolgy Unit, ISCIII, Spain

Xenodiagnosis of hares; photograph by the Medical Entomology Unit, ISCIII


Jiménez M., González E., Martín-Martín I., Hernández S., Molina R.: 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


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 autochthonous 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.

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.

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 physician 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.

10. Contributions

Contributions to this issue came from Dr. Nils Kley, Dr. Katja Schmidt and Prof. Dr. Martin H. Groschup (all Friedrich-Loeffler-Institut, Greifswald-Isle of Riems, Germany). We are grateful for critical proofreading by Dr. Carla Maia (IHMT, Lisbon, Portugal), Dr. Ricardo Molina (ISCIII, Madrid, Spain), Prof. Dr. Petr Volf (Charles University in Prague Faculty of Science, Czech Republic) and Dr. Marina Gramiccia (Istituto Superiore di Sanità, Rome, Italy). Finally, we would like to thank Andrew Lewer (Argoat Communications) for editorial assistance.

Imprint

The Public Health Telegram on published vector-borne research results by the EDENext project partners is compiled by the EDENext Public Health group and is published at regular intervals.

For more details about the project, visit www.edenext.eu

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