



Eurosurveillance

Europe's leading journal on infectious disease epidemiology, prevention and control

Vol. 15 | Weekly issue 10 | 11 March 2010

EDITORIALS

- A perspective on emerging mosquito and phlebotomine-borne diseases in Europe** 2
by G Hendrickx, R Lancelot

REVIEW ARTICLES

- West Nile virus in Europe: understanding the present to gauge the future** 4
by P Reiter
- Yellow fever and dengue: a threat to Europe?** 11
by P Reiter
- Rift Valley fever - a threat for Europe?** 18
by V Chevalier, M Pépin, L Plée, R Lancelot
- Leishmaniasis emergence in Europe** 29
by PD Ready
- Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review** 40
by J Depaquit, M Grandadam, F Fouque, P Andry, C Peyrefitte

PERSPECTIVES

- Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness** 48
by HC Maltezou, L Andonova, R Andraghetti, M Bouloy, O Ergonul, F Jongejan, N Kalvatchev, S Nichol, M Niedrig, A Platonov, G Thomson, K Leitmeyer, H Zeller

A perspective on emerging mosquito and phlebotomine-borne diseases in Europe

G Hendrickx (info@avia-gis.be)¹, R Lancelot²

1. Avia-GIS, Agro-Veterinary Information and Analysis, Zoersel, Belgium

2. CIRAD, French Agricultural Research Centre for International Development, UMR CMAEE (Control of emerging and exotic animal diseases), Montpellier, France

Citation style for this article:

Citation style for this article: Hendrickx G, Lancelot R. A perspective on emerging mosquito and phlebotomine-borne diseases in Europe. Euro Surveill. 2010;15(10):pii=19503. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19503>

This article has been published on 11 March 2010

Emerging infectious diseases are of increasing concern worldwide and in particular in Europe. In a review, Jones *et al.* have shown that between 1940 and 2004, the majority of emerging infectious diseases occurred in areas with both a high mobility and high density of population, notably in Western Europe. Furthermore, nearly a third (29%) of the recorded events related to emerging infectious diseases were due to vector-borne diseases during the decade 1990-2000[1].

This issue of Eurosurveillance presents a series of review articles with a particular focus on arthropod-borne diseases transmitted by mosquitoes and phlebotomine sandflies. Each of the papers addresses a series of issues of common interest such as the relevance of the disease in the given context, its transmission and epidemiology, including the current geographical distribution, and clinical symptoms, diagnostic methods, treatment strategies and prevention methods. Furthermore, the papers describe factors triggering changes in distribution of the vectors and disease and risk prediction models.

A review on West Nile virus by Reiter includes a fresh and innovative viewpoint on the epidemiology and transmission of the disease [2], and the same author contributed further with a twin-review on two diseases which have much in common: yellow fever and dengue [3]. Most importantly, both have a history of occurrence in Europe and vectors and pathogens are spreading through increased movement of persons and transport of goods. Chevalier *et al.* present a review on Rift Valley fever a mosquito-borne disease at Europe's fringes. The epidemiology of Rift Valley fever is fascinating because of its complex cycle where the virus may remain dormant for many years and outbreaks involve both vector related and direct transmission. The disease may become a risk in the future in countries bordering the Mediterranean Sea, mainly through increased livestock trade.

Two authors have contributed reviews on viruses transmitted by phlebotomine sandflies. Ready has written

about *Leishmania*, a parasite of particular relevance to Europe because it is currently established around the Mediterranean Sea, but known to be spreading north [4]. Depaquit *et al.* have contributed a review on a number of less well known Phlebo-, Vesiculo- and Orbiviruses such as Sandfly fever Sicilian and Naples virus, Toscana virus Chandipura virus and others that are transmitted by sandflies in Europe and more specifically around the Mediterranean Sea [5]. The paper summarizes the current knowledge on these viruses which have a potential to spread throughout the distribution zone of their phlebotomine vectors in Europe. Both reviews provide a series of maps displaying country based information on the distribution of the disease.

In addition to these papers, the issue features a perspective paper by Maltezos *et al.* presenting the present situation of Crimean-Congo hemorrhagic fever, a tick-borne disease, in Europe and emphasizing relevant aspects for preparedness concerning the potential spread of this disease in Europe in the future [6].

As will become clear from reading these reviews, often crucial knowledge is still missing which is needed to anticipate, prevent or prepare for the establishment and spread of vector-borne diseases. One of these is reliable information on the continent wide distribution of potential disease vectors. National presence-absence maps, as shown by Ready [4] and Depaquit [5] are a first step in this direction and need further refinement.

In 2007-08, the European Centre for Disease Prevention and Control funded the V-borne project with the aim to identify and document vector-borne diseases relevant for public health in Europe, provide an overview of the existing relevant resources, carry out a qualitative multi-disciplinary risk assessment within the limits of the available information and data, and identify priorities for future prevention and control of vector borne diseases in Europe. Building on the expertise from this network, ECDC created a European network for

arthropod vector surveillance for human public health, the VBORNET in 2009 [7]. The network will establish pan-European state of the art maps of validated vector distributions that can be used as basis for risk assessment studies thus contributing to preparedness for the emerge or re-emerge of vector-borne disease in Europe.

References

1. Jones KE, Pate NG, Levy MA, Storeygard A, Balk D, Gittleman JL et al. Global trends in emerging infectious diseases. *Nature*. 2008;451:990-3. Available from: <http://dx.doi.org/10.1038/nature06536>
2. Reiter P. West Nile virus in Europe: understanding the present to gauge the future. *Euro Surveill*. 2010;15(10):pii=19508. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19508>
3. Paul Reiter Dengue and Yellow fever *Eurosurveillance*. *Euro Surveill*. 2010;15(10):pii=19509. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19509>
4. Chevalier V, Pépin M, Plée L, Lancelot R. Rift Valley fever - a threat for Europe?. *Euro Surveill*. 2010;15(10):pii=19506. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19506>
5. Depaquit J, Grandadam M, Fouque F, Andry P, Peyrefitte C. Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review . *Euro Surveill*. 2010;15(10):pii=19507. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19507>
6. Maltezos HC, Andonova L, Andraghetti R, Bouloy M, Ergonul O, Jongejan F, Kalvatchev N, Nichol S, Niedrig M, Platonov A, Thomson G, Leitmeyer K, Zeller H. Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness. *Euro Surveill*. 2010;15(10):pii=19504. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19504>
7. European Centre for Disease prevention and Control (ECDC). Network of medical entomologists and public health experts (VBORNET). Available from: <http://www.ecdc.europa.eu/en/activities/diseaseprogrammes/Pages/VBORNET.aspx>

West Nile virus in Europe: understanding the present to gauge the future

P Reiter (paul.reiter@pasteur.fr)¹

1. Insects and Infectious Disease Unit, Institut Pasteur, Paris, France

Citation style for this article:

Citation style for this article: Reiter P. West Nile virus in Europe: understanding the present to gauge the future. *Euro Surveill.* 2010;15(10):pii=19508. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19508>

This article has been published on 11 March 2010

The appearance of West Nile virus in New York in 1999 and the unprecedented panzootic that followed, have stimulated a major research effort in the western hemisphere and a new interest in the presence of this virus in the Old World. This review considers current understanding of the natural history of this pathogen, with particular regard to transmission in Europe.

Background

West Nile virus (WNV) is by far the most widely distributed arbovirus. It belongs to the Japanese encephalitis antigenic complex of the family *Flaviviridae*, transmitted in an avian cycle by ornithophilic mosquitoes, chiefly of the genus *Culex* [1]. Mammals can also be infected, but are considered dead end hosts because viraemia is generally too low to infect mosquitoes [2].

Mosquitoes acquire infection by feeding on a viraemic host. Virus passes through the gut wall into the haemolymph, the 'blood' of the insect, after which replication occurs in most of the internal tissues. When the salivary glands are infected, the virus can pass to a new host via saliva injected into the skin by the insect when it takes a blood meal. The period from the infective blood meal to infectivity, the extrinsic incubation period, lasts 10-14 days depending on temperature. Ornithophilic vectors that also bite and infect mammals, including humans, are termed bridge vectors.

Human infections attributable to WNV have been reported in many countries in the Old World for more than 50 years [3-5]. In recent years these have included Algeria 1994 (eight deaths) [6], Romania 1996-2000 (21 deaths) [7], Tunisia 1997 (eight deaths) [8], Russia 1999 (40 deaths) [9], Israel 2000 (42 deaths) [10], and Sudan 2004 (four deaths) [11]. By far the largest outbreaks occurred in Bucharest in 1996 (393 hospitalised cases, 17 deaths) and Volgograd in 1999 (826 hospitalised cases, 40 deaths). Both occurred in urban areas and were associated with cellars flooded with sewage-polluted water in poorly maintained apartment blocks, a highly productive breeding site for an effective vector, *Culex pipiens* [7,9,12]. Outbreaks on this scale have also occurred in Israel [13]. All three sites are on major migratory routes of birds that overwinter in Africa.

In its original range, WNV is enzootic throughout Africa, parts of Europe, Asia and Australia, but it received little attention until 1999, when a topotype circulating in Tunisia and Israel appeared in the Bronx, New York [14,15], probably imported in a live bird. The epizootic that followed was spectacular and unprecedented: within five years, the virus appeared ubiquitous, sometimes common, in nearly all counties of every state east of the Rocky Mountains, as well as parts of western Nevada and southern California. Sizeable outbreaks were also observed in six Canadian provinces. It is now widely established from Canada to Venezuela. To date (1999-2009), 29,606 clinical cases and 1,423 deaths have been reported in humans, and more than 27,000 cases in horses, with a case fatality rate of about 33% [16]. Two thirds of the horse population in the United States are now vaccinated, but no vaccine is available for humans.

The virus

Two lineages of WNV are widely recognised that are about 30% divergent [14]. Lineage I includes WNV strains from Africa, the Middle East, Europe, India, Australia (formerly Kunjin virus) and the Americas. The close relationship between isolates from Kenya, Romania and Senegal are evidence of the geographic mobility of the virus in migratory birds [17]. The virus isolated in the Bronx, New York in 1999 was closely related to Lineage I strains circulating in Israel and Tunisia a year earlier [18] and most probably imported in a wild bird. Until recently, all isolates of Lineage II were from Sub-Saharan Africa and Madagascar, but in 2004, it was isolated from a goshawk in Hungary, and from several birds of prey in 2005 [19].

At least five new lineages have been proposed for strains isolated in central Europe, Russia and India [20-23]. This is not surprising, given that the original range of the virus spans Europe, Africa, Asia and Australia, the increasing accessibility of sequencing technology, and the enormous interest in the virus since its appearance in North America. Lastly, a new genotype was identified in the US in 2003 and may now be the dominant strain in North America [24,25].

Pathology

Only a small portion of human infections are symptomatic, with the headache, tiredness, body aches and swollen lymph glands typical of many febrile diseases. Occasionally there is an abdominal rash. About one in 150 patients develop one or multiple indicators of neuro-invasive disease; neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, and paralysis. This can occur in people of any age, but those over 50 years are at highest risk [26]. In the past five years, 4.8% of laboratory-confirmed clinical infections reported in the US were fatal. Symptomatic infections in horses are also rare and generally mild, but can cause neurologic disease including fatal encephalomyelitis [27].

In the Old World, mortality in birds associated with WNV infection is rare [28], although significant numbers of storks and domestic geese died during epizootics in Israel [29]. In striking contrast, the virus is highly pathogenic for New World birds; the appearance of large numbers of dead or dying birds is often an indicator of local transmission [30]. In the early days following appearance of the virus in the US, it appeared that members of the crow family (*Corvidae*) were particularly susceptible, but virus has been detected in dead and dying birds of more than 250 species – with viraemia as high as 109 pfu/ml – as well as various species of mammals and even in alligators [1]. In rural Europe, in the absence of large-scale bird mortality, neurologic symptoms in horses are often the sole indication of local presence of the virus.

Vectors

Mosquitoes of the genus *Culex* are generally considered the principal vectors of WNV, both in the Old World and in the Americas. Studies with bird-baited traps in the wetlands of Mediterranean Europe indicate four such species are dominant. For example, in a region of the Danube delta that has enormous populations of resident and migrant birds, 82% of mosquito captures in 2008 (>10,000 mosquitoes, 17 species) were of three species: *Cx. pipiens* (44%), *Cx. torrentium* (27%) and *Cx. modestus* (11%). *Coquilleltidia richardii* (14%) and *Anopheles maculipennis* (3%) made up all but 1% of the remaining species (F-L Prioteasa, personal communication). In contrast, *Cx. modestus* and *C. richardii* were the dominant species captured on humans in the same area (35% and 34%, respectively), while *Cx. pipiens* was one of five species that contributed less than 2% of the catch. On the other hand, 93% of all mosquitoes captured by bird-baited traps in a village were *Cx. pipiens*, 5% were *Cx. torrentium*, and neither *Cx. modestus* nor *C. richardii* were present. Similarly, in many urban areas, *Cx. pipiens* is the dominant species, and blood-meal analysis confirms that it is highly ornithophilic [31]. These data illustrate the complex relationship between abundance, species composition, host preference and vector competence. It has been suggested that a decline in bird populations in the autumn migration season augments the incidence of mammal-biting,

but this is not borne out by field studies in Chicago, Illinois, an area of intense transmission [32].

In a study in the Danube delta study WNV was indicated by RAMP kit (Response Biomedical Corporation, Canada; a commercial kit based on WNV-specific antibodies with high specificity and sensitivity, [33,34]) in 14 pools of mosquitoes: 11 of *Cx. pipiens*, two of *Cx. torrentium* and one of *An. maculipennis* (F-L Prioteasa, personal communication). In a laboratory study of mosquitoes from the Rhone delta, France, infection and transmission rates were 89.2% and 54.5%, respectively, for *Cx. modestus*, and 38.5% and 15.8%, respectively, for *Cx. pipiens* [35,36]. Coupled with this high potential as a vector, *Cx. modestus* is abundant in reed-beds that are very probably an important ecotope for WNV transmission.

In New York following the appearance of the virus in the US, WNV RNA was detected in three pools of overwintering *Cx. pipiens*, and virus was isolated from one of these [37]. In the Czech Republic, virus was detected in overwintering *Cx. pipiens* by PCR, but not confirmed by isolation (Z Hubalek and Iwo Rolf, personal communication), and in the Danube delta region, four pools of *Cx. pipiens* and one of *An. maculipennis* tested positive by RAMP (F-L Prioteasa, personal communication). These results, although not confirmed by virus isolation, are particularly interesting because a field study of *Culex* species in Massachusetts, US, confirmed that females do not feed on blood before overwintering (P. Reiter, unpublished data). This implies that these insects acquire their infection by vertical transmission between generations via the egg stage. Moreover, in the spring of the year of the study, a few days after mosquitoes had exited their overwintering sites, a number of WNV-positive crows were collected in a neighbouring states, circumstantial evidence that infected overwintering females had transmitted virus to these birds in their first (post-winter) blood meal. Lastly, WNV has been isolated from male *Cx. pipiens* in Connecticut, US, further evidence of vertical transmission [38], and from larvae of *Cx. univittatus* s.l. in the Rift Valley, Kenya [39].

Transmission between vertebrates

In a landmark study, 25 bird species representing 17 families and 10 orders were exposed to WNV by infectious mosquito bite. Only four of 87 individuals did not develop a detectable viraemia [40]. The most competent species, judged by magnitude and duration of viraemia, were passerines (perching birds, 11 species, including members of the crow family) and charadriiformes (a wader and a gull). In surviving birds, the infection persisted in certain organs in 16 of 41 infected birds until euthanised on day 14 after infection. In addition, five of 15 species (representing 11 families) became infected when virus was placed in the back of the oral cavity (either in suspension or as a single infected mosquito) and crows were infected

when fed a dead infected sparrow. Furthermore, virus was observed in the faeces of 17 of 24 species and in the oral cavity of 12 of 14 species for up to 10 days after infection. Moreover, contact transmission between cage mates was observed in four species. In summary, birds can be infected by a variety of routes other than mosquito bites, and different species may have different potential for maintaining the transmission cycle.

In the light of this complexity, the spectacular conquest of the New World by WNV demands attention. Mosquito-borne transmission involves both the extrinsic and intrinsic incubation periods; even at high ambient temperatures this takes a minimum of 10–14 days, so it is hard to imagine that the virus could have traversed an entire continent in a period of four or five summers by this mechanism alone. Importation by infected migrant birds returning from their overwintering grounds could explain the distribution. Indeed, a new region of transmission, separate from the northern states, did appear in Florida and adjoining states two years after the initial New York infestation, presumably introduced by infected migrant birds, but thereafter the virus progressed rapidly westward along a broad front stretching from Canada to the Gulf of Mexico [41]. By 2003, by far the majority of counties east of the Rocky Mountains had reported confirmed WNV-positive dying birds.

An alternative explanation for dispersal rests on oral infection: crows are scavengers and feed on carrion, including dead crows. They are social birds, roost in large crowded colonies, have a wide daily dispersal range of up to 20 km in all directions, and their feeding grounds overlap with crows from other roosts. They also exhibit “kin-based cooperative breeding” in which grown offspring remain with their parents to rear new young [42]. It is conceivable that: (i) crows that die away from their roost relay virus by oral infection to birds from neighbouring roosts; (ii) faecal-oral transmission is significant in crowded roosts, (iii) crows feeding on carcasses of other infected species/animals introduce the infection to others in their roosts, and (iv) viraemic adult and juvenile birds infect nestlings per os. In this way, bird-to-bird transmission, particularly among social birds, could be a major, even the principal driver of amplification and dispersal, with mosquito-borne transmission active at the local level. Modelling studies give some support for this hypothesis [43].

There is also good evidence that oral and faecal-oral infection may be important in transmission dynamics in other species. In the New World, mortality in many species of raptors is out of all proportion to their abundance in nature [44–46]. Fatal infections in Imperial Eagles in Spain and goshawks in Hungary [47], high seroprevalence in kestrels in Egypt [48], and high mortality in flocks of domestic geese in Israel [49] and Hungary [47] point to the same mechanism.

Oral infection is not limited to consumption of dead or dying birds. For example, adult hamsters are readily infected by ingestion of infected material as well as by mosquito bite [50]. In these animals, virus is rapidly cleared from the blood, but can survive in the central nervous system for at least 86 days [51]. Moreover, as a chronic renal infection, virus is excreted in the urine for at least eight months [52]. Thus, even if viraemia in mammals is insufficient to infect mosquitoes, it may still contribute to infection of scavengers and raptors. Circumstantial evidence for this may be the high mortality of owls, which largely feed on nocturnal rodents and other small mammals. For example, an epizootic of 64 dead or dying Great Horned Owls received by a wildlife rehabilitation centre in Ohio in the space of six weeks was attributed to WNV [53], and there are similar reports from other sites in the US. Lastly, large die-off in an alligator farm in Georgia has been attributed to the alligators’ diet of horse meat [54].

In the 1950s, up to 100% of hooded crows (*Corvus corone sardonius*) and more than 80% of the human population sampled in a group of villages at the southern end of the Nile delta, 50 km north of Cairo, Egypt, were seropositive for WNV, and more than 80% of the human population were also seropositive [55]. Laboratory studies confirmed that the birds were highly susceptible to WNV infection with consistently high titres of viraemia. The African species is not markedly social in habits, but it may be that, as in the New World, carrion feeding contributes to the high infection rate, and it is tempting to speculate that the virus is particularly adapted to corvids and raptors. Moreover, these birds feed by tearing shreds of meat from carrion or prey and packing them into a large storage bolus in the crop, after which fragments of the bolus are moved, piece by piece, to the stomach. Virus will be destroyed by the low pH of the stomach, but presumably until then, infection can occur by contact with the walls of the crop.

The contrast in pathogenicity between the Old and the New World is indicative of a long association between the virus and its avian hosts in its original range. Indeed, bird species with low mortality in the Americas are those that, like the virus, are exotics imported from the Old World. In this context, there is a clear parallel with another Old World flavivirus, yellow fever virus, which was transported to the Americas from Africa in the slave trade. In its original range, infections in wild primates, the enzootic hosts, are asymptomatic, but in the Americas, the virus is lethal to monkeys; local inhabitants recognise an epizootic when the rain forest goes ‘silent’ because of mass mortality among Howler monkeys. In both cases, the introduction of an exotic zoonotic virus that is not pathogenic in its original range (presumably because it has a long history of contact with its hosts) has had a catastrophic impact on the local fauna in its new habitat. This is an important point: it is probably inappropriate to suggest that WNV will emerge as a serious pathogen in the Old

World on the basis of what has happened in the past decade in the Americas.

Bird migration

In a serosurvey along the entire Nile valley, from southern Sudan to the Nile delta, seropositive humans were present at 39 of 40 locations [48]. The river is one of the world's major routes for migrating birds, and the continuation of this flyway into Europe, via the Levant, the Bosphorus and into eastern Europe, is a pathway with a consistent history of equine and human cases of WNV. Indeed, more than 130 records of suspected and confirmed WNV infection, dating back to the 1950s, have recently been collected from archives of health reports in Romania (G. Nicolescu, personal communication). It is interesting, however, that although the seasonal pattern of West Nile fever cases in Egypt and Romania is roughly synchronous, the seroprevalence data suggest a much higher and more consistent rate of transmission south of the Mediterranean. Moreover, the Nile valley study, there was little indication of significant mortality in humans; WNV appeared to be a childhood infection and the majority of people in older cohorts, who are more vulnerable to central nervous system complications, were already immune.

In a study of 25 species of birds captured in the Guadalquivir delta, southern Spain, trans-Saharan migrant species had higher seroprevalence and higher antibody titres than resident and short-distance migrants, evidence that the migrants are primarily exposed to WNV in areas with higher circulation of virus, rather than in Europe. Indeed, a study in Senegal, where several of these species overwinter, revealed seroprevalence in horses as high as 90% [56], recalling the high seroprevalence observed along the river Nile [48]. An interesting point regards infections in horses: morbidity and mortality has not been documented in Egypt or Senegal, perhaps an indication of innate immunocompetence in areas of high circulation. The same may apply to Romania, which has a population of about a million horses but little evidence of symptomatic infections.

Transport of virus

As already stated, commonality between viral sequences in different geographic areas is clear evidence of transportation in birds. This raises the question: how is it possible that a bird, in which viraemia lasts at most seven or eight days, can carry virus over distances of thousands of kilometres in a flight that lasts many weeks? The simplest explanation is that migrants *en route* have refuelling stops where they rest and feed before continuing their journey; at these sites, virus could be transmitted between migrants, and to local resident species, so that stopovers become foci of infection. This is plausible at certain sites, for example at desert oases, but transmission in, for example, the Nile valley occurs in mid-summer, after the passage of spring migrants [48]. An alternative explanation is that ectoparasites, such as hippoboscids and

ticks, may constitute the real reservoir, carrying the virus on their avian hosts, and somehow transferring it to new birds at the migration destination. Lastly, it has been suggested that migration is stressful, and that this stress may cause a recrudescence of virus in birds with chronic infection. There is no physiological evidence for such stress, and indeed corticosterone levels rise after migration is complete [57]. Moreover, it is unlikely that viraemia in immunocompromised birds would attain levels sufficient to infect mosquitoes. A more likely possibility is that latent virus enters the transmission cycles when migrants are consumed by scavengers or raptors, or when feeding their young.

Vector control

In the US, ultra-low-volume fogging with adulticidal aerosols of insecticides delivered from road vehicles is widely used to combat WNV vectors and nuisance mosquitoes in residential areas. Unfortunately, the efficacy of this technique is affected by spacing between buildings, distance between roads, amount and type of vegetation, wind, convection and many other factors, and realistic field evaluations have given markedly variable results [58,59]. Moreover, aerosols do not affect the aquatic stages of the insects, and mortality of adults is restricted to those that are in flight and exposed in the short time, a matter of minutes, that the aerosol is airborne in lethal concentrations. For this and many other reasons, the epidemiological impact of fogging is hard to assess [60], and may be minor at best.

In Europe, most transmission is associated with wetland areas of high biodiversity where, apart from difficulty of access, the use of insecticides is undesirable. In urban areas, a logical approach is the elimination of larval habitat. Graded drainage systems and other measures of basic sanitation are key to eliminating the problem at source, but this is not always straightforward. For example, water in catch-basins (settling tanks below street-drains) can be a major source of *Cx. pipiens* during dry weather, but they are difficult to treat effectively because they are flushed by rainfall. The problem in poorly constructed apartment buildings, such as occurred in Bucharest, will require major reconstruction.

Weather and WNV recrudescence

A great deal of attention has been paid to the potential impact of climate change on the prevalence and incidence of mosquito-borne disease [61]. Given that WNV is rarely evident in the Old World, however, it is hard to assess the role of climatic factors in its transmission. In this context it is therefore pertinent to review knowledge about Saint Louis encephalitis virus (SLEV), a closely related counterpart in the New World that has been the subject of research in the Americas since the 1930s, for the similarities to WNV are striking:

- Both are flaviviruses in the Japanese encephalitis complex.

- Both are transmitted between birds by ornithophilic mosquitoes, mainly of the genus *Culex*.
- Transmission of SLEV is rarely evident because infections in birds are asymptomatic, as is the case with WNV in the Old World.
- As in Europe, urban epidemics of Saint Louis encephalitis have occurred in areas of poor sanitation, where sewage-polluted ditches and other collections of organically rich water lead to large numbers of *Culex* mosquitoes.
- Infection of humans and horses can cause encephalitis, sometimes fatal.
- Mammals appear to be dead-end hosts; viraemia is insufficient to infect mosquitoes.

In common with WNV and indeed many other arboviruses, SLEV can remain undetected over long periods of time. It is only at erratic intervals, sometimes separated by several decades, that a sudden recrudescence is observed, occasionally developing into a significant epizootic. For example, the last major outbreak of Saint Louis encephalitis in North America was in 1976, yet despite a massive increase in surveillance of mosquitoes and birds for WNV (with simultaneous testing for SLEV), only a few small outbreaks have been documented in the past 25 years.

Attempts have been made to associate Saint Louis encephalitis outbreaks with specific weather conditions. In regions of the US where *Cx. pipiens* and a second species, *Cx. restuans*, are the principal vectors, a pattern of mild winters, cold wet springs and hot dry summers has been associated with epizootics and human cases [62]. In the period since the first major epidemics to be recognised (more than 1,000 cases in St. Louis, Missouri, in neighbourhoods with primitive sanitation and extensive sewage-polluted ditches in 1932 and 1933) the pattern holds true for some, but by no means all outbreaks, nor for years with such conditions but no outbreaks. Hot dry summers are liable to result in large accumulations of organically polluted stagnant water, favoured breeding habitat for *Cx. pipiens*, but although a number of summers have fitted this description since the appearance of WNV, and despite an enormous increase in vigilance for WNV, evidence of SLEV transmission has been unusually low. Similarly in Europe, the summers of 1996 and 1999 were unusually hot and dry and coincided with outbreaks in Bucharest and Volgograd, but even hotter and drier years have occurred since then without any accompanying transmission. In short, the causes for recrudescence of both viruses remain enigmatic, and it may well be impossible to associate periods of transmission with specific patterns of weather. Indeed, given that the cradle of transmission is almost certainly south of the Sahara, we may need to look to the African continent for clues; transmission in Europe may represent the tip of the iceberg which has its main mass in the tropics.

Future of WNV in Europe

As already stated, the spectacular panzootic of WNV in the Americas has drawn attention to this virus, and it has been suggested that it is also an emerging pathogen in the Old World. It is important to put this into perspective: even if we include the urban outbreaks in Romania and Russia, less than 200 deaths in humans have been recorded over the past decade, and the number of equine cases is in the same order of magnitude. While it is true that an increasing number of small outbreaks, mainly among horses, have been reported, at least part of this increase was probably due to increased awareness of the virus, and major improvements in surveillance and diagnostic facilities.

One point is clear: the importation and establishment of vector-borne pathogens that have a relatively low profile in their current habitat is a serious danger to Europe and throughout the world. It is a direct result of the revolution of transport technologies and increasing global trade that has taken place in the past three decades. Modern examples include the global circulation of dengue virus serotypes [63], the intercontinental dissemination of *Aedes albopictus* and other mosquitoes in used tires [64,65], the epidemic of chikungunya virus in Italy [66], and the importation of bluetongue virus and trypanosomiasis into Europe [67,68]. Thus, if for example SLEV were to be introduced into the Old World, there is every reason to believe that it would spark a panzootic analogous to that of WNV in the western hemisphere. In short, globalisation is potentially a far greater challenge to public health in Europe than any future changes in climate [69].

Acknowledgements

I am grateful to Guy Hendrickx and Sarah Randolph for helpful suggestions. This review is based on a literature study conducted as part of the European Centre for Disease Prevention and Control funded V-borne project "Assessment of the magnitude and impact of vector-borne diseases in Europe", tender n° OJ/2007/04/13-PROC/2007/003. It was compiled, edited and reviewed according to the required Euro-Surveillance scientific review format using EDEN funding, EU grant GOCE-2003-010284 EDEN. The paper is catalogued by the EDEN Steering Committee as EDEN0196 (<http://www.eden-fp6project.net/>). The contents of this publication are the responsibility of the authors and do not necessarily reflect the views nor of the European Centre for Disease Prevention and Control, nor of the European Commission.

References

1. Hayes E, Komar N, Nasci R, Montgomery S, O'Leary D, Campbell G. Epidemiology and transmission dynamics of West Nile virus disease. *Emerg Infect Dis.* 2005;11(8):1167-73.
2. Dauphin G, Zientara S, Zeller H, Murgue B. West Nile: worldwide current situation in animals and humans. *Comp Immunol Microbiol Infect Dis.* 2004;27(5):343-55.
3. Bernkopf H, Levine S, Nerson R. Isolation of West Nile virus in Israel. *J Infect Dis.* 1953;93(3):207-18.
4. Hubalek Z, Halouzka J, Juricova Z. West Nile fever in Czechland. *Emerg Infect Dis.* 1999;5(4):594-5.
5. Murgue B, Murri S, Triki H, Deubel V, Zeller HG. West Nile in the Mediterranean basin: 1950-2000. *Ann N Y Acad Sci.* 2001;951:117-26.

6. Le Guenno B, Bougermouh A, Azzam T, Bouakaz R. West Nile: a deadly virus? *The Lancet*. 1996;348(9037):1315.
7. Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI. West Nile encephalitis epidemic in southeastern Romania. *Lancet*. 1998;352(9130):767-71.
8. Feki I, Marrakchi C, Ben Hmida M, Belahsen F, Ben Jemaa M, Maaloul I, et al. Epidemic West Nile virus encephalitis in Tunisia. *Neuroepidemiology*. 2005;24(1-2):1-7.
9. Platonov AE, Shipulin GA, Shipulina OY, Tyutyunnik EN, Frolochkina TI, Lanciotti RS, et al. Outbreak of West Nile virus infection, Volgograd Region, Russia, 1999. *Emerg Infect Dis*. 2001;7(1):128-32.
10. Green MS, Weinberger M, Ben-Ezer J, Bin H, Mendelson E, Gandacu D, et al. Long-term Death Rates, West Nile virus epidemic, Israel, 2000. *Emerg Infect Dis*. 2005;11(11):1754-7.
11. Depoortere E, Kave J, Keus K, Zeller H, Murri S, Legros D. Outbreak of West Nile virus causing severe neurological involvement in children, Nuba Mountains, Sudan, 2002. *Trop Med Int Health*. 2004;9(6):730-6.
12. Savage H, Ceianu C, Nicolescu G, Karabatsos N, Lanciotti R. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. *Am J Trop Med Hyg*. 1999;61(4):600-11. Erratum. *Am J Trop Med Hyg*. 2000;62(1):162.
13. Weinberger M, Pitlik SD, Gandacu D, Lang R, Nassar F, Ben David D, et al. West Nile fever outbreak, Israel, 2000: epidemiologic aspects. *Emerg Infect Dis*. 2001;7(4):686-91.
14. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science*. 1999;286(5448):2333-7.
15. Hayes CG. West Nile virus: Uganda, 1937, to New York City, 1999. *Ann N Y Acad Sci*. 2001;951:25-37.
16. Centers for Disease Control and Prevention; December 2009, available from http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCountog_detailed.htm.
17. Charrel RN, Brault AC, Gallian P, Lemasson JJ, Murgue B, Murri S, et al. Evolutionary relationship between Old World West Nile virus strains. Evidence for viral gene flow between Africa, the Middle East, and Europe. *Virology*. 2003;315(2):381-8.
18. Lanciotti R, Ebel G, Deubel V, Kerst A, Murri S. Complete genome sequences and phylogenetic analysis of West Nile virus strains isolated from the United States, Europe, and the Middle East. *Virology*. 2002;298(1):96-105.
19. Erdelyi K, Ursu K, Ferenczi E, Szeredi L, Ratz F, Skare J, et al. Clinical and pathologic features of lineage 2 West Nile virus infections in birds of prey in Hungary. *Vector Borne Zoonotic Dis*. 2007 Summer;7(2):181-8.
20. Bakonyi T, Hubalek Z, Rudolf I, Nowotny N. Novel flavivirus or new lineage of West Nile virus, central Europe. *Emerg Infect Dis*. 2005;11(2):225-31.
21. Lvov D, Butenko A, Gromashevsky V, Kovtunov A, Prilipov A, Kinney R, et al. West Nile virus and other zoonotic viruses in Russia: examples of emerging reemerging situations. *Arch Virol Suppl*. 2004;(18):85-96.
22. Bondre VP, Jadi RS, Mishra AC, Yergolkar PN, Arankalle VA. West Nile virus isolates from India: evidence for a distinct genetic lineage. *J Gen Virol*. 2007;88(Pt 3):875-84.
23. Kramer LD, Styer LM, Ebel GD. A global perspective on the epidemiology of West Nile virus. *Annu Rev Entomol*. 2008;53:61-81.
24. Davis C, Ebel G, Lanciotti R, Brault A, Guzman H. Phylogenetic analysis of North American West Nile virus isolates, 2001-2004: evidence for the emergence of a dominant genotype. *Virology*. 2005;342(2):252-65.
25. Snapinn KW, Holmes EC, Young DS, Bernard KA, Kramer LD, Ebel GD. Declining growth rate of West Nile virus in North America. *J Virol*. 2007;81(5):2531-4.
26. Hayes EB, Sejvar JJ, Zaki SR, Lanciotti RS, Bode AV, Campbell GL. Virology, pathology, and clinical manifestations of West Nile virus disease. *Emerg Infect Dis*. 2005;11(8):1174-9.
27. Cantile C, Di Guardo G, Eleni C, Arispici M. Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. *Equine Vet J*. 2000;32(1):31-5.
28. Hubalek Z, Halouzka J. West Nile fever--a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis*. 1999;5(5):643-50.
29. Malkinson M, Banet C, Weisman Y, Pokamunski S, King R, Drouet MT, et al. Introduction of West Nile virus in the Middle East by migrating white storks. *Emerg Infect Dis*. 2002;8(4):392-7.
30. Steele KE, Linn MJ, Schoepp RJ, Komar N, Geisbert TW, Manduca RM, et al. Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Vet Pathol*. 2000;37(3):208-24.
31. Hamer GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, et al. *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. *J Med Entomol*. 2008;45(1):125-8.
32. Loss SR, Hamer GL, Walker ED, Ruiz MO, Goldberg TL, Kitron UD, et al. Avian host community structure and prevalence of West Nile virus in Chicago, Illinois. *Oecologia*. 2009;159(2):415-24.
33. Burkhalter KL, Lindsay R, Anderson R, Dibbernardo A, Fong W, Nasci RS. Evaluation of commercial assays for detecting West Nile virus antigen. *J Am Mosq Control Assoc*. 2006;22(1):64-9.
34. Stone WB, Therrien JE, Benson R, Kramer L, Kauffman EB, Eldson M, et al. Assays to detect West Nile virus in dead birds. *Emerg Infect Dis*. 2005;11(11):1770-3.
35. Balenghien T, Vazeille M, Grandadam M, Schaffner F, Zeller H, Reiter P, et al. Vector competence of some French *Culex* and *Aedes* mosquitoes for West Nile virus. *Vector Borne Zoonotic Dis*. 2008;8(5):589-95.
36. Balenghien T, Vazeille M, Reiter P, Schaffner F, Zeller H, Bicot DJ. Evidence of laboratory vector competence of *Culex modestus* for West Nile virus. *J Am Mosq Control Assoc*. 2007;23(2):233-6.
37. Nasci RS, Savage HM, White DJ, Miller JR, Cropp BC, Godsey MS, et al. West Nile virus in overwintering *Culex* mosquitoes, New York City, 2000. *Emerg Infect Dis*. 2001;7(4):742-4.
38. Anderson JF, Andreadis TG, Main AJ, Ferrandino FJ, Vossbrinck CR. West Nile virus from female and male mosquitoes (Diptera: Culicidae) in subterranean, ground, and canopy habitats in Connecticut. *J Med Entomol*. 2006;43(5):1010-9.
39. Miller BM, Nasci RS, Godsey MS, Savage HM, Lutwama JJ, Lanciotti RS, et al. First field evidence for natural vertical transmission of West Nile virus in *Culex univittatus* complex mosquitoes from Rift Valley Province, Kenya. *Am J Trop Med Hyg*. 2000;62(2):240-6.
40. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, et al. Experimental infection of North American birds with the New York 1999 Strain of West Nile virus. *Emerg Infect Dis*. 2003;9(3):311-22.
41. Rappole JH, Hubalek Z. Migratory birds and West Nile virus. *J Appl Microbiol*. 2003;94 Suppl:47S-58S.
42. Baglione V, Canestrari D, Marcos JM, Griesser M, Ekman J. History, environment and social behaviour: experimentally induced cooperative breeding in the carrion crow. *Proc Biol Sci*. 2002;269(1497):1247-51.
43. Hartemink NA, Davis SA, Reiter P, Hubalek Z, Heesterbeek JA. Importance of bird-to-bird transmission for the establishment of West Nile virus. *Vector Borne Zoonotic Dis*. 2007 Winter;7(4):575-84.
44. Nemeth NM, Kratz GE, Bates R, Scherpelz JA, Bowen RA, Komar N. Naturally induced humoral immunity to West Nile virus infection in raptors. *Ecohealth*. 2008;5(3):298-304.
45. Nemeth N, Kratz G, Edwards E, Scherpelz J, Bowen R, Komar N. Surveillance for West Nile virus in clinic-admitted raptors, Colorado. *Emerg Infect Dis*. 2007 Feb;13(2):305-7.
46. Joyner PH, Kelly S, Shreve AA, Snead SE, Sleeman JM, Pettit DA. West Nile virus in raptors from Virginia during 2003: clinical, diagnostic, and epidemiologic findings. *J Wildl Dis*. 2006;42(2):335-44.
47. Glavits R, Ferenczi E, Ivanics E, Bakonyi T, Mato T, Zarka P, et al. Co-occurrence of West Nile Fever and circovirus infection in a goose flock in Hungary. *Avian Pathol*. 2005;34(5):408-14.
48. Taylor RM, Work TH, Hurlbut HS, Rizk F. A study of the ecology of west nile virus in Egypt. *American Journal of Tropical Medicine and Hygiene*. 1956;5:579-620.
49. Banet-Noach C, Simanov L, Malkinson M. Direct (non-vector) transmission of West Nile virus in geese. *Avian Pathol*. 2003;32(5):489-94.
50. Sbrana E, Tonry JH, Xiao SY, da Rosa AP, Higgs S, Tesh RB. Oral transmission of West Nile virus in a hamster model. *Am J Trop Med Hyg*. 2005;72(3):325-9.
51. Siddharthan V, Wang H, Motter NE, Hall JO, Skinner RD, Skirpstunas RT, et al. Persistent West Nile virus associated with a neurological sequela in hamsters identified by motor unit number estimation. *J Virol*. 2009;83(9):4251-61.
52. Tesh RB, Siirin M, Guzman H, Travassos da Rosa AP, Wu X, Duan T, et al. Persistent West Nile virus infection in the golden hamster: studies on its mechanism and possible implications for other flavivirus infections. *J Infect Dis*. 2005;192(2):287-95.
53. Castalia, Ohio; October 2002, available from <http://backtothewild.com/birdofpreyproblem.html>

54. Miller DL, Mauel MJ, Baldwin C, Burtle G, Ingram D, Hines ME, et al. West Nile virus in farmed alligators. *Emerg Infect Dis.* 2003;9(7):794-9.
55. Taylor RM, Work TH, Hurlbut HS, Rizk F. A study of the ecology of west nile virus in Egypt. *American Journal of Tropical Medicine and Hygiene.* 1956;5:579-620.
56. Chevalier V, Lancelot R, Diaite A, Mondet B, Sall B, De Lamballerie X. Serological assessment of West Nile fever virus activity in the pastoral system of Ferlo, Senegal. *Ann N Y Acad Sci.* 2006;1081:216-25.
57. Reneerkens J, Morrison RI, Ramenofsky M, Piersma T, Wingfield JC. Baseline and stress-induced levels of corticosterone during different life cycle substages in a shorebird on the high arctic breeding grounds. *Physiol Biochem Zool.* 2002;75(2):200-8.
58. Reiter P, Eliason DA, Francy DB, Moore CG, Campos EG. Apparent influence of the stage of blood meal digestion on the efficacy of ground applied ULV aerosols for the control of urban *Culex* mosquitoes. I. Field evidence. *J Am Mosq Control Assoc.* 1990;6(3):366-70.
59. Reddy MR, Spielman A, Lepore TJ, Henley D, Kiszewski AE, Reiter P. Efficacy of resmethrin aerosols applied from the road for suppressing *Culex* vectors of West Nile virus. *Vector Borne Zoonotic Dis.* 2006 Summer;6(2):117-27.
60. Newton EA, Reiter P. A model of the transmission of dengue fever with an evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. *Am J Trop Med Hyg.* 1992;47(6):709-20.
61. Reiter P. A field trial of expanded polystyrene balls for the control of *Culex* mosquitoes breeding in pit latrines. *J Am Mosq Control Assoc.* 1985;1(4):519-21.
62. Reiter P. Weather, vector biology and arboviral recrudescence. Monath TP, editor. Boca Raton, FL: CRC Press; 1988.
63. Gubler DJ. The global pandemic of dengue/dengue haemorrhagic fever: current status and prospects for the future. *Ann Acad Med Singapore.* 1998;27(2):227-34.
64. Hawley WA, Reiter P, Copeland RS, Pumpuni CB, Craig GB, Jr. *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. *Science.* 1987;236(4805):1114-6.
65. Reiter P. *Aedes albopictus* and the world trade in used tires, 1988-1995: the shape of things to come? *J Am Mosq Control Assoc.* 1998;14(1):83-94.
66. Angelini R, Finarelli AC, Angelini P, Po C, Petropulacos K, Macini P, et al. An outbreak of chikungunya fever in the province of Ravenna, Italy. *Euro Surveill.* 2007;12(36). pii=3260. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3260>
67. Meroc E, Faes C, Herr C, Staubach C, Verheyden B, Vanbinst T, et al. Establishing the spread of bluetongue virus at the end of the 2006 epidemic in Belgium. *Vet Microbiol.* 2008;131(1-2):133-44.
68. Moretti G. [African trypanosomiasis detected in France. Difficulties of diagnosis]. *Presse Med.* 1969;77(41):1404. [French].
69. Tatem AJ, Hay SI, Rogers DJ. Global traffic and disease vector dispersal. *Proc Natl Acad Sci U S A.* 2006;103(16):6242-7.

Yellow fever and dengue: a threat to Europe?

P Reiter (paul.reiter@pasteur.fr)¹

1. Insects and Infectious Disease Unit, Institut Pasteur, Paris, France

Citation style for this article:

Citation style for this article: Reiter P. Yellow fever and dengue: a threat to Europe?. Euro Surveill. 2010;15(10):pii=19509. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19509>

This article has been published on 11 March 2010

The introduction and rapidly expanding range of *Aedes albopictus* in Europe is an iconic example of the growing risk of the globalisation of vectors and vector-borne diseases. The history of yellow fever and dengue in temperate regions confirms that transmission of both diseases could recur, particularly if *Ae. aegypti*, a more effective vector, were to be re-introduced. The article is a broad overview of the natural history and epidemiology of both diseases in the context of these risks.

Background

There is logic in dealing with yellow fever and dengue together, for they have much in common:

- Both are caused by viruses of the family *Flaviviridae*, genus *Flavivirus*.
- Both viruses are strictly primatophilic – they only infect primates, including man.
- In their original habitat, both are zoonotic infections transmitted by forest-dwelling mosquitoes.
- Both can cause haemorrhagic illness in humans, often with fatal consequences.
- Both owe their importance as human pathogens to two forest mosquitoes that have become closely associated with the peridomestic environment.
- The viruses and their urban vectors owe their worldwide distribution to transportation of goods and people.
- Both diseases have a history of transmission in temperate regions, including Europe.

According to the World Health Organization, there are currently 200,000 worldwide cases and 30,000 deaths from yellow fever per year, 90% of them in Africa [1], and as many as 50 million cases of dengue [2].

Epidemics of yellow fever, sometimes catastrophic, were once common in North America as far north as New York and Boston (Table), and in European ports as far north as Cardiff and Dublin [3]. Large epidemics of dengue occurred in the same regions from the 18th century onwards. A massive epidemic, estimated at one million cases, with at least 1,000 deaths, occurred in Greece in 1927–28 [4,5]

Aedes aegypti, the primary urban vector for both viruses, was once established as far north in Europe as Brest and Odessa (Figure 1). It disappeared from the entire Mediterranean region in the mid-20th century, for reasons that are not clear. *Ae. albopictus*, generally regarded as a less important vector of dengue [7], is also capable of transmitting yellow fever. It was introduced to Europe in the 1970s, is well established in at least twelve countries (Figure 2) [8], and is likely to spread northwards, perhaps as far as Scandinavia.

The number of persons who visit countries endemic for dengue and yellow fever is continually rising [11,12]. It is therefore cogent to consider whether introduction of these viruses is likely to lead to autochthonous and even endemic transmission in Europe.

Transmission

Five factors are key to the epidemiology of vector-borne diseases: the ecology and behaviour of the host, the ecology and behaviour of the vectors, and the degree of immunity in the population. A holistic view of this complexity is key to assessing the likelihood of transmission in Europe [13].

Origin of the viruses

There is little doubt that the yellow fever virus (YFV) originated in Africa, and that viruses circulating in the New World are of African origin. Curiously, yellow fever has never been recorded in Asia, although *Ae. aegypti* is widespread there.

There are four antigenically distinct DENV serotypes that cause very similar disease in humans. It is widely accepted that all four are of Asian origin [14], although DENV-2 is enzootic in Africa [15].

Zoonotic vectors and hosts

In the Old World, the sylvatic vectors of yellow fever and dengue are canopy-dwelling mosquitoes of the genus *Aedes* and three subgenera, *Stegomyia*, *Finlaya*, and *Diceromyia*, that feed exclusively on monkeys. In the Americas, the principal zoonotic vectors of yellow fever are *Sabethes* and *Haemagogus* species; both are also strictly primatophilic [3].

Sylvatic transmission to humans

Sylvatic infections are acquired when humans enter woodland where there is zoonotic transmission. In recent years, a number of unvaccinated tourists have died of yellow fever after visiting enzootic areas [16,17].

Vector-host specificity

Host specificity is a characteristic of many vectors; it is conceivable that it improves the chances of locating hosts. This may be particularly useful in the sylvatic environment, where bands of monkeys roam between established sleeping sites.

The specificity of DENV and YFV to primatophilic vectors may have evolved to exploit this relationship, and/or to surmount barriers to infection in the insect.

Whatever the reason, given the absence of wild primates, it is unlikely that any vector species native to Europe is able to transmit these viruses.

Peridomestic transmission

Neither YFV nor DENV would have major importance as human pathogens in the absence of two mosquito species, *Ae. (Stegomyia) aegypti* and *Ae. (S.) albopictus*, both of which have become closely associated with the peridomestic environment. Infected humans returning from an enzootic area may initiate transmission to humans in human settlements if either of these species is present (although to date, no yellow fever infections have been attributed to *Ae. albopictus*).

TABLE

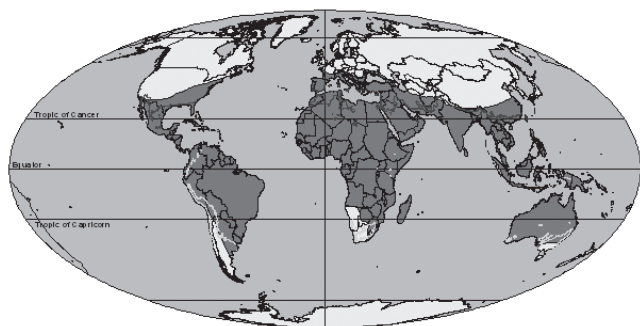
Major epidemics of yellow fever in North America, north of Mexico

Year		Year	
1668	New York, Philadelphia and other settlements	1803	Boston, Philadelphia
1690	Charleston	1804	Philadelphia
1691	Boston	1805	Philadelphia
1693	Charleston, Philadelphia, Boston	1807	Charleston
1694	Philadelphia, New York, Boston	1811	New Orleans, Florida, New Jersey
1699	Charleston, Philadelphia	1817	New Orleans, Charleston, Baltimore
1702	New York	1819	New Orleans, Charleston, Baltimore, Philadelphia, New York
1703	Charleston	1820	New Orleans, Philadelphia
1728	Charleston	1821	New Orleans, Mississippi Valley, Alabama, Charleston, Baltimore, Philadelphia, New York, Boston
1732	Charleston	1822	New Orleans, New York
1734	Charleston, Philadelphia, New York, Albany, Boston	1823	Key West
1737	Virginia	1824	New Orleans, Charleston
1739	Charleston	1825	Mobile, Natchez, Washington
1741	Virginia, Philadelphia, New York	1827	New Orleans, Mobile
1743	Virginia, New York	1828	New Orleans, Memphis
1745	Charleston, New York	1829	Key West, Mobile, Natchez
1747	New Haven	1837	New Orleans, Mobile, Natchez
1748	Charleston	1839	Galveston, Mobile, Charleston
1751	Philadelphia, New York	1841	Key West, New Orleans
1762	Philadelphia	1843	Galveston, Mobile, Mississippi Valley, Charleston
1778	Philadelphia	1847	New Orleans, Mobile, Natchez
1780	Philadelphia	1852	Charleston
1783	Baltimore	1853	New Orleans
1791	Philadelphia, New York	1854	New Orleans, Mobile, Alabama, Charleston
1792	Charleston	1855	Mississippi Valley, Norfolk
1793	Philadelphia	1856	New Orleans, Charleston
1794	Philadelphia	1858	Charleston
1795	Philadelphia	1867	Key West, Galveston, New Orleans, Mobile, Philadelphia
1796	Philadelphia	1870	New York
1797	Philadelphia	1873	New Orleans, Mississippi Valley, Alabama, Memphis
1798	Philadelphia	1876	Charleston
1799	Philadelphia	1877	Port Royal SC
1800	Philadelphia	1878	New Orleans, Memphis, Mississippi Valley to St Louis, Chattanooga, many other cities
1801	Norfolk, New York, Massachusetts	1879	Memphis
1802	Philadelphia	1905	New Orleans

Reproduced from [6] with permission from *Environmental Health Perspectives*.

FIGURE 1

Historical distribution of *Aedes aegypti*



Dark grey areas: maximum range distribution of *Ae. aegypti*, black lines: January 10°C isotherm in the northern hemisphere; mid grey lines: the July 10°C isotherm in the southern hemisphere. The distribution limit broadly fits the 10°C isotherm in the southern hemisphere, but far less so in the northern hemisphere. Source: adapted from a map published by Christophers [9].

Dengue is endemic in many urban and rural populations throughout the tropics. ‘Virgin soil’ epidemics in large cities are often explosive. In 1988, for example, there were an estimated 420,000 cases in four months in the coastal city of Guayaquil, Ecuador [18]

The large urban outbreaks of yellow fever that were common until the early 20th century remain a real and constant danger in enzootic countries that do not enforce routine vaccination. Moreover, it is reasonable to assume that areas that are prone to dengue transmission are equally prone to yellow fever, so areas without history of the latter, including those in south-east Asia, may well be at risk.

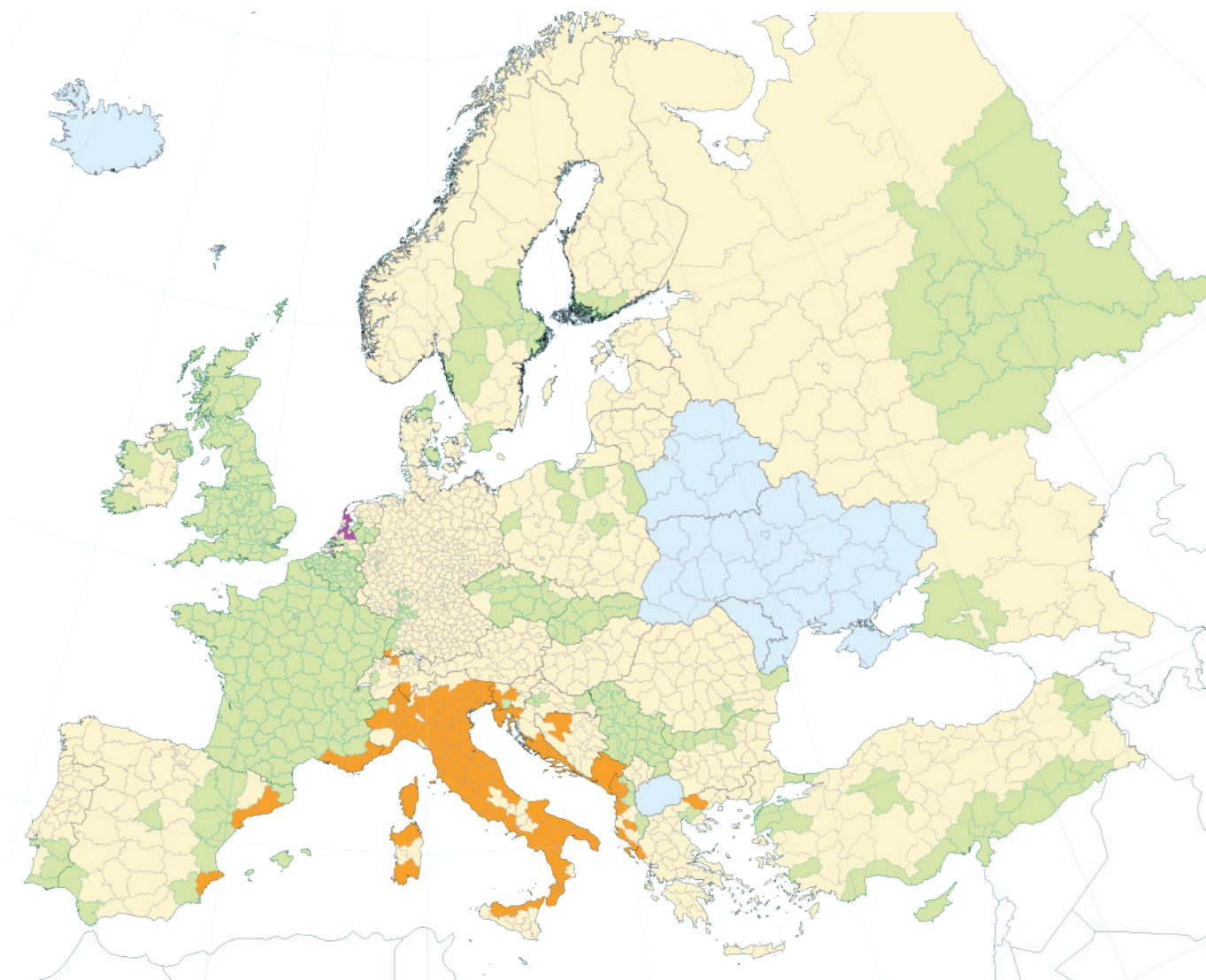
Vectors

The yellow fever mosquito, *Aedes aegypti*

Ae. aegypti is the quintessential urban vector of yellow fever and dengue. It is a remarkable species because

FIGURE 2

Current (2009) distribution of *Aedes albopictus* in Europe by administrative unit



Orange: overwintering expanding populations; purple: populations only observed indoors (in glass houses); green: not detected in past 5 years; pale yellow: no recent data on mosquito fauna; blue: no information on any mosquito studies; white: not included in this study. Source: [10].

the 'domesticated' form is rarely found more than 100 m from human habitation and feeds almost exclusively on human blood. Nevertheless, like its forest ancestor, it remains day-active with a preference for heavy shade. It freely enters homes and other buildings and spends much of its time hidden in dark places, often among clothing, a stable microclimate with few predators. Its human host is abundant and lives under the same roof, an arrangement that minimises the hazards of questing for a blood meal. It lays eggs in man-made objects that contain water, from discarded tires and buckets to the saucers under flowerpots and water-storage barrels. In short, humans are the perfect host: they provide safe shelter, plentiful food and abundant sites for procreation. Indeed, in most cities of the tropics, homes are so close together and breeding sites so abundant that they can be regarded as a single factory for mosquitoes in an urban jungle. In the past three decades, attempts to reduce populations of the species have rarely been successful and never sustained [19,20].

The Asian Tiger mosquito, *Aedes albopictus*

Ae. albopictus is often abundant in the peridomestic environment, particularly in areas with plentiful vegetation. However, in addition to humans, it feeds freely on animals and birds, and so can exist far from human habitation. Since non-primates are not susceptible to the viruses, such blood meals do not contribute to the transmission cycle, and for this reason, *Ae. albopictus* has generally been regarded as a secondary vector [7]. Nevertheless, dengue epidemics have been recorded in places where *Ae. albopictus* is the only vector [21], and in recent years, the species has proved highly effective in urban transmission of another African sylvatic virus, chikungunya virus [22,23].

Globalisation of vectors and viruses

Aedes aegypti

Ae. aegypti and yellow fever arrived in the New World together, as passengers in the slave trade. Slave ships generally made the passage from Africa to the Americas in four to six weeks. The virus was enzootic in regions where the slave caravans captured local inhabitants, and urban transmission was rife in the ports of dispatch. The casks used for shipboard storage of water must have been prolific breeding sites for the mosquito, and the slaves were an abundant source of blood. With the slaves and the mosquito came the virus, and it was not uncommon for ships to arrive in port with large numbers of dying persons aboard, hence the yellow flag of quarantine.

In the United States, the species has been recorded from 21 states (Alabama, Arkansas, Florida, District of Columbia, Georgia, Illinois, Indiana, Kansas, Kentucky, Louisiana, Maryland, Missouri, Mississippi, New York, North Carolina, Ohio, Oklahoma, South Carolina, Tennessee, Texas, and Virginia) [24]. In many of these, winter temperatures below -20°C are not unusual. Presumably the mosquitoes survive in sheltered sites,

for they are not resistant to freezing. Thus there is no obvious climatic reason why the species, were it to be re-introduced, could not survive in most areas in Europe.

Aedes albopictus

In its original range, *Ae. albopictus* was present from Beijing and northern Japan to tropical Asia [25]. In 1983, however, the mosquito was found in Memphis, Tennessee [26], and, two years later, a survey revealed that it was widely distributed, often common, in the southern United States. Investigation revealed a global trade in used tyres that were frequently infested with eggs and larvae of the species [27]. Japan was the principal exporter, and a study of winter diapause at various latitudes in Asia confirmed that the day-length that triggered diapause was identical in the southern United States and in southern Japan [28]. The mosquito is now widespread in the United States, and is a major nuisance species as far north as Nebraska and Illinois, where winter snowfall can be well above 200 cm, average January night-time temperatures are -10°C, and temperatures as low as -33°C have been recorded. It is also established in Mexico and all the countries of Central and South America except Chile. In Africa it is well established in Nigeria, Gabon, Equatorial Guinea and Cameroon [29,30], and in Europe it has been reported from 16 countries [8]. Recent infestations in the Netherlands have been traced to imports of 'lucky bamboo' from sub-tropical China [31], but these mosquitoes do not appear to have survived the winter, perhaps because they have no winter diapause.

Clinical features

Yellow fever

As with most viral diseases, yellow fever can present with a wide spectrum of symptoms, from mild to fatal. In clinical cases, there is generally a sudden onset of fever with severe headache, arthralgias, and myalgia. The striking yellowing of the eyes and skin, caused by hepatic dysfunction, may appear on the third day and indicates a poor prognosis. The fever often follows a 'saddleback' curve, with a brief drop in temperature and symptoms after the third day, followed by a return with increased severity that can lead to spontaneous haemorrhage ('coffee ground' vomit), delirium, renal failure, coma and death. Fatality rates of clinical cases can be as high as 80% [3], on a par with Ebola, Marburg and other haemorrhagic viral infections.

Dengue

As many as 80% of all dengue infections are asymptomatic. Among clinical cases, early stages are similar to those of yellow fever, although with excruciating arthralgia and myalgia, hence the term 'break-bone fever'. Fever and other symptoms rarely last more than seven days, but convalescence can be prolonged and debilitating. The later stages of the illness often include a widespread rash [32].

A portion of dengue cases, usually less than 5%, can be severe and a fraction of these may be fatal [33]. Severe dengue, commonly referred to as dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) to distinguish it from 'classic' dengue, is associated with spontaneous haemorrhage and an increase of vascular permeability that can lead to life-threatening hypovolemic shock. The causes of this condition have been debated for decades, but remain unresolved [34-36]. A widely held but hotly contested hypothesis is that after infection with one serotype, secondary infections by one or more of the others can precipitate the syndrome by a process referred to as antibody-dependent enhancement, but the occurrence of severe dengue in epidemics of primary infection, such as the Greek epidemic and a recent epidemic in Cape Verde [37], contradicts this hypothesis. An associated controversy is the validity of graded sets of criteria to categorise severity that are recommended by the World Health Organization, and these have been revised several times in recent years [38]. Both issues are of prime importance for the management and treatment of patients.

It is a common misconception that DHF/DSS first appeared in the 1950s in south-east Asia. It is certainly true that the syndrome became a serious public health problem in that period, but it was not a new phenomenon: significant mortality associated with haemorrhagic symptoms had been described in the earliest epidemic of dengue-like disease on record, in Philadelphia in 1789, as well as in later epidemics in East Africa and in Australia [14,39]. Moreover, as already mentioned, at least 1,000 people died in the Greek epidemic in 1927-28. In the years after the Second World War, however, rapid expansion of densely populated urban areas, coupled with enormous infestations of *Ae. aegypti*, led to a massive increase in the prevalence and incidence of the disease in south-east Asia, so a plausible explanation for the emergence of this 'new' syndrome is that escalating numbers of classic infections simply led to an increased awareness of the relatively rare manifestations – the 'iceberg effect'.

Treatment

There is no specific treatment for yellow fever or dengue virus infections; supportive therapy is the only option, although there is active research into antiviral drugs against these diseases [40]. For dengue fevers, intravenous fluids are used to counter haemoconcentration, and platelet transfusions in the event of severe thrombocytopaenia [41]. Strict avoidance of anticoagulants, including aspirin, is important.

Prevention Vaccination

Yellow fever

A safe, effective yellow fever vaccine, based on a live attenuated strain, has been available for more than half a century, and mass vaccination is a highly effective approach to prevent urban transmission, but the incidence of the disease, particularly in Africa, confirms

that coverage is inadequate, and there is a real and present danger of a major urban epidemic. Moreover, there is good reason to believe that the 2.5 billion people who live in regions at risk of dengue infection are also at risk of yellow fever; if so, then, given the lax attitude towards vaccination of travellers in most countries, the danger of a catastrophic epidemic beyond regions generally associated with transmission is also real, and this could include parts of Europe infested with *Ae. albopictus*. If such an event were to occur, current stocks of vaccine would probably be inadequate to respond to worldwide demand.

Dengue

No vaccine against dengue is available, but attenuated virus vaccines and second-generation recombinant vaccines are in active development [42]. A large-scale trial (phase IIb) of a chimeric tetravalent vaccine [43] has been under way since February 2009 [44]. If successful, then a vaccine might be licenced within five years.

Vector control

At the beginning of the 20th century, urban yellow fever was eliminated from many countries by energetic campaigns to eliminate *Ae. aegypti* breeding sites. After the Second World War, focal application of the synthetic pesticide dichlorodiphenyltrichloroethane (DDT) to infested containers and their surroundings was an outstanding success; according to the Pan American Health Organization, the species was eradicated from 22 countries in the Americas [45]. The reason for the efficacy of this method has only recently become apparent: 'skip-oviposition' (the deposition of small numbers of eggs in many different sites) made it highly probable that they would encounter treated sites [19]. No substitute for DDT is currently available, so many authorities resort to spraying insecticidal aerosols (ultra-low-volume) of organophosphates or pyrethroids from hand-held machines, road vehicles or aircraft. Unfortunately, the method is expensive and generally ineffective, at least against *Ae. aegypti*, because the species spends much of its time indoors at sites that are inaccessible to the aerosol [20,46]. Moreover, even if a large number of mosquitoes were to be eliminated by this treatment, the impact on adult mosquito populations would probably be too short for an effective impact on transmission [47]. Although the World Health Organization recommends that health authorities evaluate the technique under local circumstances [6], their principal strategy is community-based source reduction, the elimination of breeding sites by the community. Unfortunately, there is no evidence that this approach has been successful in any part of the world.

Control of *Ae. albopictus* is probably even more difficult than for *Ae. aegypti*, given its ability to breed away from human habitation, but insecticidal aerosols may be more effective for *Ae. albopictus* because the mosquito tends to rest outdoors.

The future in Europe

Dengue is essentially an urban disease because of the urban ecology of its vectors and the behaviour of its hosts. Rapid urbanisation has made it an increasingly serious public health problem in the tropics [48]. Millions of people travel from the tropics to Europe and North America each year (for example, 1.2 million people who live in the UK visit the Indian subcontinent, with average stays of 29 days) and, after malaria, dengue infection is the second most frequent reason for hospitalisation after their return [11,12].

The history of dengue and yellow fever in Europe is evidence that conditions are already suitable for transmission. The establishment of *Ae. albopictus* has made this possible, and the possibility will increase as the species expands northwards, or if *Ae. aegypti* is re-established. The epidemic of chikungunya in northern Italy in 2007 [8,49] confirms that *Ae. albopictus* is capable of supporting epidemic transmission, although laboratory studies indicate that the strain of virus involved was particularly adapted to this species [50,51]. Nevertheless, it is not unreasonable to assume that climatic conditions that permit malaria transmission will also support transmission of yellow fever and dengue, in which case transmission could extend into northern Europe [52].

Lastly, it is widely stated that the incidence of vector-borne diseases will increase if global temperatures increase. While there is no doubt that temperature and rainfall play a role in their transmission, it is clear that many other factors are involved [6]. A more urgent emerging problem is the quantum leap in the mobility of vectors and pathogens that has taken place in the past four decades, a direct result of the revolution of transport technologies and global travel [53]. The potential impact of this globalisation of vector-borne diseases is a challenge for the future.

Acknowledgements

This review is based on a literature study conducted as part of the European Centre for Disease Prevention and Control funded V-borne project "Assessment of the magnitude and impact of vector-borne diseases in Europe", tender n° OJ/2007/04/13-PROC/2007/003. It was compiled, edited and reviewed according to the required Euro-Surveillance scientific review format using EDEN funding, EU grant GOCE-2003-010284 EDEN. The paper is catalogued by the EDEN Steering Committee as EDEN0198 (<http://www.eden-fp6project.net/>). The contents of this publication are the responsibility of the authors and don't necessarily reflect the views nor of the European Centre for Disease Prevention and Control, nor of the European Commission.

References

1. World Health Organization (WHO). Yellow Fever. Geneva, December 2009. Fact sheet No 100. Available from: <http://www.who.int/mediacentre/factsheets/fs100/en/>
2. World Health Organization (WHO). Dengue and dengue haemorrhagic fever. Geneva, March 2009. Fact sheet No 117. Available from: <http://www.who.int/mediacentre/factsheets/fs117/en/index.html>
3. Monath TP. Yellow fever as an endemic/epidemic disease and priorities for vaccination. *Bull Soc Pathol Exot*. 2006;99(5):341-7.
4. Papaevangelou G, Halstead SB. Infections with two dengue viruses in Greece in the 20th century. Did dengue hemorrhagic fever occur in the 1928 epidemic? *J Trop Med Hyg*. 1977;80(3):46-51.
5. Rosen L. Dengue in Greece in 1927 and 1928 and the pathogenesis of dengue hemorrhagic fever: new data and a different conclusion. *Am J Trop Med Hyg*. 1986;35(3):642-53.
6. Reiter P. Climate change and mosquito-borne disease. *Environ Health Perspect*. 2001;109 Suppl 1:141-61.
7. Reiter P, Fontenille D, Paupy C. *Aedes albopictus* as an epidemic vector of chikungunya virus: another emerging problem? *Lancet Infect Dis*. 2006;6(8):463-4.
8. Scholte E-J, Schaffner F. Waiting for the tiger: establishment and spread of the *Aedes albopictus* mosquito in Europe. Takken WaKB, editor. Wageningen, The Netherlands: Wageningen Academic Publishers; 2007.
9. Christophers SR. *Aedes aegypti* (L.) the yellow fever mosquito, its life history, bionomics and structure. 1960. Cambridge University Press Cambridge, MA.
10. *Aedes albopictus* distribution maps. European Centre for Disease Prevention and Control (ECDC). Stockholm; March 2010. Available from: http://ecdc.europa.eu/en/activities/pages/programme_on_emerging_and_vector-borne_diseases_maps.aspx
11. Wichmann O, Gascon J, Schunk M, Puente S, Siikamäki H, Gjorup I, et al. Severe dengue virus infection in travelers: risk factors and laboratory indicators. *J Infect Dis*. 2007;195(8):1089-96.
12. Wilder-Smith A, Schwartz E. Dengue in travelers. *N Engl J Med*. 2005;353(9):924-32.
13. Reiter P. Climate change and mosquito-borne disease: knowing the horse before hitching the cart. *Rev Sci Tech*. 2008;27(2):383-98.
14. Gubler D. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. Gubler D, Kuno G, editors. *Dengue and dengue hemorrhagic fever*. Wallingford, Oxon, UK; New York: CAB International; 1997. p. 478.
15. Diallo M, Ba Y, Sall AA, Diop OM, Ndione JA, Mondo M, et al. Amplification of the sylvatic cycle of dengue virus type 2, Senegal, 1999-2000: entomologic findings and epidemiologic considerations. *Emerg Infect Dis*. 2003;9(3):362-7.
16. McFarland JM, Baddour LM, Nelson JE, Elkins SK, Craven RB, Cropp BC, et al. Imported yellow fever in a United States citizen. *Clin Infect Dis*. 1997;25(5):1143-7.
17. World Health Organization (WHO). One imported case of confirmed yellow fever detected in Belgium. *Wkly Epidemiol Rec*. 2001;76:357.
18. Centers for Disease Control and Prevention (CDC). International Notes Dengue Epidemic -- Ecuador, 1988. *MMWR Morb Mortal Wkly Rep*. 1989;38(24):419-421. Available from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/00001411.htm>
19. Reiter P. Oviposition, dispersal and survival in *Aedes aegypti*; implications for the efficacy of control strategies. *Vector Borne Zoonotic Dis*. 2007; Summer;7(2):261-73.
20. Reiter P, Gubler DJ. Surveillance and control of urban dengue vectors. Gubler DJ, Kuno G, editors. *Dengue and Dengue Hemorrhagic Fever*. New York: CAB International; 1997. p. 425-62.
21. Effler PV, Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, et al. Dengue fever, Hawaii, 2001-2002. *Emerg Infect Dis*. 2005;11(5):742-9.
22. Pages F, Peyrefitte CN, Mve MT, Jarjaval F, Brisse S, Iteman I, et al. *Aedes albopictus* mosquito: the main vector of the 2007 Chikungunya outbreak in Gabon. *PLoS ONE*. 2009;4(3):e4691.
23. Renault P, Solet JL, Sissoko D, Balleydier E, Larrieu S, Filleul L, et al. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. *Am J Trop Med Hyg*. 2007;77(4):727-31.
24. Darsie R, Ward R. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Salt Lake City, Utah, USA: American Mosquito Control Association; 1981.
25. Hawley WA. The biology of *Aedes albopictus*. *J Am Mosq Control Assoc Suppl*. 1988;1:1-39.
26. Reiter P, Darsie R. *Aedes albopictus* in Memphis, Tennessee (USA): An achievement of modern transportation? *Mosquito News*. 1984;44:396-9.
27. Reiter P, Sprenger D. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. *J Am Mosq Control Assoc*. 1987;3(3):494-501.

28. Hawley WA, Reiter P, Copeland RS, Pumpuni CB, Craig GB, Jr. *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. *Science*. 1987;236(4805):1114-6.
29. Paupy C, Ollomo B, Kamgang B, Moutailler S, Rousset D, Demanou M, et al. Comparative Role of *Aedes albopictus* and *Aedes aegypti* in the Emergence of Dengue and Chikungunya in Central Africa. *Vector Borne Zoonotic Dis*. 2009 Sep 2.
30. Simard F, Nchoutpouen E, Toto JC, Fontenille D. Geographic distribution and breeding site preference of *Aedes albopictus* and *Aedes aegypti* (Diptera: culicidae) in Cameroon, Central Africa. *J Med Entomol*. 2005;42(5):726-31.
31. Scholte E, Jacobs F, Linton Y, Dijkstra E, Fransen J, Takken W. First record of *Aedes* (*Stegomyia*) *albopictus* in the Netherlands. *European Mosquito Bulletin*. 2007;22:5-9. Available from: http://e-m-b.org/sites/e-m-b.org/files/European_Mosquito_Bulletin_Publications811/EMB22/EMB22_02.pdf
32. Farrar J. Clinical features of dengue. Halstead SB, editor. *Dengue*. London: Imperial College Press; 2008. p. 171-91.
33. Morens DM. Dengue fever and dengue hemorrhagic fever. *Pediatr Infect Dis J*. 2009;28(7):635-6.
34. Halstead SB, Lan NT, Myint TT, Shwe TN, Nisalak A, Kalyanaraj S, et al. Dengue hemorrhagic fever in infants: research opportunities ignored. *Emerg Infect Dis*. 2002;8(12):1474-9.
35. Rosen L. The Emperor's New Clothes revisited, or reflections on the pathogenesis of dengue hemorrhagic fever. *Am J Trop Med Hyg*. 1977;26(3):337-43.
36. Murgue B. Severe dengue: questioning the paradigm. *Microbes Infect*. 2010;12(2):113-8.
37. World Health Organization (WHO). Dengue fever in Cape Verde - update 1. Geneva, November 2009. Available from: http://www.who.int/csr/don/2009_11_18/en/index.html
38. Deen JL, Harris E, Wills B, Balmaseda A, Hammond SN, Rocha C, et al. The WHO dengue classification and case definitions: time for a reassessment. *Lancet*. 2006;368(9530):170-3.
39. Carey DE. Chikungunya and dengue: a case of mistaken identity? *J Hist Med Allied Sci*. 1971;26(3):243-62.
40. Monath TP. Treatment of yellow fever. *Antiviral Res*. 2008;78(1):116-24.
41. Wills B. Management of dengue. Halstead SB, editor. *Dengue*. London: Imperial College Press; 2008. p. 193-217.
42. Webster DP, Farrar J, Rowland-Jones S. Progress towards a dengue vaccine. *Lancet Infect Dis*. 2009;9(11):678-87.
43. Guy B, Nougarede N, Begue S, Sanchez V, Souag N, Carre M, et al. Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects. *Vaccine*. 2008;26(45):5712-21.
44. Efficacy and Safety of Dengue Vaccine in Healthy Children. Washington: National Institutes of Health; February 2010, available from: <http://clinicaltrials.gov/ct2/show/NCT00842530>
45. PAHO. Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control. Washington, DC: Pan American Health Organization; 1994.
46. Reiter P, Nathan M. Guidelines for assessing the efficacy of insecticidal space sprays for control of the dengue vector, *Aedes aegypti*. Geneva 2001. World Health Organization (WHO).
47. Newton EA, Reiter P. A model of the transmission of dengue fever with an evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. *Am J Trop Med Hyg*. 1992;47(6):709-20.
48. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol*. 2002;10(2):100-3.
49. European Centre for Disease prevention and Control (ECDC). Mission Report: Chikungunya in Italy. Joint ECDC/WHO visit for a European risk assessment 17-21 September 2007; Available from: http://ecdc.europa.eu/en/publications/Publications/0709_MIR_Chikungunya_in_Italy.pdf.
50. Tsatsarkin KA, McGee CE, Volk SM, Vanlandingham DL, Weaver SC, Higgs S. Epistatic roles of E2 glycoprotein mutations in adaption of chikungunya virus to *Aedes albopictus* and *Ae. aegypti* mosquitoes. *PLoS One*. 2009;4(8):e6835.
51. Vazeille M, Moutailler S, Coudrier D, Rousseaux C, Khun H, Huerre M, et al. Two Chikungunya isolates from the outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito, *Aedes albopictus*. *PLoS One*. 2007;2(11):e1168.
52. Reiter P. From Shakespeare to Defoe: malaria in England in the Little Ice Age. *Emerg Infect Dis*. 2000;6(1):1-11.
53. Reiter P. A mollusc on the leg of a beetle: human activities and the global dispersal of vectors and vectorborne pathogens. Infectious disease movement in a borderless world. Washington, DC: The National Academies Press; 2010. p. 150.

Rift Valley fever - a threat for Europe?

V Chevalier (veronique.chevalier@cirad.fr)¹, M Pépin², L Plée³, R Lancelot⁴

1. Centre International de Recherche Agronomique pour le Développement (CIRAD, International Centre of Agricultural Research for Development), Unit for animal and integrated risk management (UR AGIRs), Montpellier, France
2. Agence française pour la sécurité sanitaire des aliments (AFSSA, French Agency for Food Safety), Lyon, France
3. Agence française pour la sécurité sanitaire des aliments (AFSSA, French Agency for Food Safety), Unit for the evaluation of risks associated with food and animal health, Maisons-Alfort, France
4. Centre International de Recherche Agronomique pour le Développement (CIRAD, International Centre of Agricultural Research for Development), Unit for the control of exotic and emerging animal diseases (UMR CMAEE), Montpellier, France

Citation style for this article:

Citation style for this article: Chevalier V, Pépin M, Plée L, Lancelot R. Rift Valley fever - a threat for Europe?. *Euro Surveill.* 2010;15(10):pii=19506. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19506>

This article has been published on 11 March 2010

Rift Valley fever (RVF) is a severe mosquito-borne disease affecting humans and domestic ruminants, caused by a *Phlebovirus* (*Bunyaviridae*). It is widespread in Africa and has recently spread to Yemen and Saudi Arabia. RVF epidemics are more and more frequent in Africa and the Middle East, probably in relation with climatic changes (episodes of heavy rainfall in eastern and southern Africa), as well as intensified livestock trade. The probability of introduction and large-scale spread of RVF in Europe is very low, but localised RVF outbreaks may occur in humid areas with a large population of ruminants. Should this happen, human cases would probably occur in exposed individuals: farmers, veterinarians, slaughterhouse employees etc. Surveillance and diagnostic methods are available, but control tools are limited: vector control is difficult to implement, and vaccines are only available for ruminants, with either a limited efficacy (inactivated vaccines) or a residual pathogenic effect. The best strategy to protect Europe and the rest of the world against RVF is to develop more efficient surveillance and control tools and to implement coordinated regional monitoring and control programmes.

Relevance of Rift Valley fever to public health in the European Union

Rift Valley fever (RVF) is a zoonotic disease of domestic ruminants and humans caused by an arbovirus belonging to the *Phlebovirus* genus (family *Bunyaviridae*). It causes high mortality rates in newborn ruminants, especially sheep and goats, and abortion in pregnant animals. Human infection by the RVF virus (RVFV) may result from mosquito bites, exposure to body fluids of livestock or to carcasses and organs during necropsy, slaughtering, and butchering [1].

The public health impact of RVF can be severe. In Egypt in 1976, 200,000 people were infected and 600 fatal cases officially reported, among others in the River Nile delta [2]. Over 200 human deaths were reported in Mauritania in 1987 [3]. In 2007-2008, 738 human cases were officially reported in Sudan, including 230 deaths

[4]. It is likely that the number of cases was underreported because RVF mostly affects rural populations living far from public health facilities. The occurrence of RVF in northern Egypt is evidence that RVF may occur in Mediterranean countries, thus directly threatening Europe. In the Indian Ocean, RVF has been introduced in the French island of Mayotte, with several clinical cases reported in humans [5]

Transmission, epidemiology and clinical symptoms

The RVFV transmission cycle involves ruminants and mosquitoes. Host sensitivity depends on age and animal species [6] (Table 1). Humans are dead-end hosts. The epidemiological cycle is made more complex by direct transmission from infected ruminants to healthy ruminants or humans, by transovarian transmission in some mosquito species, and by a large number of potential vectors with different bio-ecology [6]. The existence of wild reservoir hosts has not been clearly demonstrated to date (Figure 1).

Transmission mechanisms

The bite of infected mosquitoes is the main transmission mechanism of RVF in ruminants during inter-epizootic periods. More than 30 mosquito species were found to be infected by RVFV [6,7] (Table 2), belonging to seven genera of which *Aedes* and *Culex* are considered as the most important from the point of view of vector competence (other genera are *Anopheles*, *Coquillettidia*, *Eretmapodite*, *Mansonia* and *Ochlerotatus*).

In mosquitoes, transovarian RVFV transmission has been observed in *Aedes mcintoshi*. It appears to be a likely phenomenon in several other species, including the widespread *Ae. vexans* species complex. In some of these *Aedes* species, infected, diapaused eggs may survive in dried mud during inter-epizootic and/or dry/cold periods [8] and hatch infected imagoes.

Ruminant-to-human transmission is the main infection route for humans, although they can also be infected

by mosquito bites [9]. Body fluids such as the blood (during slaughtering and butchering), foetal membranes and amniotic fluid of viraemic ruminants are highly infective for humans. Fresh and raw meat may be a source of infection for humans, but the virus is destroyed rapidly during meat maturation. Empirical

field observations indicate that ruminants can also become infected by contact with material containing virus (e.g. fetus and fetal membranes after abortion), however, this route of transmission has not yet been confirmed [10].

TABLE 1
Species susceptibility and sensibility to the Rift Valley fever virus

Mortality >70%	Mortality 10-70%	Severe disease with low fatality rate (<10%)	Antibody production	Not susceptible
Lamb	Sheep	Human	Camel	Bird
Kid	Calf	Cattle	Horse	Reptile
Puppy	Some rodents	Goat	Cat	Amphibian
Kitten		African Buffalo	Dog	
Mouse		Asian Buffalo	Swine	
Rat		Monkey	Donkey	
			Rabbit	

Reproduced from Lefèvre et al. [5] with permission from the publisher (Lavoisier, France)

FIGURE 1
Epidemiological cycle of Rift Valley fever

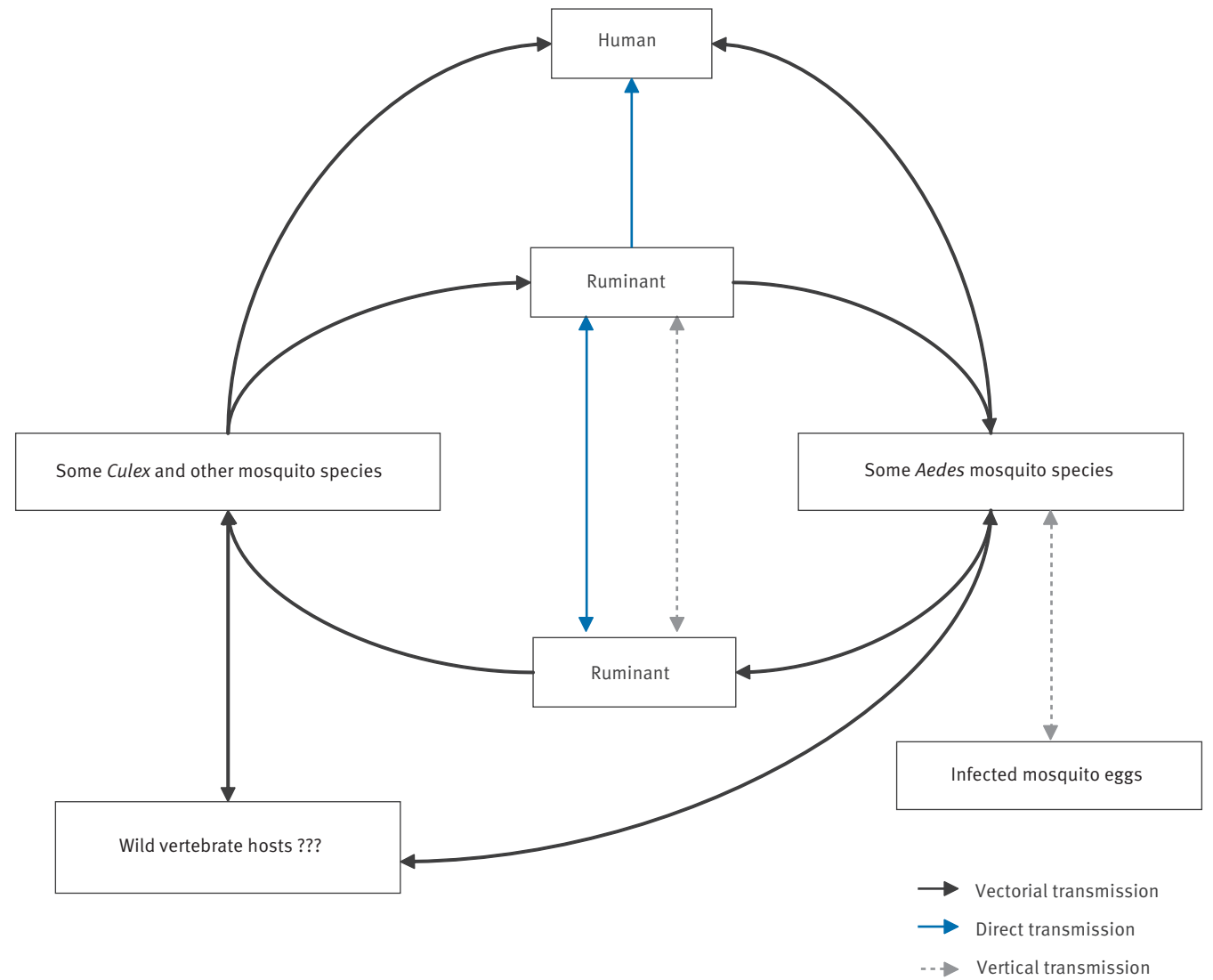


TABLE 2

Arthropods naturally infected by Rift Valley fever virus

Genus	Species	Country (year)
<i>Aedes (Aedimorphus)</i>	<i>cumminsii</i>	Kenya (1981-1984)
		Burkina Faso (1983)
	<i>dalzieli</i>	Senegal (1974, 1983)
	<i>dentatus</i>	Zimbabwe (1969)
	<i>durbanensis</i>	Kenya (1937)
	<i>ochraceus</i>	Senegal (1993)
	<i>tarsalis</i>	Uganda (1944)
	<i>vexans arabiensis</i>	Senegal (1993)
		Saudi Arabia (2000)
<i>Aedes (Neomelaniconion)</i>	<i>circumluteolus</i>	Uganda (1955)
		South Africa (1955, 1981)
	<i>mcintoshi</i>	Zimbabwe (1969)
		South Africa (1974-1975)
		Kenya (1981-1984)
	<i>palpalis</i>	Central African Republic (1969)
<i>Ochlerotatus (Ochlerotatus)</i>	<i>caballus</i>	South Africa (1953)
	<i>caspius</i>	Suspected, Egypt (1993)
	<i>juppi</i>	South Africa (1974-1975)
<i>Aedes (Stegomyia)</i>	<i>africanus</i>	Uganda (1956)
	<i>demeilloni</i>	Uganda (1944)
<i>Aedes (Diceromyia)</i>	<i>furcifer group</i>	Burkina Faso (1983)
<i>Anopheles (Anopheles)</i>	<i>coustani</i>	Zimbabwe (1969)
		Madagascar (1979)
	<i>fuscicolor</i>	Madagascar (1979)
<i>Anopheles (Cellia)</i>	<i>chrityi</i>	Kenya (1981-1984)
	<i>cinereus</i>	South Africa (1974-1975)
	<i>pauliani</i>	Madagascar (1979)
	<i>pharoensis</i>	Kenya (1981-1984)
<i>Culex (Culex)</i>	<i>spp.</i>	Madagascar (1979)
	<i>antennatus</i>	Nigeria (1967-1970)
		Kenya (1981-1984)
	<i>neavi</i>	South Africa (1981)
	<i>pipiens</i>	Egypt (1977)
	<i>poicilipes</i>	Senegal (1998, 2003)
	<i>theileri</i>	South Africa (1970)
		Zimbabwe (1969)
	<i>tritaeniorhynchus</i>	Saudi Arabia (2000)
	<i>vansomeri</i>	Kenya (1981-1984)
	<i>zombaensis</i>	South Africa (1981)
		Kenya (1981-1984, 1989)
<i>Culex (Eumelanomyia)</i>	<i>rubinotus</i>	Kenya (1981-1984)
<i>Eretmapodites</i>	<i>chrysogaster</i>	Uganda (1944)
	<i>quinquevittatus</i>	South Africa (1971)
		Kenya (1981-1984)
<i>Coquillettidia</i>	<i>fuscopennata</i>	Uganda (1959)
	<i>grandidieri</i>	Madagascar (1979)
<i>Mansonia (Mansoniodes)</i>	<i>africana</i>	Uganda (1959, 1968)
		Central African Republic (1969)
		Kenya (1989)
	<i>uniformis</i>	Uganda (1959)
		Madagascar (1979)
<i>Other diptera</i>	<i>Culicoides spp.</i>	Nigeria (1967)

Adapted from [1].

Direct human-to-human transmission has not been reported, and RVF is not considered to be a nosocomial disease. Transplacental RVFV transmission may occur in vertebrates, including humans. It results in abortion and high newborn mortality rates [11].

Rodents may be infected during epizootic periods [12-15] but their epidemiological role in virus transmission and maintenance is not clear. Bat species also have been suspected [16]. Finally, wild ruminants may play a role in the epidemiology of RVF in areas where their population density is high [17].

Clinical features

Animals

Clinical manifestations vary depending on age and animal species. In sheep, a fever of up to 41-42°C is observed after a short incubation period. Newborn lambs (and sometimes kids) usually die within 36 to 40 hours after the onset of symptoms, with mortality rates sometimes reaching 95%. Older animals (from two weeks to three months-old) either die or develop only a mild infection. In pregnant ewes, abortions are frequent, ranging from 5% to 100%. Twenty per cent of the aborting ewes die. Vomiting may be the only clinical sign presented by adult sheep and lambs older than three months. However, these animals may experience fever with depression, haemorrhagic diarrhoea, blood-stained muco-purulent nasal discharge, and icterus. Case-fatality rates vary between 20% and 30%. Adult goats develop a mild form of the disease, but abortions are frequent (80%). Mortality rates are generally low [10]. Calves often develop acute illness, with fever, fetid diarrhoea, and dyspnoea. Mortality rates may vary from 10% to 70%. Abortion is often the only clinical sign and mortality rates are low (10-15%).

Humans

In most cases, human infections remain unapparent, or with mild, influenza-like symptoms. However, infected people may experience an undifferentiated, severe, influenza-like syndrome and hepatitis with vomiting and diarrhoea. Complications may occur. Severe forms are manifested in three different clinical syndromes. The most frequent one is a maculo-retinitis, with blurred vision and a loss of visual acuity due to retinal haemorrhage and macular oedema. Encephalitis may also occur, accompanied by confusion and coma. This form is rarely fatal but permanent sequelae are encountered. The third and most severe form is a haemorrhagic fever, with hepatitis, thrombocytopenia, icterus, and multiple haemorrhages. This form is often fatal [10,18,19]. Human case-fatality rates have been lower than 1% in the past, however, an increase has been reported since 1970 [19]. In the RVF epidemic in Saudi Arabia in the year 2000, the fatality rate reached 14% [20].

Diagnostic methods

RVFV presents a high biohazard for livestock farmers, veterinarians, butchers, slaughterhouse employees, and laboratory staff handling infected biological samples. International public health agencies have set a bio-safety level (BSL) of BSL₃ for facilities in Europe handling the virus and of BSL₄ for facilities in the United States (US).

Appropriate diagnostic samples are peripheral blood collected on EDTA, plasma or serum of infected animals or patients, and the liver, brain, spleen or lymph nodes of dead animals. When samples can be conveyed rapidly to a diagnostic laboratory (<48 hours), they should be stored at a temperature below +4 °C. When this is not the case, samples should be frozen at -20 °C (or below). Small fragments of organs may be stored in a 10-20% glycerol solution

Virus isolation can be performed in suckling or weaned mice by intracerebral or intraperitoneal inoculation or in a variety of cell cultures including Vero, BHK21, or mosquito line cells. RVFV can be identified in cell cultures by immunofluorescence, virus neutralisation test, reverse transcriptase polymerase chain reaction (RT-PCR), and/or genome sequencing. Virus isolation is the gold standard for RVF diagnosis. However, its sensitivity is rather low: RVFV isolation is not easy to achieve. Alternatively, the detection of RVFV ribonucleic acid (RNA) can be done using RT-PCR performed on RNA extracted directly from biological samples [21]. Results are available within a few hours, which makes RT-PCR the priority test when a case of RVF is suspected.

Serological tests to detect antibodies against RVFV include the virus neutralisation test (VNT), and enzyme-linked immunosorbent assays (ELISA). VNT is very specific, cross reactions with other Phleboviruses being limited [22;23]. It is the gold standard serological test. However, it is costly, time consuming, and requires a BSL₃ or 4 laboratory.

(Indirect) immunoglobulin (Ig) detection ELISAs are quick, sensitive and specific. They are progressively replacing VNT [24]. A competition ELISA (cELISA) is also commercially available to detect IgG and IgM. It allows serological diagnosis in ruminants and humans. At the earliest, it can detect antibodies as soon as four days following infection or vaccination in animals reacting very early, and eight days post-vaccination for 100% of animals [25]. More recently, another indirect ELISA based on a recombinant RVFV nucleoprotein has been developed. Its sensitivity is 98.7% and specificity 99.4% [26-28].

The cELISA has been evaluated with human and animal sera collected in Africa, and also with sera from French livestock (cattle, sheep and goats) to check their specificity with European ruminant breeds which turned out to be excellent with a predictive negative value of

100% (n = 502), 95% confidence interval: 99.3 to 100% [29].

Treatments

There is no specific treatment for either humans or animals.

Prevention

Vaccines

A human vaccine (inactivated with beta-propiolactone) has been produced in the US and was used to protect laboratory staff and military troops. However, its production has been stopped [30].

Given that domestic ruminants are involved in the epidemiological cycle and that humans mostly become infected after contact with viraemic animals, the vaccination of ruminants is the method of choice to prevent human disease. Both live and inactivated vaccines are available for livestock.

The Smithburn vaccine is a live attenuated vaccine. It is inexpensive to prepare and immunogenic for sheep, goats, and cattle. It protects these species against abortion caused by a wild RVFV, and post-vaccinal immunity is life long. However, it has a residual pathogenic effect and may induce foetal abnormalities and/or abortion in ruminants. It is also pathogenic for humans (febrile syndrome). Despite these drawbacks, it is recommended by the Food and Agriculture Organization of the United Nations (FAO) [31] and remains the most widely used vaccine against RVF in Africa.

The inactivated RVF vaccine provides a lower level of protection and its production is more expensive. Moreover, it requires at least two inoculations and frequent booster shots to induce the desired level of protection, rendering it inappropriate in countries where large portions of ruminant herds are nomadic. However, it was used by the Israeli veterinary services to prevent RVF introduction to Israel after the 1977-1978 epidemic in Egypt [32], as well as by the Egyptian veterinary services to prevent re-introduction of RVF from Sudan after an epidemic hit that country in 2007.

Other candidate vaccines are being evaluated such as the so-called “clone 13” which is an attenuated strain of RVFV that was isolated from a moderately ill patient in the Central African Republic [33]. This vaccine induces neutralising antibodies against RVFV. New-generation vaccines are also under study: recombinant vaccines using a poxvirus or an *Alphavirus*-based vector [34,35] and DNA vaccines [34*,36*]. However, these vaccines are still in the preliminary stages of development.

Smithburn and inactivated vaccines are produced and commercially available in Egypt, South Africa, and Kenya. There is no Community pharmaceutical legislation prohibiting companies from producing RVF vaccines on EU territory and there is no obligation to notify such production to the European Commission.

Moreover, quoting Council Directive 2001/82/EC (EC 2001b), “in the event of serious epizootic diseases, Member States may provisionally allow the use of immunological veterinary medicinal products without a marketing authorisation, in the absence of a suitable medicinal product and after informing the Commission of the detailed conditions of use (article 8)” [37*].

Insecticide treatments

Larvicide treatments may provide a control alternative where mosquito breeding sites are well identified and cover limited surface areas. Both Methoprene, a hormonal larval growth inhibitor, and *Bacillus thuringiensis israeliensis* (BTI) preparations, a microbial larvicide, are commercially available and can be used successfully to treat temporary ponds and watering places where mosquitoes proliferate. Adulticide treatments (e.g. using pyrethroids) are expensive and difficult to implement. Moreover, because this usually involves treating large areas, the environmental and ecological consequences may be important.

Other measures

Preventive measures should also include restrictions on animal movements, the avoidance or control of the slaughter and butchering of ruminants, the use of insect repellents and bed nets during outbreaks, information campaigns, and increased and targeted surveillance of animals, humans and vectors.

Current geographical distribution

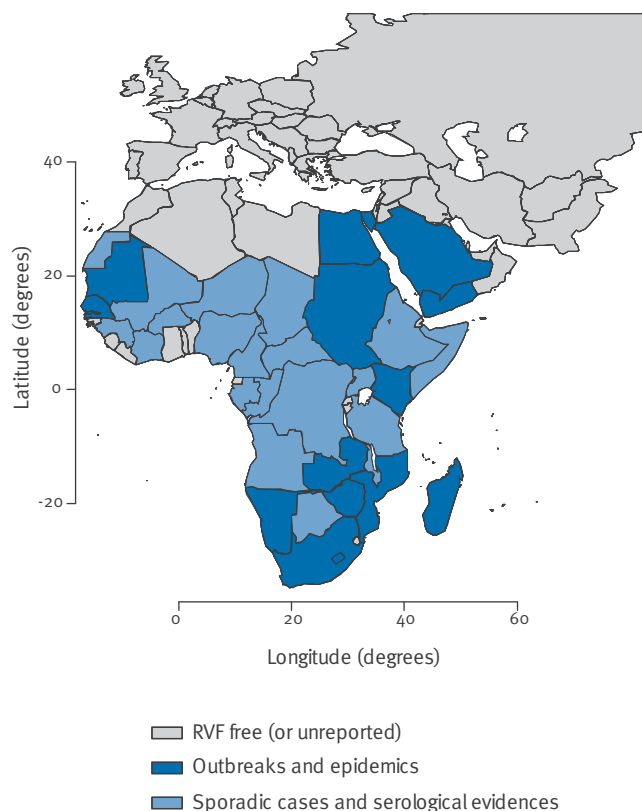
RVF is either enzootic, or is reported in most sub-Saharan African countries, Egypt and Madagascar (Figure 2).

During the first large epidemic, reported in Egypt in 1977-1978, over 600 people died of RVF [39]. The epidemic reached the Mediterranean shore (Nile delta) but did not spread to neighbouring countries. In September 2000, RVF was detected for the first time outside of the African continent in Saudi Arabia and Yemen, and led to human deaths and major livestock losses [40]. By the end of 2006, the disease had re-emerged in Kenya [41], followed by Tanzania and Somalia [42]. Another large epidemic hit the Sudan in 2007 in the Nile Valley around Khartoum [4]. In May 2007, RVF was diagnosed on the French island of Mayotte in a young boy who had been evacuated from Anjouan, one of the other islands of the Comoros archipelago. The RVFV was probably introduced there by the trade of live ruminants imported from Kenya or Tanzania during the 2006-2007 epidemics. Studies conducted after this first human case was reported have shown that 10% of cattle had antibodies against RVFV (ELISA, IgG and/or IgM) - without any clinical suspicions reported by the public and private veterinary services. A retrospective study was then conducted in 2008, using blood samples collected from clinically suspected human cases of dengue or chikungunya illness who had tested negative for these two diseases, between 1 September 2007 and 31 May 2008. Ten human RVF cases were

found (including IgM- and/or RT-PCR-positive samples), seven of them (70%) occurring from January to April, during the hot, rainy season [5]. This study has

FIGURE 2

Geographical distribution of Rift Valley fever



Source: United States Centers of Disease Control and Prevention [38*].

demonstrated that RVF had been circulating in Mayotte at least since early 2007, probably introduced there by the illegal importation of live infected ruminants from other Comoros islands.

In 2008, a RVF epidemic occurred in Madagascar with over 500 human cases [43]. Several outbreaks were reported in South Africa in late 2007 and 2008 without any reported human cases [44].

Factors of change

Factors that could cause a change in the epidemiology of RVF are summarised in Table 3. Irrigated areas, including rice fields, constitute favourable breeding sites for many mosquito species. Dambos are temporary surface water bodies found in semi-arid eastern Africa. With heavy rainfall and consecutive flooding, considerable mosquito proliferation may occur (mostly *Aedes* and *Culex* spp.). Wadi are temporary rivers encountered in arid areas (e.g. Yemen or Saudi Arabia): when they stop flowing, surface water remains available in ponds and mosquitoes may proliferate.

Livestock trade and the Mediterranean region

Livestock trade and transport may affect the geographical distribution of RVF and contribute to a large scale – sometimes continental - spread of the disease and to the introduction of the virus into disease-free areas via livestock movements. RVF cases were reported in irrigated areas of the Sudan during the 1970s. Antibodies were detected in camels that crossed the border from Sudan to Egypt, suggesting that infected camels may have introduced RVFV into Egypt [39].

TABLE 3

Main outbreaks of Rift Valley fever and factors causing them

Year	Country	Ecosystem	Vector	Hosts	Triggering factor
1975	South Africa	?	?	?	?
1976	Sudan	Irrigated area	?	Small ruminants	Irrigation (?)
1977	Egypt	Irrigated area	<i>Culex pipiens</i>	Small ruminants, camels, humans	Irrigation, cattle trade
1987	Mauritania, Senegal	Irrigated area	<i>Culex pipiens</i>	Small ruminants, cattle, camels, humans	?
1993	Egypt	Irrigated area	?	Small ruminants, humans	Irrigation
1997	Egypt	Irrigated area	?	Small ruminants, humans	Irrigation
1997-1998	Kenya	Dambos	<i>Aedes</i> spp. <i>Culex zombaensis</i>	Small ruminants	Rainfall
2000	Yemen, Saudi Arabia	Wadi	<i>Aedes vexans</i> <i>Culex tritaeniorhynchus</i>	Small ruminants, cattle, camels, humans	Rainfall and virus introduction
2006-2007	Kenya, Tanzania, Somalia	Dambos	?	Small ruminants, cattle, humans	Rainfall
2007	Sudan	Irrigated area	?	Small ruminants, cattle, humans	?
2007-2008	Mayotte	Island	?	Small ruminants, cattle, humans	Virus introduction
2008	Madagascar	Rice field in highlands	<i>Culex?</i> <i>Anopheles?</i>	Small ruminants, cattle, humans	?

During the outbreak in Saudi Arabia in 2000, six viral strains of RVFV were isolated from *Aedes* mosquitoes. These strains were genetically close to the strain isolated in Kenya (1997-1998), suggesting that the virus was probably introduced into Saudi Arabia from the Horn of Africa by ruminants [45]. It remains unknown whether the virus has survived in Saudi Arabia since 2000. In any event, the risk of re-introduction from the Horn of Africa is high. During the period of religious festivals in Mecca, 10 to 15 million small ruminants are imported from there to Saudi Arabia.

A similar pattern in sheep trade is observed between sub-Saharan Africa and northern Africa. In the coming years, the Muslim feasts of *Eid-ul-Fitr* and *Eid al-Adha* will occur between September and November, i.e. when the activity of mosquito populations is high (end of the rainy season in Sahelian Africa) [46].

Therefore, the introduction of RVF-infected animals on the eastern and southern shores of the Mediterranean Sea is a likely event. Once introduced there, RVFV may find ruminant hosts, as well as competent mosquito species [47]. However, because livestock trade from northern Africa and the Middle East to Europe is forbidden, the introduction of RVF-infected animals to Europe looks unlikely [48].

Climate

Climate warming is likely to have an impact on the geographical distribution of RVF. Higher temperatures increase mosquito feeding frequency and egg production and decrease the duration of their development cycle, as well as the extrinsic incubation period of RVFV in mosquitoes. Therefore, higher temperatures associated with increased rainfall may result in higher vector densities and vector competence and, subsequently, a higher RVFV transmission rate. In addition, transovarian transmission processes could be altered.

If the virus were introduced to northern Africa or southern Europe, mosquitoes such as *Ae. vexans* could play a role as vectors in many Mediterranean countries. Several *Ochlerotatus* species, which breed in wetlands, might also be able to transmit the virus. *Culex pipiens*, a ubiquitous species, is locally abundant (in wetlands, rice fields, irrigated crops, sewers etc) and may act as an amplifier in the biological cycle. Increased temperatures could also have an impact on the vector competence and capacity of other endemic European mosquito species [49], although this is difficult to quantify (it has already been proved in controlled conditions with other arboviruses). Indeed, if introduced, several potential vector species that have so far not been investigated may become involved in the transmission of the RVFV.

In East Africa, RVFV causes major epidemics at irregular intervals of 5-15 years. Climate models for this region predict an increase in the mean annual rainfall as well as an increase in the frequency and intensity

of extreme rainfall events [50]. These changes may induce more severe and more frequent outbreaks in East Africa, which would thus represent a high risk area for neighbouring regions with livestock trade relationships such as the Indian Ocean islands.

Vectors

The flight capacities of *Aedes* and *Culex* mosquitoes are somewhat limited, ranging from a few hundred meters to more than 10 km [51,52]. However, these distances are long enough to allow a local spread of RVF.

Wind transportation of infected mosquitoes has been reported for other arboviruses [53,54]. Presently, no information is available for RVFV vectors. Passive transportation of infected mosquitoes in boats or planes travelling from Africa has been reported for *Anopheles* mosquitoes infected by *Plasmodium* parasites [55]. However, for RVFV to be introduced this way, such infected mosquitoes would need to find susceptible hosts to initiate a local cycle. This event looks unlikely.

Predictive models

Risk mapping

East Africa (Kenya)

In Kenya, a correlation has been demonstrated between heavy rainfall events and the occurrence of RVF outbreaks. Maps of remotely sensed rainfall as well as vegetation index maps have been used together with ground data to monitor and predict vector population dynamics and RVFV activity and have established a correlation between these two parameters. The main advantage of remote sensing for the prediction of RVF occurrence in East Africa is the relatively low cost. It is readily available on a country and regional basis and its use may allow preventive measures to be taken such as the vaccination of susceptible livestock and the control of mosquito larvae [56,57].

Predictive models have been improved over the past decade through the addition of Pacific and Indian Ocean surface temperature anomalies and rainfall and normalised difference vegetation index (NDVI) data. An accuracy of 95-100% was estimated for the prediction of Kenyan epizootics of RVF, with a lead time of two to five months [57]. The FAO has used the technology to warn countries facing an increased risk of RVF. However, the geographic scope of these models is limited because ecological and epidemiological processes are different in other areas of Africa [58]. The outlook for the use of these models is even worse for the Mediterranean basin and Europe where climate determinants differ significantly from those of East Africa and the potential ecological and epidemiological processes are unknown as the disease has never been reported in these areas.

West Africa (Senegal)

RVF is endemic in the Ferlo area (northern Senegal) [59]. This area is characterised by a temporary pond ecosystem. These ponds are filled at the beginning

of the rainy season (July) and dry up from October to January, according to their size and the intensity of rainfall, and are favourable environment to the development of *Aedes* mosquito populations.

However, the East African model can not be applied in West Africa: abundant rainfall is not often associated with RVF outbreaks. The epidemiological process leading to RVF epidemics looks much more complex,

TABLE 4

Competent mosquito vectors of Rift Valley fever virus with known distribution in the European Union and candidate countries

Country	<i>Aedes vexans vexans</i>	<i>Ochlerotatus caspius</i>	<i>Culex theileri</i>	<i>Culex pipiens</i>	<i>Culex perexiguus</i>
Austria	X	X	?	X	?
Belgium	X	X	?	X	?
Bulgaria	X	X	X	X	X
Croatia ¹	X	X	?	X	?
Cyprus	?	X	?	X	?
Czech Republic	X	X	?	X	?
Denmark	X	X	?	X	?
Estonia	X	X	?	X	?
Finland	X	X	?	X	?
France (mainland)	X	X	X	X	?
France (Corsica)	X	X	X	X	?
Germany	X	X	?	X	?
Greece	X	X	X	X	X
Hungary	X	X	X	X	?
Ireland	?	X	?	X	?
Italy (mainland)	X	X	X	X	X
Italy (Sardinia)	X	X	X	X	?
Italy (Sicily)	X	X	X	X	X
Latvia	X	X	?	X	?
Lithuania	X	X	?	X	?
Luxembourg	?	?	?	?	?
Former Yugoslav Republic of Macedonia ¹	X	X	X	X	X
Malta	?	X	?	X	?
The Netherlands	X	?	?	X	?
Poland	X	X	?	X	?
Portugal	X	X	X	X	X
Romania	X	X	X	X	?
Slovakia	X	X	X	X	?
Slovenia	X	X	?	X	?
Spain (mainland)	X	X	X	X	X
Spain (Balearic Islands)	X	?	?	X	?
Sweden	X	X	?	X	?
Turkey ¹	X	X	X	X	X
United Kingdom	X	X	?	X	?

X: vector present; ?: unknown to the authors, or not found yet.

¹EU candidate country.

Adapted from [1].

involving the joint dynamics of hosts movements (transhumance), host immunity, and a vector population with brief activity during the rainy season.

In this region, the risk of transmission was shown to be heterogeneous and linked to pond type [59]. A very high spatial resolution remote sensing image was used to characterise the temporary ponds and their environment and derive indices linked to mosquito biology [60]. However, this work is not advanced enough to be used in surveillance programmes.

Risk analysis for Europe

A detailed, qualitative risk analysis was performed in 2005 by The European Food Safety Authority (EFSA) [1]. The main conclusions of this study are summarised below.

Ruminant importations

The importation of infected ruminants is the greatest hazard for RVF introduction to the European Union (EU). Clinical signs may not be observed rapidly in livestock living in remote, humid areas such as the Camargue region in France or the Danube delta in Romania. Such a scenario would allow RVFV to amplify and endemic foci to develop, if suitable ecological and entomological conditions were met [1].

Official RVF-free status is required for a country to export livestock and livestock meat to the EU. Such a status depends on a country's ability – relying on observable evidences – to implement an efficient disease surveillance system and willingness to report possible RVF outbreaks. These constraints are the same as for foot-and-mouth disease and other epizootic diseases. They were instituted in 1972 (directive 72/462/CEE [61], later modified to be more stringent). The practical consequence is that any introduction of live ruminants and their products from Africa and the Middle East to the European Union is forbidden. However, illegal and unknown ruminant importations probably occur between the Middle East and central Europe, and between northern Africa and southern Europe. This is also a major component of the risk of introduction of many other important animal and zoonotic diseases, like peste des petits ruminants, foot-and-mouth disease, bluetongue disease, Crimean-Congo haemorrhagic fever, etc. For instance, a risk analysis has recently been conducted to assess the risk of introduction of peste des petits ruminants virus (a *Morbillivirus*) from Maghreb to France. The conclusion was that the risk was extremely low, ranging from 0 to 2 on a scale from 0 (impossible event) to 9 (certain event) [48].

Vectors

Several potential RVFV vectors are present in the EU (Tables 4 and 5). Differences in climate, seasonal variations of vector and host density, and genetic drift may result in differences in vector competence (the biological suitability of the vector to transmit the pathogen) and vectorial capacity (external factors such as

number and lifespan of the vector, feeding preferences of the host) compared with the situation in Africa. Nevertheless, there is almost no doubt that several of the mosquito species in the EU, e.g. *Cx. pipiens*, would be competent vectors for RVF [62]. Moreover, the introduction and spread of new vector species represents a further risk. For example, *Ae. albopictus* can transmit RVFV [62–64], and many epidemiological concerns arise from this species' current distribution in Europe: Albania, Bosnia and Herzegovina, Croatia, Italy (including Sicily and Sardinia), south eastern continental France and Corsica, limited areas of Germany (north of the Alps), Greece, Monaco, Montenegro, the Netherlands (green houses), San Marino, Slovenia, eastern Spain, southern Switzerland, and the Vatican city [65].

Virus survival

Blood, organs, fresh meat, fetal fluids and tissues as well as hides all represent a serious hazard to at-risk occupational groups (farmers, veterinarians, slaughterhouse employees, butchers, etc). The virus persists in the liver, spleen and kidneys, but rapidly disappears from meat as the pH decreases with meat maturation. The importance of blood, bone and offal meal products as a vehicle for RVFV has not been evaluated [4]. Milk is not considered to constitute a risk. However, due to a lack of data, transmission by ingestion of milk can not be definitively ruled out.

Accidental RVF infections have been recorded in laboratory staff handling blood and tissues from infected animals.

Conclusion

Several national and Commission-supported analyses have been conducted to assess the risk of the introduction and spread of RVF within the EU. The conclusions have been that the overall risk was low. However, the recent reappearance of RVF in East Africa, including Sudan, the Nile Valley, and the Indian Ocean, has shown that the RVFV is very active and sensitive to climate and other environmental as well as socio-economic changes. These changes, together with growing human populations and an associated increased demand for meat, will promote greater controlled and uncontrolled movements of livestock. Consequently, the Mediterranean basin, central Europe, and the Middle East will probably be increasingly exposed to the risk of introduction of RVF. It is important to promote risk analyses that rely on accurate estimations of livestock movements between endemic and RVF-free areas. Moreover, high-risk ecosystems should be catalogued and the data updated on a regular basis to account for environmental changes. This latter activity has been initiated under the EU-funded Emerging Diseases in a changing European environment (EDEN) project and should be continued once the project ends in 2010. Research programmes are needed to better characterise the bionomics of RVFV vectors in Europe and to develop RVFV introduction, installation, and

spread models to improve disease surveillance and provide more efficient decision-making tools.

Furthermore, more efficient vector and disease control methods are needed to enable the implementation of efficient contingency plans:

- For vector control, a systematic assessment of existing methods and tools should be undertaken (laboratory and field experiments) and research programmes developing new technologies should be supported, including options for the development of genetically modified mosquitoes designed either to reduce population sizes or to replace existing populations with vectors unable to transmit the disease.

- For disease control in European ruminants, the existing vaccines should be tested, preferably in collaboration with pharmaceutical companies. Because the cheapest and most efficacious existing vaccine (the Smithburn RVFV strain) has residual pathogenic effects in ruminants and humans, research on new-generation vaccines (e.g. recombinant, or reverse-genetic vaccines) should also be supported, both for human and animal populations.

- Because a large-scale RVF epidemic appears unlikely in Europe (where a low proportion of people have direct contact with ruminants and their body fluids), human vaccination should target the population subgroups at high risk of exposure (farmers, veterinarians, slaughterhouse employees, butchers etc), once human vaccines have been developed.

- Finally, the most relevant long term strategy is to control RVF where it is endemic. A substantial effort is needed to better understand the bio-ecology of RVFV vectors and viruses and epidemiological processes in Africa, to develop predictive and quantitative risk models and maps, and to implement risk-based surveillance and control methods.

Acknowledgements

This review was conducted in the framework of the European Centre for Disease Prevention and Control funded V-borne project "Assessment of the magnitude and impact of vector-borne diseases in Europe", tender no. OJ/2007/04/13-PROC/2007/003. It was compiled, edited and reviewed using EDEN and Arbozoonet funding, (EU grant GOCE-2003-010284 EDEN; EU grant agreement 211757). The paper is catalogued by the EDEN steering committee as EDEN0197 (<http://www.eden-fp6project.net/>). The contents of this publication are the responsibility of the authors and do not necessarily reflect the views of either the European Centre for Disease Prevention and Control or the European Commission.

* Erratum: This reference was corrected on 18 March.

References

1. European Food Safety Authority (EFSA). Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) on a request from the Commission related to "The Risk of a Rift Valley Fever Incursion and its Persistence within the Community". EFSA-Q-2004-050. The EFSA Journal. 2005;3(10):1-128. Available from: <http://www.efsa.europa.eu/fr/efsajournal/doc/238.pdf>
2. Meegan JM, Hoogstraal H, Moussa MI. An epizootic of Rift Valley fever in Egypt in 1977. *Vet Rec.* 1979;105(6):124-125.
3. Jouan A, Le Guenno B, Digoutte JP, Philippe B, Riou O, Adam F. An RVF epidemic in southern Mauritania. *Ann Inst Pasteur Virol.* 1988;139(3):307-308.
4. World Health Organization (WHO). Rift Valley fever in Sudan--Update 4. Available from: http://www.who.int/csr/don/2007_11_05/
5. Sissoko D, Giry C, Gabrie P, Tarantola A, Pettinelli F, Collet L et al. Rift Valley fever, Mayotte, 2007-2008. *Emerg Infect Dis.* 2009;15(4):568-70.
6. Lefèvre PC, Blancou J, Chermette R. Principales maladies infectieuses et parasitaires du bétail. Europe et régions chaudes. Vol. 1. Lavoisier, editor. Généralités. Maladies virales. Londres, Paris, New York; 2003. [French].
7. McIntosh BM, Jupp PG. Epidemiological aspects of Rift Valley fever in South Africa with reference to vectors. *Contrib Epidemiol Biostat.* 1981;3:92-9.
8. Linthicum KJ, Davis FG, Kairo A, Bailey, CL. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. *J Hyg (Lond).* 1985;95(1):197-209.
9. Davies FG, Martin V. Recognizing Rift Valley fever. *Vet Ital.* 2006; 42(1):31-35.
10. Gerdes GH. Rift Valley fever. *Rev Sci Tech.* 2004;23(2):613-23.
11. Arishi HM, Aqeel AY, Al Hazmi MM. Vertical transmission of fatal Rift Valley fever in a newborn. *Ann Trop Pediatr.* 2006;26(3):251-3.
12. Gora D, Yaya T, Jocelyn T, Didier F, Maoulouth D, Amadou S et al. The potential role of rodents in the enzootic cycle of Rift Valley fever virus in Senegal. *Microbes infect.* 2000;2(4):343-6.
13. Weinbren MP, Masson PJ. Rift Valley fever in a wild field rat (*Arvicanthis abyssinicus*): a possible natural host. *S Afr Med J.* 1957;31(18):427-30.
14. Youssef BZ, Donia HA. The potential role of *Rattus rattus* in enzootic cycle of Rift Valley Fever in Egypt 2-application of reverse transcriptase polymerase chain reaction (RT-PCR) in blood samples of *Rattus rattus*. *J Egypt Public Health Assoc.* 2002; 77(1-2):133-41.
15. Pretorius A, Oelofsen MJ, Smith MS, van der Ryst E. Rift Valley fever virus: a seroepidemiologic study of small terrestrial vertebrates in South Africa. *Am J Trop Med Hyg.* 1997; 57(6):693-8.
16. Oelofsen M.J., Van der Ryst E. Could bats act as reservoir hosts for Rift Valley fever virus? *Onderstepoort J Vet Res.* 1999;66(1):51-4.
17. Evans A, Gakuya F, Paweska JT, Rostal M, Akoolo L, Van Vuren PJ et al. Prevalence of antibodies against Rift Valley fever virus in Kenyan wildlife. *Epidemiol Infect.* 2008;136(9):1261-9.
18. Daubney R, Hudson JR, Garnham PC. Enzootic hepatitis or Rift Valley fever: an undescribed disease of sheep, cattle and man from east Africa. *J. Pathol Bacteriol.* 1931;89:545-579.
19. Gubler DJ. The global emergence/resurgence of arboviral diseases as public health problems. *Arch Med Res.* 2002;33(4):330-42.
20. Balkhy HH, Memish ZA. Rift Valley Fever: an uninvited zoonosis in the Arabian peninsula. *Int J Antimicrob Agents.* 2003;21(2):153-7.
21. Sall A.A. et al. Single-tube and nested reverse transcriptase-polymerase chain reaction for detection of Rift Valley fever virus in human and animal sera. *J Virol Methods.* 2001; 91(1):85-92.
22. Tesh RB, Peters CJ, Meegan JM. Studies on the antigenic relationship among phleboviruses. *Am J Trop Med Hyg.* 1982;31(1):149-55.
23. Xu F, Liu D, Nunes MR, DA Rosa AP, Tesh RB, Xiao SY. Antigenic and genetic relationships among Rift Valley fever virus and other selected members of the genus Phlebovirus (Bunyaviridae). *Am J Trop Med Hyg.* 2007;76(6):1194-200.
24. Paweska JT, Barnard BJ, Williams R. The use of sucrose-acetone-extracted Rift Valley fever virus antigen derived from cell culture in an indirect enzyme-linked immunosorbent assay and haemagglutination-inhibition test. *Onderstepoort J Vet Res.* 1995;62(4):227-33.
25. Paweska JT, Smith SJ, Wright IM, Williams R, Cohen AS, Van Dijk AA et al. Indirect enzyme-linked immunosorbent assay for the detection of antibody against Rift Valley fever virus in domestic and wild ruminant sera. *Onderstepoort J Vet Res.* 2003;70(1):49-64.
26. Jansen van Vuren P, Potgieter AC, Paweska JT, van Dijk AA. Preparation and evaluation of a recombinant Rift Valley fever virus N protein for the detection of IgG and IgM antibodies in humans and animals by indirect ELISA. *J Virol Methods.* 2007;140(1-2):106-14.

27. Fafetine JM, Tijhaar E, Paweska JT, Neves LC, Hendriks J, Swanepoel R et al. Cloning and expression of Rift Valley fever virus nucleocapsid (N) protein and evaluation of a N-protein based indirect ELISA for the detection of specific IgG and IgM antibodies in domestic ruminants. *Vet Microbiol*. 2007;121(1-2):29-38.
28. Paweska JT, van Vuren PJ, Kemp A, Buss P, Bengis RG, Gakuya F et al. Recombinant nucleocapsid-based ELISA for detection of IgG antibody to Rift Valley fever virus in African buffalo. *Vet Microbiol*. 2009;127(1-2):21-8.
29. Cêtre-Sossah C, Billecocq A, Lancelot R, Defernez C, Favre J, Bouloy M, et al. Evaluation of a commercial ELISA for the detection of antibodies to Rift Valley fever virus in sera of domestic ruminants in France. *Prev Vet Med*. 2009;90(1-2):146-9.
30. Geisbert TW, Jahrling PB. Exotic emerging viral diseases: progress and challenges. *Nat Med*. 2004;10(12 Suppl):S110-121.
31. Geering W, Davis FG, Martin V. Préparations des plans d'intervention contre la fièvre de la Vallée du Rift. Manuel FAO de Santé Animale. 2003;15. [French].
32. Shimshony A, Klopfer-Orgad U, Bali S. The influence of information flow on the veterinary policy of Rift Valley fever prevention in Israel, 1978-1979. *Contributions to Epidemiology and Biostatistics*. 1981; 3:159-171.
33. Muller R, Saluzzo JF, Lopez N, Dreier T, Turell M, Smith J et al. Characterization of clone 13, a naturally attenuated avirulent isolate of Rift Valley fever virus, which is altered in the small segment. *Am J Trop Med Hyg*. 1995;53(4):405-11.
34. Wallace DB, Ellis CE, Espach A, Smith SJ, Greyling RR, Viljoen GJ. Protective immune responses induced by different recombinant vaccine regimes to Rift Valley fever. *Vaccine*. 2006;24(49-50):7181-9.
35. Gorchakov R, Volkova E, Yun N, Petrakova O, Linde NS, Paessler S et al. Comparative analysis of the alphavirus-based vectors expressing Rift Valley fever virus glycoproteins. *Virology*. 2007;366(1):212-25.
36. Wang QH, Wang XJ, Hu S, Ge JY, Bu ZG. Study on DNA immune of envelope protein gene of Rift Valley Fever Virus. *Wei Sheng Wu Xue Bao*. 2007;47(4):677-81.
37. Directive 2004/28/EC of the European Parliament and the Council of the 31 March 2004 amending Directive 2001/82/EC on the Community code relating to veterinary medical products. OJ L 136. 2004 April 30. p. 58 -84. Available from: http://www.echamp.eu/fileadmin/user_upload/Regulation/Directive_2001-82-EC___Consolidated_Version_.pdf
38. Centers for Disease Control and Prevention (CDC). [Internet]. Rift Valley Fever Distribution Map. Available from: <http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/rvfmap.htm>
39. Abd el-Rahim IH, el-Hakim U, Hussein M. An epizootic of Rift Valley fever in Egypt in 1997. *Rev Sci Tech*. 1999;18(3):741-8.
40. Ahmad K. More deaths from Rift Valley fever in Saudi Arabia and Yemen. *Lancet*. 2000;356(9239):1422.
41. Centers for Disease Control and Prevention (CDC). Rift Valley fever outbreak--Kenya, November 2006-January 2007. *MMWR Morb Mortal Wkly Rep*. 2007;56(4):73-6.
42. World Health Organization (WHO). Outbreaks of Rift Valley fever in Kenya, Somalia, and United Republic of Tanzania, December 2006-April 2007. *Wkly Epidemiol Rec*. 2007;82:169-80.
43. Rift Valley fever - Madagascar (02). In: ProMED-mail [online]. Boston US: International Society for Infectious Diseases; 16 July 2008. Archive number 20080716.2157. Available from: http://www.promedmail.org/pls/otn/f?p=2400:1001:4293294425104239:::F2400_P1001_BACK_PAGE,F2400_P1001_ARCHIVE_NUMBER,F2400_P1001_USE_ARCHIVE:1001,20080716.2157,Y
44. Rift Valley fever, buffalo-South Africa: (Mpulalanga). In: ProMED-mail [online]. Boston US: International Society for Infectious Diseases; 20 September 2009. Archive number 20090920.3297. Available from: http://www.promedmail.org/pls/otn/f?p=2400:1001:50943:::F2400_P1001_BACK_PAGE,F2400_P1001_ARCHIVE_NUMBER,F2400_P1001_USE_ARCHIVE:1001,20090920.3297,Y
45. Shoemaker T, Boulianne C, Vincent MJ, Pezzanite L, Al-Qahtani MM, Al-Mazrou Y et al. Genetic analysis of viruses associated with emergence of Rift Valley fever in Saudi Arabia and Yemen, 2000-01. *Emerg Infect Dis*. 2002;8(12):1415-20.
46. Chevalier V, de la Rocque S, Baldet T, Vial L, Roger F. Epidemiological processes involved in the emergence of vector-borne diseases: West Nile fever, Rift Valley fever, Japanese encephalitis and Crimean-Congo haemorrhagic fever. *Rev Sci Tech*. 2005;23(2):535-55.
47. Moutailler S, Krida G, Schaffner F, Vazeille M, Failloux AB. Potential vectors of Rift Valley fever virus in the Mediterranean region. *Vector Borne Zoonotic Dis*. 2008;8(6):749-53.
48. Miller M, Etter E, Dufour B, Libeau G, Lancelot R. Analyse qualitative du risque d'introduction de la peste des petits ruminants en France. *Epidémiol Santé Anim*. 2009;56:217-26. [French].
49. Turell MJ. Effect of environmental temperature on the vector competence of *Aedes fowleri* for Rift Valley fever virus. *Res Virol*. 1989;140(2):147-54.
50. Intergovernmental Panel on Climate Change (IPCC). Climate change 2007: the physical basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt et al, editors. United Kingdom and New York, Cambridge University Press;2007. Available from: http://www.ipcc.ch/publications_and_data/publications_ipcc_fourth_assessment_report_wg1_report_the_physical_science_basis.htm
51. Ba Y, Diallo D, Dia I, Diallo M. Feeding pattern of Rift Valley fever virus vectors in Senegal. Implications in the disease epidemiology. *Bull Soc Pathol Exot*. 2006;99(4):283-9.
52. Bogoević MS, Hengl T, Merdić E. Spatiotemporal monitoring of floodwater mosquito dispersal in Osijek, Croatia. *J Am Mosq Control Assoc*. 2007;23(2):99-108.
53. Kay BH, Farrow RA. Mosquito (Diptera: Culicidae) dispersal: implications for the epidemiology of Japanese and Murray Valley encephalitis viruses in Australia. *J Med Entomol*. 2000;37(6):797-801.
54. Chapman HF, Hughes JM, Ritchie SA, Kay BH. Population structure and dispersal of the freshwater mosquitoes *Culex annulirostris* and *Culex palpalis* (Diptera: Culicidae) in Papua New Guinea and northern Australia. *J Med Entomol*. 2003;40(2):165-9.
55. Guillet P, Germain M, Giacomini T, Chandre F, Akogbeto M, Faye O et al. Origin and prevention of airport malaria in France. *Trop Med Int Health*. 1998;3(9):700-5.
56. Linthicum KJ, Bailey CL, Tucker CJ, Mitchell KD, Logan TM, Davies FG et al. Application of polar-orbiting, meteorological satellite data to detect flooding of Rift Valley fever virus vector mosquito habitats in Kenya. *Med Vet Entomol*. 1990;4(4):433-8.
57. Linthicum KJ, Anyamba A, Tucker CJ, Kelley PW, Myers MF, Peters CJ. Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. *Science*. 1999;285(5426):397-400.
58. Martin V, De Simone L, Lubroth J, Ceccato P, Chevalier V. Perspectives on using remotely sensed imagery in predictive veterinary epidemiology and global early warning systems. *Geospat Health*. 2007;2(1):3-14.
59. Chevalier V, Lancelot R, Thiongane Y, Sall B, Diatté A, Mondet B. Rift Valley fever in small ruminants, Senegal, 2003. *Emerg Infect Dis*. 2005;11(11):1693-700.
60. Soti V, Chevalier V, Maura J, Tran AL, Etter E, Lelong C et al. Landscape characterization of Rift Valley fever risk areas using very high spatial resolution imagery: case study in the Ferlo area, Senegal. Proceedings of the GISVet Conference 20-24 august 2007. Copenhagen - Denmark.
61. Council Directive 72/462/EEC of 12 December 1972 on health and veterinary inspection problems upon importation of bovine animals and swine and fresh meat from third countries. OJ L 302. 1972 Dec 31. p. 28. Available from: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31972Lo462:EN:HTML>
62. Moutailler S, Bouloy M, Failloux AB. Short report: efficient oral infection of *Culex pipiens quinquefasciatus* by Rift Valley fever virus using a cotton stick support. *Am J Trop Med Hyg*. 2007;76(5):827-9.
63. Turell MJ, Bailey CL, Beaman JR. Vector competence of a Houston, Texas strain of *Aedes albopictus* for Rift Valley fever virus. *J Am Mosq Control Assoc*. 1988;4(1):94-6.
64. Rodhain F. *Aedes albopictus*: a potential problem in France. *Parassitologia*. 1995;37(2-3):115-9.
65. European Centre on Disease Prevention and Control (ECDC). Areas of possible establishment of *Aedes albopictus* (the tiger mosquito) in Europe for 2010 and 2030. In : Impacts of Europe's changing climate - 2008 indicator-based assessment, E.R.N. 4/2008, p. 155. Editor. 2008. The European Environment Agency (EEA), Copenhagen.

Leishmaniasis emergence in Europe

P D Ready (P.Ready@nhm.ac.uk)¹

1. Department of Entomology, Natural History Museum, London, United Kingdom

Citation style for this article:

Citation style for this article: Ready PD. Leishmaniasis emergence in Europe. Euro Surveill. 2010;15(10):pii=19505. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19505>

This article has been published on 11 March 2010

Leishmaniasis emergence in Europe is reviewed, based on a search of literature up to and including 2009. Topics covered are the disease, its relevance, transmission and epidemiology, diagnostic methods, treatment, prevention, current geographical distribution, potential factors triggering changes in distribution, and risk prediction. Potential factors triggering distribution changes include vectorial competence, importation or dispersal of vectors and reservoir hosts, travel, and climatic/environmental change. The risk of introducing leishmaniasis into the European Union (EU) and its spread among Member States was assessed for the short (2-3 years) and long term (15-20 years). There is only a low risk of introducing exotic *Leishmania* species because of the absence of proven vectors and/or reservoir hosts. The main threat comes from the spread of the two parasites endemic in the EU, namely *Leishmania infantum*, which causes zoonotic visceral and cutaneous leishmaniasis in humans and the domestic dog (the reservoir host), and *L. tropica*, which causes anthroponotic cutaneous leishmaniasis. The natural vector of *L. tropica* occurs in southern Europe, but periodic disease outbreaks in Greece (and potentially elsewhere) should be easily contained by surveillance and prompt treatment, unless dogs or other synanthropic mammals prove to be reservoir hosts. The northward spread of *L. infantum* from the Mediterranean region will depend on whether climate and land cover permit the vectors to establish seasonal biting rates that match those of southern Europe. Increasing dog travel poses a significant risk of introducing *L. infantum* into northern Europe, and the threat posed by non-vectorial dog-to-dog transmission should be investigated.

Leishmaniasis

Leishmaniasis (or 'leishmaniosis') is a complex of mammalian diseases caused by parasitic protozoans classified as *Leishmania* species (Kinetoplastida, Trypanosomatidae) [1,2]. Natural transmission may be zoonotic or anthroponotic, and it is usually by the bite of a phlebotomine sandfly species (order Diptera, family Psychodidae; subfamily Phlebotominae) of the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World) [2,3]. Primary skin infections (cutaneous leishmaniasis) sometimes resolve without treatment, with the host developing acquired immunity

through cellular and humoral responses [4], but the infection can spread to produce secondary lesions in the skin (including diffuse cutaneous leishmaniasis), the mucosa (muco-cutaneous leishmaniasis) and the spleen, liver and bone marrow (visceral leishmaniasis, which is usually fatal if untreated) [1]. Worldwide, at least 20 *Leishmania* species cause cutaneous and/or visceral human leishmaniasis (HumL) [1,5]. Most foci occur in the tropics or subtropics, and only zoonotic *L. infantum* is transmitted in both the eastern and western hemispheres [5] (Table 1).

Worldwide and European relevance of leishmaniasis

The World Health Organization (WHO) reports that the public health impact of leishmaniasis worldwide has been grossly underestimated for many years [1]. In 2001 and 2004, Desjeux reported that in the previous decade endemic regions had spread, prevalence had increased and the number of unrecorded cases must have been substantial, because notification was compulsory in only 32 of the 88 countries where 350 million people were at risk [5,6]. About two million new cases of HumL (half a million visceral) are considered to occur every year in the endemic zones of Latin America, Africa, the Indian subcontinent, the Middle East and the Mediterranean region [1].

Risks of emergence or re-emergence of leishmaniasis in Europe are associated with three main scenarios:

- 1) the introduction of exotic *Leishmania* species or strains into Europe via the increasing worldwide travelling of humans [6] and domestic dogs [7],
- 2) the natural spread of visceral and cutaneous leishmaniasis caused by *L. infantum* and *L. tropica* from the Mediterranean region of Europe, where these species are endemic [1,8,9], to neighbouring temperate areas where there are vectors without disease [2],
- 3) the re-emergence of disease in the Mediterranean region of Europe caused by an increase in the number of immunosuppressed people.

The high prevalence of asymptomatic human carriers of *L. infantum* in southern Europe [10-13] suggests that this parasite is a latent public health threat. This was demonstrated by the increase of co-infections with

human immunodeficiency virus (HIV) and *Leishmania* that has been observed since the 1980s [14], with leishmaniasis becoming the third most frequent opportunistic parasitic disease after toxoplasmosis and cryptosporidiosis [15].

Disease transmission and epidemiology

Visceral leishmaniasis (VL) is usually fatal if untreated, and so it is distinguished from cutaneous leishmaniasis (CL) in all sections of the current review. If untreated, uncomplicated CL is often disfiguring, but not fatal. In contrast, muco-cutaneous and diffuse cutaneous disease can lead to fatal secondary infections even if treated. Patient immunodeficiency is one factor for this, but in Latin America these diseases are associated with regional strains of the *L. braziliensis* and *L. mexicana* species complexes [1,5].

A female sandfly ingests *Leishmania* while blood-feeding, and then transmits the infective stages (usually accepted to be the metacyclic promastigotes) during a subsequent blood meal [16]. The infective promastigotes inoculated by the sandfly are phagocytosed in the mammalian host by macrophages and related cells, in which they transform to amastigotes and often provoke a cutaneous ulcer and lesion at the site of the bite.

There are only two transmission cycles with proven long-term endemism in Europe [2,17]: zoonotic visceral and cutaneous HumL caused by *L. infantum* throughout the Mediterranean region; and, anthroponotic cutaneous HumL caused by *L. tropica* now occurring sporadically in Greece (Table 1, Figure 1).

Worldwide, most transmission cycles are zoonoses, involving reservoir hosts such as rodents, marsupials, edentates, monkeys, domestic dogs and wild canids [2,5,6,18] (Table 1). However, leishmaniasis can be anthroponotic, with sandflies transmitting parasites between human hosts without the involvement of a reservoir host. Anthroponotic transmission is characteristic of species of the *L. tropica* complex and, except for *L. infantum*, of the *L. donovani* complex. One species (*L. donovani sensu stricto*) or two species (*L. donovani* and *L. archibaldi*) cause periodic epidemics of anthroponotic visceral leishmaniasis ('Kala-azar') in India and northeast Africa, respectively [19]. Sandfly vectors of both complexes (*L. donovani* and *L. tropica*) are abundant in southern Europe.

The domestic dog is the only reservoir host of major veterinary importance, and in Europe there is a large market for prophylactic drugs and treatment of canine leishmaniasis (CanL) caused by *L. infantum* [2]. Domestic cats might be secondary reservoir hosts of *L. infantum* in southern Europe [13], because they are experimentally infectious to sandflies [20] and natural infections can be associated with feline retroviruses [21].

Fewer than 50 of the approximately 1,000 species of sandflies are vectors of leishmaniasis worldwide [3]. This is due to the inability of some sandfly species to support the development of infective stages in their gut [16] and/or a lack of ecological contact with reservoir hosts [22]. Our understanding of the fundamentals of leishmaniasis epidemiology has been challenged in the last 20 years. Firstly, HIV/*Leishmania* co-infections were recorded in 35 countries worldwide, and widespread needle transmission of *L. infantum* was inferred in southwest Europe [15], where Cruz *et al.* demonstrated *Leishmania* in discarded syringes [23]. Secondly, leishmaniasis has become more apparent in northern latitudes where sandfly vectors are either absent or present in very low densities, such as in the eastern United States (US) and Canada [24] as well as in Germany [25-27]. Most infections involve CanL, not HumL, and this might be explained by dog importation from, or travel to, endemic regions, followed by vertical transmission from bitch to pup or horizontal transmission by biting hounds [24]. Vertical transmission of HumL from mother to child has rarely been reported [28].

Diagnostic methods

Most diagnoses are only genus-specific, being based on symptoms, the microscopic identification of parasites in Giemsa-stained smears of tissue or fluid, and serology [18,29]. Consequently, the identity of some causative agents has only been known relatively recently, following typing performed during limited eco-epidemiological surveys. For example, it was thought that all cutaneous leishmaniasis cases in Europe were caused by *L. tropica*, until Rioux and Lanotte reported *L. infantum* to be the causative agent in the western Mediterranean region [30].

Rioux and Lanotte used multi-locus enzyme electrophoresis (MLEE) to identify *Leishmania* species and strains [30], which remains the gold standard [1,18]. However, MLEE requires axenic culture [31] in which one strain can overgrow others in mixed infections. It is therefore more practical to identify the isoenzyme strains (or zymodemes) by directly characterising the enzyme genes [32]. Other molecular tests have been used to identify *Leishmania* infections in humans, reservoir hosts and sandfly vectors [33], including in the Mediterranean region [34], but there has been no international standardisation [29]. However, PCR of the internal transcribed spacer of the multi-copy nuclear ribosomal genes is often used [34,35]. A set of carefully chosen criteria must accompany PCR-based diagnosis, especially for immunocompromised patients [14,15]. Monoclonal antibodies have long been available for the identification of neotropical species [36] and the serotyping of Old World species [37] but they are not widely used.

Most sensitive molecular techniques indicate only the presence of a few recently living *Leishmania*, not that the parasites were infectious. Therefore, serology is

often more informative [29]. However, antigens prepared in different laboratories can cause test variation for the frequently used methods [29]: the indirect fluorescent antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA), the indirect haemagglutination assay (IHA) and the direct agglutination test (DAT). Some antigens are stable and produced commercially, such as the recombinant (r) K39 for a dipstick or strip test. Multi-centre studies of ‘Kala-azar’ diagnostics [38] showed that both the freeze-dried DAT and the rK39 strip test could exceed the 95% sensitivity and 90% specificity target, but only for the strains found in some regions. Antibody detection tests should complement other diagnostic tests, because they do not usually distinguish between acute disease, asymptomatic infections, relapses and cured cases [38].

Delayed hypersensitivity is an important feature of all forms of human leishmaniasis [4] and is often measured by the leishmanin skin test (or Montenegro reaction) [29]. False-positivity is approximately 1% in otherwise healthy people. Other problems with this test include the absence of commercially available leishmanins, that there is complete cross-reactivity among most species and strains of *Leishmania*, and that for VL its applicability is limited to the detection of past infections, because a complete anergy is found during active disease.

Treatment

Pentavalent antimonials were the first-choice drugs for leishmaniasis worldwide [39,40]. Miltefosine, Paromomycin and liposomal Amphotericin B are gradually replacing antimonials and conventional Amphotericin B in some regions [40,41], especially where there is drug resistance or the need to develop combination therapy to prevent the emergence of resistance to new drugs [41].

Highly active anti-retroviral therapy (HAART) treatment has reduced the incidence of co-infections with *Leishmania* and HIV by preventing an asymptomatic infection with *L. infantum* from becoming symptomatic, but unfortunately it is not good at preventing visceral leishmaniasis relapses. The benefits of treatment are not as clear-cut as they are for other opportunistic diseases [42].

Prevention

Most research on vaccines is strategic, not applied, for example targeting secretory-gel glycans of *Leishmania* [43] and some sandfly salivary peptides [44], both of which are injected into the mammalian host by the female sandfly during blood feeding. Therapeutic vaccine trials continue to use killed cultured parasites (often with BCG as adjuvant) in combination with anti-leishmanial drugs but with only 0-75% efficacy [45]. One second generation recombinant vaccine contains a trifusion recombinant protein (Leish-111f), and some of its epitopes are shared by *L. donovani* and *L. infantum* [46].

Research and development of vaccines against CanL has been stimulated by the economic importance of dogs and their role as reservoirs of HumL caused by *L. infantum* in the Americas and the Mediterranean region. Leishmune is the first licensed vaccine against CanL. It contains the fucose-mannose ligand (FML) antigen of *L. donovani* and has a reported efficacy of 76-80% [47]. The industrialised formulation of FML-saponin underwent safety trials in Brazil [48]. The vaccine LiESAp-MDP (excreted/secreted antigens with adjuvant) was reported to have an efficacy of 92% when tested on naturally exposed dogs in the south of France [49,50]. More recently, a modified vaccinia virus Ankara (MVA) vaccine expressing recombinant *Leishmania* DNA encoding trypanothione peroxidase (TRYP) was found to be safe and immunogenic in outbred dogs [51].

One means of controlling transmission is to reduce the biting rate of peri-domestic sandfly vectors of visceral HumL and CanL. This has been effective locally, by using repellents [52], insecticide-impregnated nets and bednets [52], topical applications of insecticides [53] and deltamethrin-impregnated dog collars [54,55]. The latter are favoured by many dog owners in southern Europe.

Current geographical distribution Outside the European Union

Table 1 (updated from Ready [2]) relates the distributions of each form of HumL to causative species and known reservoir hosts [1,5,6,17,18]. Most VL foci occur in India and neighbouring Bangladesh and Nepal, and in Africa (Sudan and neighbouring Ethiopia and Kenya), where anthroponotic ‘Kala-azar’ is caused by *L. donovani* and in north-eastern Brazil and parts of Central America, where zoonotic infantile visceral leishmaniasis is caused by *L. infantum*. Most CL foci occur in Latin America, North Africa, and the Middle East, and muco-cutaneous and diffuse cutaneous disease are frequent in South America [56].

Inside the European Union: main biomes

Only two transmission cycles have been endemic in the European Union (EU) for a long time, and both are widespread in the adjoining Middle East and in North Africa: zoonotic cutaneous and visceral leishmaniasis caused by *L. infantum* throughout the Mediterranean region and anthroponotic cutaneous leishmaniasis caused by *L. tropica*, which occurs sporadically in Greece and probably neighbouring countries and poses a high risk of introduction by migrants and travellers into the rest of the EU [2,6,17] (Table 1, Figure 1). The former is endemic and sandfly-borne only in the Mediterranean region of the EU (‘Mediterranean forests’ biome), where its epidemiological significance is clear from published serological surveys [7,8]. However, the vectors of *L. infantum* [57] (Figure 2, Table 2, updated from Ready [2]) are also abundant in the adjoining parts of the temperate region (Temperate broadleaf forests’ biome), in northern Spain [58] and central France [59],

TABLE 1

European distribution of parasites causing most human leishmaniasis worldwide up to 2009

Human disease	(Diffuse and muco-) cutaneous leishmaniasis	Cutaneous leishmaniasis	Cutaneous leishmaniasis	Visceral leishmaniasis	Visceral leishmaniasis	Cutaneous leishmaniasis	Muco-cutaneous leishmaniasis	Diffuse cutaneous leishmaniasis
<i>Leishmania</i> species	<i>L. tropica</i> species complex	<i>L. major</i>	<i>L. infantum</i> (= <i>L. chagasi</i> in Neotropics)	<i>L. infantum</i> (= <i>L. chagasi</i> in Neotropics)	Other members of <i>L. donovani</i> species complex	<i>L. braziliensis</i> species complex; <i>L. mexicana</i> species complex	<i>L. braziliensis</i>	<i>L. mexicana</i> species complex
Reservoir hosts (zoonosis) or anthroponosis	Often anthroponotic	Rodents	Domestic dogs, wild canids	Domestic dogs, wild canids	Anthroponotic	Edentates, primates, rodents, marsupials	Rodents, marsupials	Rodents, marsupials
World ecozone	Palaeartic, Afrotropical, Indo-Malayan	Palaeartic, Afrotropical, Indo-Malayan	Palaeartic, Afrotropical, Neotropical	Palaeartic, Afrotropical, Neotropical	Afrotropical, Indo-Malayan	Neotropical, Nearctic	Neotropical	Neotropical
EU biome	Mediterranean forests	Absent	Mediterranean forests, temperate broadleaf forest	Mediterranean forests, temperate broadleaf forest	Absent	Absent	Absent	Absent
EU: Cyprus	Absent	Absent	Present	Present	Present	Absent	Absent	Absent
EU: France	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Germany	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
EU: Greece	Sporadic	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Hungary	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
EU: Italy	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Malta	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Portugal	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Romania	Formerly sporadic? (untyped parasites)	Absent	Formerly sporadic? (untyped parasites)	Formerly present	Absent	Absent	Absent	Absent
EU: Spain	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU Overseas Territories: French Guyana	Absent	Absent	Absent	Absent	Absent	Present	Present	Present
EU candidate: Former Yugoslav Republic of Macedonia	Formerly sporadic? (untyped parasites)	Absent	Formerly sporadic? (confirmed in Croatia)	Present	Absent	Absent	Absent	Absent
EU candidate: European Turkey (Asiatic Turkey)	Absent? (present)	Absent (absent)	Absent? (present)	Absent? (present)	Absent	Absent	Absent	Absent
Other Europe: Albania	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
Other Europe: Switzerland	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Adapted from a contribution [2] published in the OIE (World Organisation for Animal Health) Scientific and Technical Review: In Climate change: the impact on the epidemiology and control of animal diseases (S. de la Roque, ed.). Rev Sci Tech Off Int Epiz. 2008;27(2):399-412.

and small numbers occur as far north as Paris [60] and the upper Rhine valley in Germany [26]. The occurrence of ‘vectors without disease’ poses a significant risk for the emergence of leishmaniasis in temperate regions of Europe [2].

Potential factors triggering changes in distribution

Climate change

Most transmission of *Leishmania* is by the bite of permissive sandflies, and so climate change might affect leishmaniasis distribution directly, by the effect of temperature on parasite development in female sandflies [16], or indirectly by the effect of environmental variation on the range and seasonal abundances of the vector species. Female sandflies seek sheltered resting sites for blood meal digestion, and in southern Europe the temperatures of these micro-habitats are buffered but vary significantly with the external air temperature [2].

Based on molecular markers, European vectors of leishmaniasis have extended their ranges northward since the last ice age (approximately 12,000 years ago) [61,62], and the mapping of statistical measures of climate has permitted transmission cycles to be loosely associated with some Mediterranean bioclimates [63]. However, bioclimate zones and their vegetation indicators vary regionally, and ongoing climate change may alter the patterns of land cover and land use. The geographic information system (GIS)-based spatial modelling of the Emerging Diseases in a changing European Environment (EDEN) project is permitting an analysis of

changes in climate and land cover [64] and their effects on sandflies.

The project ‘climate Change and Adaptation Strategies for Human health in Europe’ (cCASHh) concluded: “There is no compelling evidence, due to lack of historical data, that sand fly and VL distributions in Europe have altered in response to recent climate change” [9]. There is now a published analysis of the northward spread of CanL and its vectors in Italy [65], but an association with climate change was only surmised.

Capacity and competence of vectors in Europe

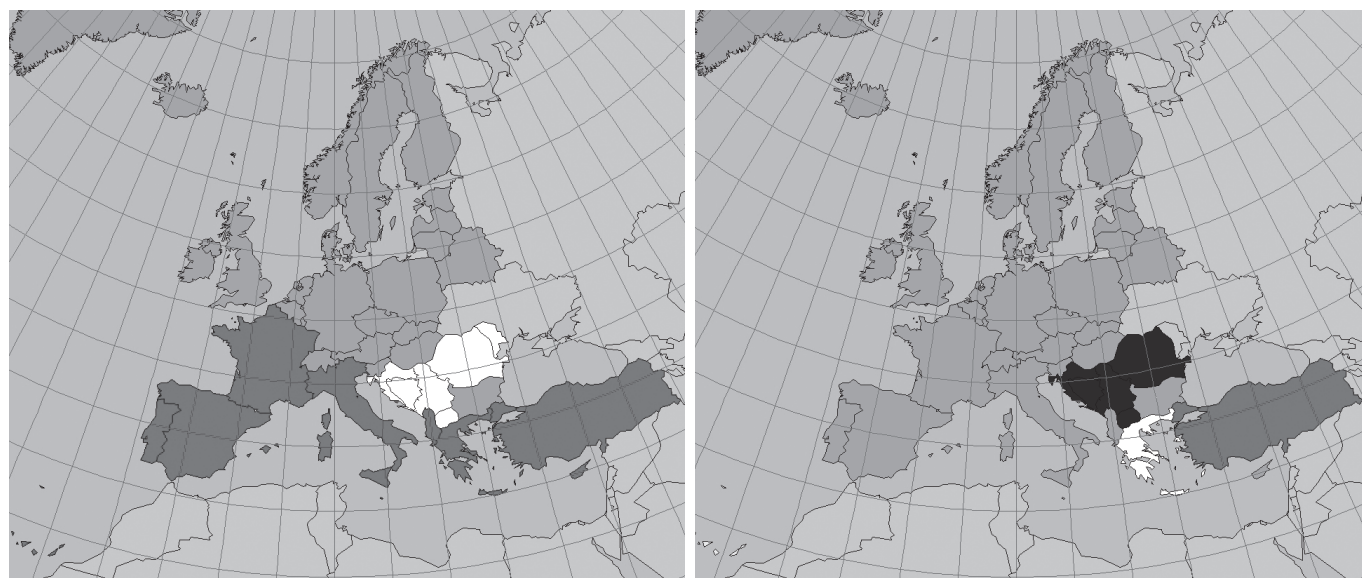
Vectorial capacity has only been calculated indirectly. The average number of gonotrophic cycles (i.e. egg development following a bloodmeal) completed by *P. ariasi* in the south of France was only a little greater than one [66]. Therefore, relatively small changes in temperature could have a large effect on vectorial capacity, because transmission occurs only during the second or subsequent bloodmeals and temperature affects the level of activity of the sandfly and therefore the frequency of the bloodmeals.

Alone, PCR detection of a natural infection of *Leishmania* in a sandfly does not identify a vector. It only indicates that the sandfly has fed on an infected mammalian host [35] because many parasites do not survive in a non-permissive sandfly after bloodmeal defecation [16].

Vectorial competence has been tested [67] or inferred based on finding naturally infected females of the more abundant human-biting species [3,57,68], from which it

FIGURE 1

Distribution by country of *Leishmania* species transmitted by phlebotomine sandflies in Europe up to 2009



Left panel: *L. infantum*; right panel: *L. tropica*.

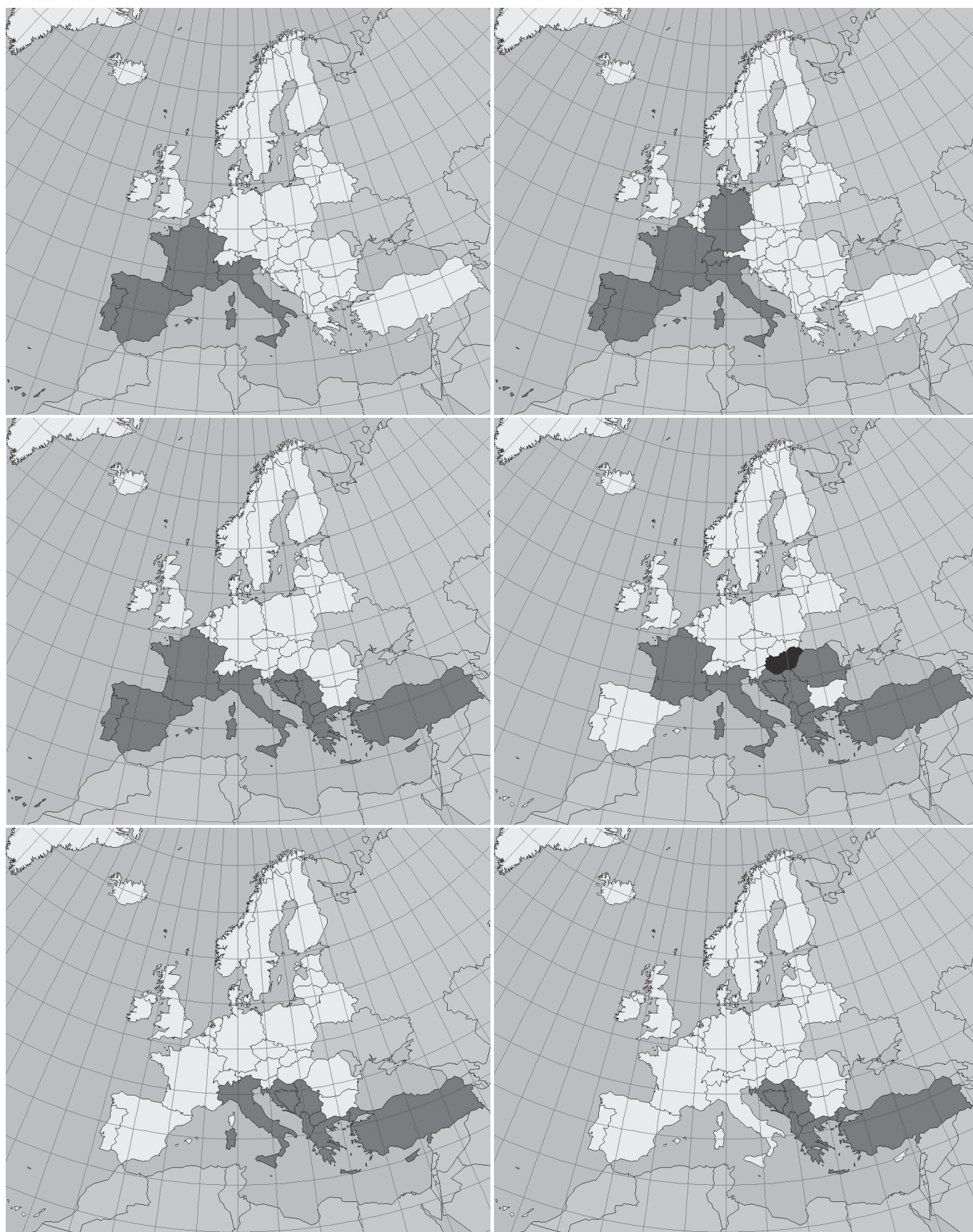
Grey: absent; dark grey: present; white: sporadic or untyped infections; black untyped infections.

Presence in North Africa and Middle East not depicted.

Source: V-borne project; reproduced with permission from the European Centre of Disease Prevention and Control.

FIGURE 2

Distribution of vectors of leishmaniasis in European countries up to 2009



From left to right and top to bottom: (a) *Phlebotomus ariasi*, (b) *P. perniciosus*, (c) *P. sergenti*, (d) *P. perfiliewi*, (e) *P. neglectus*, (f) *P. tobbi*.

Dark grey: present; light grey: absent; black: old record.

Presence in North Africa and Middle East is not depicted.

Source: V-borne project; reproduced with permission from the European Centre of Disease Prevention and Control.

is concluded that the principal vectors of *L. infantum* in the Mediterranean region are members of the subgenus *Larrousius* (Table 2, Figure 2). The vectorial competence of *Phlebotomus* (*Transphlebotomus*) *mascittii* should be tested because this species is now known to be widespread in northern France, Belgium and Germany [69]. However, low rates of biting humans and autogeny (the ability to produce eggs without a blood-meal) cast doubt on its epidemiological importance [2]. Based on distribution and vectorial competence elsewhere, *P. sergenti sensu lato* is likely to be the main vector of *L. tropica* in southern Europe [3].

Importation or dispersal of vectors and reservoir hosts

The importation or inter-continental dispersal of vectors is unlikely because sandflies are not as robust as some mosquitoes and are not known to be wind-dispersed [3]. Any importations are unlikely to be significant for several reasons: The natural vectors of Old World leishmaniasis are already abundant in Mediterranean Europe (Table 2, Figure 2); most American sandflies are believed to be poor vectors of Old World *Leishmania* species [3]; and *Leishmania* species native to the Americas have hosts that do not occur in Europe [56].

The vector of *L. major* in North Africa and the Middle East is *P. papatasi*, which is locally abundant in southern Europe. However, the natural reservoir hosts of this parasite are usually gerbil species not present in EU countries [18] and the risks of them dispersing into southern Europe or surviving accidental/deliberate release by humans have not been assessed.

Importance of travel within Europe (mainland and overseas territories) and internationally

Travel has led to increasing numbers of HumL cases that need to be treated, e.g. in France [70], Germany [25], Italy [71] and the United Kingdom [72]. Leishmaniasis in Guyana (overseas region of France) is a major source of exotic cases imported to mainland France, and *L. infantum* has travelled in the reverse direction in a dog [73]. Isoenzyme [12] and molecular markers [32,34] can sometimes identify the origins of *Leishmania* strains.

Travel poses the risk of the emergence in southern Europe of anthroponotic *L. donovani* [74] and *L. tropica* (see above), and the introduction to northern Europe of *L. infantum* in dogs taken to the Mediterranean region on holiday or rescued from there as strays [7].

TABLE 2

European distributions of sandfly vectors of human leishmaniasis up to 2009 (unproven role throughout range)

<i>Leishmania</i> species	<i>L. tropica</i> species complex - Greece only	<i>L. major</i>	<i>L. infantum</i> (= <i>L. chagasi</i> in Neotropics) - Mediterranean region only	<i>L. infantum</i> (= <i>L. chagasi</i> in Neotropics) - Mediterranean region only
Human disease	(Diffuse and muco-) cutaneous leishmaniasis	Cutaneous leishmaniasis	Cutaneous leishmaniasis	Visceral leishmaniasis
EU biome	Mediterranean forests	Absent	Mediterranean forests, Temperate broadleaf forest	Mediterranean forests, Temperate broadleaf forest
EU: Cyprus	<i>P. sergenti</i> s.l.	<i>P. papatasi</i>	<i>P. perfliewi</i> s.l., <i>P. tobbi</i>	<i>P. perfliewi</i> s.l., <i>P. tobbi</i>
EU: France	<i>P. sergenti</i> s.l.	<i>P. papatasi</i>	<i>P. ariasi</i> , <i>P. perniciosus</i> , <i>P. perfliewi</i> ?	<i>P. ariasi</i> , <i>P. perniciosus</i>
EU: Germany	No vectors	No vectors	<i>P. perniciosus</i>	<i>P. perniciosus</i>
EU: Greece	<i>P. sergenti</i> s.l.	<i>P. papatasi</i>	<i>P. perfliewi</i> s.l., <i>P. tobbi</i>	<i>P. perfliewi</i> , <i>P. tobbi</i> , <i>P. neglectus</i>
EU: Hungary	No vectors?	No vectors?	<i>P. neglectus</i> , <i>P. perfliewi</i> ?	<i>P. neglectus</i> , <i>P. perfliewi</i> ?
EU: Italy	<i>P. sergenti</i> s.l.	<i>P. papatasi</i>	<i>P. ariasi</i> , <i>P. perfliewi</i> , <i>P. perniciosus</i> , <i>P. neglectus</i>	<i>P. ariasi</i> , <i>P. perfliewi</i> , <i>P. perniciosus</i> , <i>P. neglectus</i>
EU: Malta	<i>P. sergenti</i> s.l.	<i>P. papatasi</i>	<i>P. perfliewi</i> , <i>P. perniciosus</i> , <i>P. neglectus</i>	<i>P. perfliewi</i> , <i>P. perniciosus</i> , <i>P. neglectus</i>
EU: Portugal	<i>P. sergenti</i> s.l.	<i>P. papatasi</i>	<i>P. ariasi</i> , <i>P. perniciosus</i>	<i>P. ariasi</i> , <i>P. perniciosus</i>
EU: Romania	No vectors?	<i>P. papatasi</i>	<i>P. perfliewi</i> , <i>P. neglectus</i>	<i>P. perfliewi</i>
EU: Spain	<i>P. sergenti</i> s.l.	<i>P. papatasi</i>	<i>P. ariasi</i> , <i>P. perniciosus</i>	<i>P. ariasi</i> , <i>P. perniciosus</i>
EU candidate: Former Yugoslav Republic of Macedonia	<i>P. sergenti</i> s.l.	<i>P. papatasi</i>	<i>P. perfliewi</i> , <i>P. tobbi</i> , <i>P. neglectus</i>	<i>P. perfliewi</i> , <i>P. tobbi</i> , <i>P. neglectus</i>
EU candidate: European Turkey (Asiatic Turkey)	(<i>P. sergenti</i> s.l.)	(<i>P. papatasi</i>)	(<i>P. perfliewi</i> s.l., <i>P. tobbi</i> , <i>P. neglectus</i>)	(<i>P. perfliewi</i> s.l., <i>P. tobbi</i> , <i>P. neglectus</i>)
Other Europe: Albania	<i>P. sergenti</i> s.l.	<i>P. papatasi</i>	<i>P. perfliewi</i> , <i>P. tobbi</i> , <i>P. neglectus</i>	<i>P. perfliewi</i> , <i>P. tobbi</i> , <i>P. neglectus</i>
Other Europe: Switzerland	No vectors	No vectors	<i>P. perniciosus</i>	<i>P. perniciosus</i>

Adapted from a contribution [2] published in the OIE (World Organisation for Animal Health) Scientific and Technical Review: In Climate change: the impact on the epidemiology and control of animal diseases (S. de la Roque, ed.). Rev Sci Tech Off Int Epiz. 2008;27(2):399-412.

Changes in environments (e.g. urbanisation, deforestation) and socio-economic patterns

Deforestation and urbanisation are known to affect leishmaniasis worldwide [6] because of the associations of many vectors and reservoirs with natural or rural areas. Based on the EDEN partners' findings, most Mediterranean regions have at least one vector associated more closely with rural or peri-urban zones [64]. From 1945, most of the socio-economic changes favoured a reduction in 'infantile visceral leishmaniasis' (caused by *L. infantum*) in southern Europe, including better nutrition, widespread insecticide spraying (against malaria-transmitting mosquitoes), better housing and a reduction in the rural population. The last 20 years have seen changes that have increased contact with the Mediterranean vectors, including more holidays and second homes for northern Europeans, unforeseen modes of transmission (among intravenous drug users), and immunosuppression (HIV/*Leishmania* co-infections). The latter is highest in south-western Europe [15].

Risk prediction models

The logic of visceral leishmaniasis control

Based on compartmental mathematical (R_0) models, Dye [75] concluded that insecticides can be expected to reduce the incidence of HumL caused by *L. infantum* even more effectively than they reduce the incidence of CanL, but only where transmission occurs peridomestically and the sandfly vectors are accessible to treatment, as in parts of Latin America. For control of HumL and CanL in Europe, Dye [75] concluded that a dog vaccine is highly desirable, because sandfly vectors here are less accessible to insecticide treatment. In Europe, CanL is a veterinary problem with socio-economic importance and a vaccine is more likely to be afforded than elsewhere.

Risk assessment of introduction, establishment and spread in the European Union (EU) for the short term (2-3 years)

'Oriental sore' caused by *L. tropica* is usually anthroponotic, and it is sporadically endemic in Greece and endemic in neighbouring countries to the EU. The principal vector (*P. sergenti* s.l.) is locally abundant in southern Europe, where new foci could be initiated by people infected in North Africa and the Middle East, including members of the European armed forces based in Iraq and Afghanistan [76,77]. Recently, *L. donovani* has been introduced to Cyprus [74]. Good surveillance, followed by prompt diagnosis and treatment should be extended to all areas of high risk, in order to help prevent the emergence of anthroponotic leishmaniasis.

Cutaneous leishmaniasis caused by the Old World parasite *L. major* has a low risk of emergence as a sandfly-borne disease in southern Europe in the short and long term, even though its principal vector (*P. papatasi*) is locally abundant, because its main gerbil reservoir hosts are absent.

Cutaneous leishmaniasis caused by the American parasites of the *L. braziliensis* and *L. mexicana* complexes

have low risks of emergence as sandfly-borne diseases in southern Europe in the short and long term because of the absence of their exotic vectors and mammalian reservoir hosts.

However, all these parasites pose a significant risk of introduction to Europe by intravenous drug users (IVDUs) and the establishment of local transmission by syringe needles, especially if these patients have HIV co-infections. This is based on the experience with endemic visceral leishmaniasis caused by *L. infantum* [15,42].

Risk assessment of introduction, establishment and spread in the European Union for the long term (15-20 years)

Leishmania infantum is currently the only significant causative agent of visceral and cutaneous HumL endemic in Europe. Its high prevalence in asymptomatic humans and in the widespread reservoir host (the domestic dog) means there is a high risk of emergence in parts of Europe further north, as demonstrated in northern Italy [65]. In addition to risk factors [78] and statistical models [64] with associated risk maps, EDEN is producing R_0 mathematical models as part of research to explain why large regions of temperate Europe have sandflies without HumL in the presence of imported CanL. Some of the key data come from questionnaires to veterinary clinics, validated by prospective serological surveys of CanL, from northern and southern areas with a wide range of disease prevalence.

Increasing dog travel poses a significant risk of introduction of *L. infantum* into northern Europe from the Mediterranean region. There is also a risk of establishment of non-vector transmission and spread as has been observed in North America [24]. Non-vector transmission might explain the autochthonous cases of CanL in Germany [25, 26].

L. tropica has been isolated from both the domestic dog and the black rat [5,8], and so the risk of introduction and spread of CL caused by this parasite in the EU should be re-assessed if either these mammals or related synanthropic species were found to be reservoir hosts (rather than dead-end hosts) in the disease foci in North Africa and southwest Asia [35].

Assessment of whether the existing data sources are adequate and, if not, identification of missing key data needed for conducting risk assessment studies

Research data about leishmaniasis and its spatial distribution in Europe and the Mediterranean region are being enhanced [79] and made accessible online by EDEN and another EU-funded project, LeishRisk, which has collaborated with the WHO to produce an E-compendium, a compilation of peer-reviewed literature on leishmaniasis epidemiology [1,80].

However, public health and veterinary surveillance data are more fragmentary, which undoubtedly caused the public health impact of leishmaniasis to be underestimated for many years in Europe as well as worldwide [1]. The WHO has concluded that more surveillance is necessary in Europe to assess an emergence of leishmaniasis [9], but the partners of the EDEN leishmaniasis sub-project have stressed the need for better coordination of existing surveillance, including linking human health and veterinary data for the zoonotic disease. Currently (EDEN partners, personal communications), HumL is notifiable in Greece, Italy, Portugal and Turkey, and in endemic autonomous regions in Spain. CanL is notifiable in Greece and at municipality level in the endemic regions of the four other countries mentioned above. Neither disease is notifiable in France. At international level, WHO organised a meeting of Eurasian countries in 2009 (J. Alvar, personal communication), aimed at standardising surveillance and reporting, and CanL is reported as a listed disease ('other diseases') of the World Organisation for Animal Health (OIE) [81]. The monitoring of dog travel [7] should continue to be improved and standardised.

Acknowledgements

This review is based on an extensive literature study conducted as part of the European Centre for Disease Prevention and Control funded V-borne project: "Assessment of the magnitude and impact of vector-borne diseases in Europe", tender n^o. OJ/2007/04/13-PROC/2007/003. It was compiled using EDEN funding, EU grant GOCE-2003-010284 EDEN. The paper is catalogued by the EDEN Steering Committee as EDEN0199 (<http://www.eden-fp6project.net/>). The contents of this publication are the responsibility of the author and do not necessarily reflect the views either of the ECDC or of the European Commission. I thank all partners of the EDEN leishmaniasis sub-project for information and support.

References

- World Health Organization (WHO). Leishmaniasis: background information. A brief history of the disease. WHO. 2009. Available from: www.who.int/leishmaniasis/en/
- Ready P.D. Leishmaniasis emergence and climate change. In: S de la Roque, editor. Climate change: the impact on the epidemiology and control of animal diseases. Rev Sci Tech Off Int Epiz. 2008;27(2):399-412.
- Killick-Kendrick R. Phlebotomine vectors of the leishmaniasis: a review. Med Vet Entomol. 1990;4(1):1-24.
- Peters N, Sacks D. Immune privilege in sites of chronic infection: Leishmania and regulatory T cells. Immunol Rev. 2006;213:159-79.
- Desjeux P. Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis. 2004;27(5):305-18.
- Desjeux P. The increase in risk factors for leishmaniasis worldwide. Trans R Soc Trop Med Hyg. 2001;95(3):239-43.
- Trotz-Williams LA, Trees AJ. Systematic review of the distribution of the major vector-borne parasitic infections in dogs and cats in Europe. Vet Rec. 2003;152(4):97-105.
- Gramiccia M, Gradoni L. The leishmaniasis in Southern Europe. In: W Takken, BGJ Knols, editors. Emerging pests and Vector-Borne Diseases, Ecology and control of vector-borne diseases, Vol. 1. Wageningen Academic Publishers. 2007:75-95.
- World Health Organization (WHO). Regional Office for Europe. Vectorborne and rodentborne diseases. WHO. 29 September 2009. Available from: http://www.euro.who.int/globalchange/Assessment/20070216_10
- Moral L, Rubio EM, Moya M. A leishmanin skin test survey in the human population of l'Alacantí region (Spain): implications for the epidemiology of Leishmania infantum infection in southern Europe. Trans R Soc Trop Med Hyg. 2002;96(2):129-32.
- Martín-Sánchez J, Pineda JA, Morillas-Márquez F, García-García JA, Acedo C, Macías J. Detection of Leishmania infantum kinetoplast DNA in peripheral blood from asymptomatic individuals at risk for parenterally transmitted infections: relationship between polymerase chain reaction results and other Leishmania infection markers. Am J Trop Med Hyg. 2004;70(5):545-8.
- Pratlong F, Rioux JA, Marty P, Faraut-Gambarelli F, Dereure J, Lanotte G, et al. Isoenzymatic analysis of 712 strains of Leishmania infantum in the south of France and relationship of enzymatic polymorphism to clinical and epidemiological features. J Clin Microbiol. 2004;42(9):4077-82.
- Marty P, Izri A, Ozon C, Haas P, Rosenthal E, Del Giudice P, et al. A century of leishmaniasis in Alpes-Maritimes, France. Ann Trop Med Parasitol. 2007;101(7):563-74.
- Alvar J, Cañavate C, Gutiérrez-Solar B, Jiménez M, Laguna F, López Vélez R, et al. Leishmania and human immunodeficiency virus co-infection: the first 10 years. Clin Microbiol Rev. 1997;10(2):298-319.
- Desjeux P, Alvar J. Leishmania/HIV co-infections: epidemiology in Europe. Ann Trop Med Parasitol. 2003;97 Suppl 1:3-15.
- Bates PA. Transmission of Leishmania metacyclic promastigotes by phlebotomine sand flies. Int J Parasitol. 2007;37(10):1097-106.
- Desjeux P. Information on the epidemiology and control of the leishmaniasis by country or territory. WHO/LEISH/91.30. Geneva: World Health Organization. 1991.
- World Health Organization. Control of the Leishmaniasis. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 1990;793:1-158.
- Mauricio IL, Gaunt MW, Stothard JR, Miles MA. Glycoprotein 63 (gp63) genes show gene conversion and reveal the evolution of Old World Leishmania. Int J Parasitol. 2007;37(5):565-76.
- Maroli M, Pennisi MG, Di Muccio T, Khoury C, Gradoni L, Gramiccia M. Infection of sandflies by a cat naturally infected with Leishmania infantum. Vet Parasitol. 2007;145(3-4):357-60.
- Solano-Gallego L, Rodríguez-Cortés A, Iniesta L, Quintana J, Pastor J, Espada Y. Cross-sectional serosurvey of feline leishmaniasis in ecoregions around the Northwestern Mediterranean. Am J Trop Med Hyg. 2007;76(4):676-80.
- Ready P. Sand fly evolution and its relationship to Leishmania transmission. Mem Inst Oswaldo Cruz. 2000;95(4):589-90.
- Cruz I, Morales MA, Noguer I, Rodríguez A, Alvar J. Leishmania in discarded syringes from intravenous drug users. Lancet. 2002;359(9312):1124-5.
- Duprey ZH, Steurer FJ, Rooney JA, Kirchhoff LV, Jackson JE, Rowton ED, et al. Canine visceral leishmaniasis, United States and Canada, 2000-2003. Emerg Infect Dis. 2006;12(3):440-6.
- Harms G, Schöniar G, Feldmeier H. Leishmaniasis in Germany. Emerg Infect Dis. 2003;9(7):872-5.
- Naucke TJ, Schmitt C. Is leishmaniasis becoming endemic in Germany? Int J Med Microbiol. 2004;293 Suppl 37:179-81.
- Mettler M, Grimm F, Naucke TJ, Maasjost C, Deplazes P. [Canine leishmaniasis in Central Europe: retrospective survey and serological study of imported and travelling dogs]. Berl Munch Tierarztl Wochenschr. 2005;118(1-2):37-44. German.
- Meinecke CK, Schottelius J, Oskam L, Fleischer B. Congenital transmission of visceral leishmaniasis (Kala Azar) from an asymptomatic mother to her child. Pediatrics. 1999;104(5):e65.
- World Organisation for Animal Health (OIE). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. OIE. 2004. Available from: http://www.oie.int/fr/normes/mmanual/a_00050.htm
- Rioux JA, Lanotte G. Leishmania infantum as a cause of cutaneous leishmaniasis. Trans R Soc Trop Med Hyg. 1990;84(6):898.
- Evans D, editor. Handbook on Isolation, Characterization and Cryopreservation of Leishmania. Geneva: World Health Organization; 1989.
- Lukes J, Mauricio IL, Schöniar G, Dujardin JC, Soteriadou K, Dedet JP, et al. Evolutionary and geographical history of the Leishmania donovani complex with a revision of current taxonomy. Proc Natl Acad Sci USA. 2007;104(22):9375-80.
- Alvar J, Barker JR. Molecular tools for epidemiological studies and diagnosis of leishmaniasis and selected other parasitic diseases. Trans R Soc Trop Med Hyg. 2002;96:1-250.

34. Schönián G, Mauricio I, Gramiccia M, Cañavate C, Boelaert M, Dujardin JC. Leishmaniasis in the Mediterranean in the era of molecular epidemiology. *Trends Parasitol.* 2008;24(3):135-42.
35. Parvizi P, Mazlumi-Gavani AS, Davies CR, Courtenay O, Ready PD. Two *Leishmania* species circulating in the Kaleybar focus of 'infantile visceral leishmaniasis', northwest Iran: implications for deltamethrin dog collar intervention. *Trans R Soc Trop Med Hyg.* 2008;102(9):891-97.
36. Shaw JJ, De Faria DL, Basano SA, Corbett CE, Rodrigues CJ, Ishikawa EA, et al. The aetiological agents of American cutaneous leishmaniasis in the municipality of Monte Negro, Rondônia state, western Amazonia, Brazil. *Ann Trop Med Parasitol.* 2007;101(8):681-8.
37. Ardehali S, Moattari A, Hatam GR, Hosseini SM, Sharifi I. Characterization of *Leishmania* isolated in Iran: 1. Serotyping with species specific monoclonal antibodies. *Acta Trop.* 2000;75(3):301-7.
38. Boelaert M, El-Safi S, Hailu A, Mukhtar M, Rijal S, Sundar S, et al. Diagnostic tests for kala-azar: a multi-centre study of the freeze-dried DAT, rK39 strip test and KATex in East Africa and the Indian subcontinent. *Trans R Soc Trop Med Hyg.* 2008;102(1):32-40.
39. Ameen M. Cutaneous leishmaniasis: therapeutic strategies and future directions. *Expert Opin Pharmacother.* 2007;8(16):2689-99.
40. Gradoni L, Soteriadou K, Louzir H, Dakkak A, Toz SO, Jaffe C, et al. Drug regimens for visceral leishmaniasis in Mediterranean countries. *Trop Med Int Health.* 2008;13(10):1272-6.
41. Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. *Clin Microbiol Rev.* 2006;19(1):111-26.
42. López-Vélez R. The impact of highly active antiretroviral therapy (HAART) on visceral leishmaniasis in Spanish patients who are co-infected with HIV. *Ann Trop Med Parasitol.* 2003;97 Suppl 1:143-7.
43. Rogers ME, Sizova OV, Ferguson MA, Nikolaev AV, Bates PA. Synthetic glycovaccine protects against the bite of *Leishmania*-infected sand flies. *J Infect Dis.* 2006;194(4):512-8.
44. Valenzuela JG, Belkaid Y, Garfield MK, Mendez S, Kamhawi S, Rowton ED, et al. Toward a defined anti-*Leishmania* vaccine targeting vector antigens: characterization of a protective salivary protein. *J Exp Med.* 2001;194(3):331-42.
45. World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases (WHO). Eighteenth Programme Report. Progress 2005-2006. Geneva: WHO. 2007.
46. Reed SG, Campos-Neto A. Vaccines for parasitic and bacterial diseases. *Curr Opin Immunol.* 2003;15(4):456-60.
47. Nogueira FS, Moreira MA, Borja-Cabrera GP, Santos FN, Menz I, Parra LE, et al. Leishmune vaccine blocks the transmission of canine visceral leishmaniasis: absence of *Leishmania* parasites in blood, skin and lymph nodes of vaccinated exposed dogs. *Vaccine.* 2005;23(40):4805-10.
48. Parra LE, Borja-Cabrera GP, Santos FN, Souza LO, Palatnik-de-Sousa CB, Menz I. Safety trial using the Leishmune vaccine against canine visceral leishmaniasis in Brazil. *Vaccine.* 2007;25(12):2180-6.
49. Lemesre JL, Holzmüller P, Cavaleyra M, Gonçalves RB, Hottin G, Papierok G. Protection against experimental visceral leishmaniasis infection in dogs immunized with purified excreted secreted antigens of *Leishmania infantum* promastigotes. *Vaccine.* 2005;23(22):2825-40.
50. Lemesre JL, Holzmüller P, Gonçalves RB, Bourdoiseau G, Hugnet C, Cavaleyra M, et al. Long-lasting protection against canine leishmaniasis using LiESAP-MDP vaccine in endemic areas of France: double-blind randomised efficacy trial. *Vaccine.* 2007;25(21):4223-34.
51. Carson C, Antoniou M, Ruiz-Argüello MB, Alcamí A, Christodoulou V, Messaritakis I, et al. A prime/boost DNA/Modified vaccinia virus Ankara vaccine expressing recombinant *Leishmania* DNA encoding TRYP is safe and immunogenic in outbred dogs, the reservoir of zoonotic visceral leishmaniasis. *Vaccine.* 2009;27(7):1080-6.
52. Centers for Disease Control and Prevention (CDC). Parasitic Disease Information. *Leishmania* Infection. 2008. Atlanta: CDC. Available from: http://www.cdc.gov/ncidod/dpd/parasites/leishmania/factsht_leishmania.htm#prevent
53. Reithinger R, Teodoro U, Davies CR. Topical insecticide treatments to protect dogs from sand fly vectors of leishmaniasis. *Emerg Infect Dis.* 2001;7(5):872-6.
54. Killick-Kendrick R, Killick-Kendrick M, Focheux C, Dereure J, Puech MP, Cadiergues MC. Protection of dogs from the bites of phlebotomine sandflies by deltamethrin collars for the control of canine leishmaniasis. *Med Vet Entomol.* 1997;11(2):105-11.
55. Maroli M, Mizzon V, Siragusa C, D'Oorazi A, Gradoni L. Evidence for an impact on the incidence of canine leishmaniasis by the use of deltamethrin-impregnated dog collars in southern Italy. *Med Vet Entomol.* 2001;15(4):358-63.
56. Lainson R, Shaw JJ, Silveira FT, de Souza AA, Braga RR, Ishikawa EA. The dermal leishmaniasis of Brazil, with special reference to the eco-epidemiology of the disease in Amazonia. *Mem Inst Oswaldo Cruz.* 1994;89(3):435-43.
57. Gállego M, Pratlong F, Fisa R, Riera C, Rioux JA, Dedet JP, et al. The life-cycle of *Leishmania infantum* MON-77 in the Priorat (Catalonia, Spain) involves humans, dogs and sandflies; also literature review of distribution and hosts of *L. infantum* zymodemes in the Old World. *Trans R Soc Trop Med Hyg.* 2001;95(3):269-71.
58. Aransay AM, Testa JM, Morillas-Marquez F, Lucientes J, Ready PD. Distribution of sandfly species in relation to canine leishmaniasis from the Ebro Valley to Valencia, northeastern Spain. *Parasitol Res.* 2004;94(6):416-20.
59. Houin R, Deniau M, Puel F, Reynouard F, Barbier D, Bonnet M. [Phlebotomine sandflies of Touraine]. *Ann Parasitol Hum Comp.* 1975;50(2):233-43. French.
60. Rioux JA, Golvan YJ. [Epidemiology of the leishmaniasis in the south of France]. National Institute of Health and Medical Research (INSERM) Monography. Paris. 1969;37:1-223. French.
61. Aransay AM, Ready PD, Morillas-Marquez F. Population differentiation of *Phlebotomus perniciosus* in Spain following postglacial dispersal. *Heredity.* 2003;90(4):316-25.
62. Perrotey S, Mahamdallie S, Pesson B, Richardson KJ, Gállego M, Ready PD. Postglacial dispersal of *Phlebotomus perniciosus* into France. *Parasite* 2005;12(4):283-91.
63. Rioux JA, de la Roque S. [Climates, leishmaniasis and trypanosomiasis.] In: Rodhain F, editor. [Climate change, infectious and allergic diseases]. Éditions scientifiques et médicales, Elsevier. 2003. 41-62.
64. Martinez S, Vanwambeke SO, Ready P. Linking changes in landscape composition and configuration with sandfly occurrence in southwest France. Fourth International Workshop on the Analysis of Multi-temporal Remote Sensing Images, 2007. MultiTemp 2007.
65. Maroli M, Rossi L, Baldelli R, Capelli G, Ferroglio E, Genchi C, et al. The northward spread of leishmaniasis in Italy: evidence from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. *Trop Med Int Health.* 2008;13(2):256-64.
66. Dye C, Guy MW, Elkins DB, Wilkes TJ, Killick-Kendrick R. The life expectancy of phlebotomine sandflies: first field estimates from southern France. *Med Vet Entomol.* 1987;1(4):417-25.
67. Volf P, Hostomska J, Rohousova I. Molecular crosstalks in *Leishmania*-sandfly-host relationships. *Parasite.* 2008;15(3):237-43.
68. Rossi E, Bongiorno G, Ciolli E, Di Muccio T, Scalone A, Gramiccia M, et al. Seasonal phenology, host-blood feeding preferences and natural *Leishmania* infection of *Phlebotomus perniciosus* (Diptera, Psychodidae) in a high-endemic focus of canine leishmaniasis in Rome province, Italy. *Acta Trop.* 2008;105(2):158-65.
69. Depaquit J, Naucke TJ, Schmitt C, Ferte H, Leger N. A molecular analysis of the subgenus *Transphlebotomus* *Artemiev*, 1984 (*Phlebotomus*, Diptera, Psychodidae) inferred from ND4 mtDNA with new northern records of *Phlebotomus mascittii* Grassi, 1908. *Parasitol Res.* 2005;95(2):113-6.
70. El Hajj L, Thellier M, Carrière J, Bricaire F, Danis M, Caumes E. Localized cutaneous leishmaniasis imported into Paris: a review of 39 cases. *Int J Dermatol.* 2004;43(2):120-5.
71. Antinori S, Gianelli E, Calattini S, Longhi E, Gramiccia M, Corbellino M. Cutaneous leishmaniasis: an increasing threat for travellers. *Clin Microbiol Infect.* 2005;11(5):343-6.
72. Lawn SD, Whetham J, Chiodini PL, Kanagalingam J, Watson J, Behrens RH et al. New world mucosal and cutaneous leishmaniasis: an emerging health problem among British travellers. *QJM.* 2004;97(12):781-8.
73. Rotureau B, Ravel C, Aznar C, Carme B, Dedet JP. First report of *Leishmania infantum* in French Guiana: canine visceral leishmaniasis imported from the Old World. *J Clin Microbiol.* 2006;44(3):1120-2.
74. Antoniou M, Haralambous C, Mazeris A, Pratlong F, Dedet JP, Soteriadou K. *Leishmania donovani* leishmaniasis in Cyprus. *Lancet Infect Dis.* 2009;9(2), 76-7.
75. Dye C. The logic of visceral leishmaniasis control. *Am J Trop Med Hyg.* 1996;55(2):125-30.
76. Magill AJ, Grögl M, Gasser RA Jr, Sun W, Oster CN. Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. *N Engl J Med.* 1993;328(19):1383-7.

77. Crum NF, Aronson NE, Lederman ER, Rusnak JM, Cross JH. History of U.S. military contributions to the study of parasitic diseases. *Mil Med.* 2005;170(4 Suppl):17-29.
78. Martín-Sánchez J, Morales-Yuste M, Acedo-Sanchez C, Baron S, Díaz V, Morillas-Marquez F. Canine Leishmaniasis in southeastern Spain. *Emerg Infect Dis.* 2009; 15:795-8.
79. Dujardin JC, Campino L, Cañavate C, Dedet JP, Gradoni L, Soteriadou K, et al. Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. *Emerg Infect Dis.* 2008;14(7):1013-8.
80. LeishRisk [Internet]. Belgium: European Commission. [cited 2009 Mar 16]. Available from: <http://www.leishrisk.net/leishrisk/>
81. World Organisation for Animal Health (OIE) [Internet]. OIE Listed diseases. France: OIE. [updated 2007 Mar 14; cited 2009 Mar 16]. Available from: www.oie.int/eng/maladies/en_classification2007.htm?e1d7

Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review

J Depaquit (jerome.depaquit@univ-reims.fr)¹, M Grandadam^{2,3}, F Fouque⁴, PE Andry¹, C Peyrefitte⁵

1. Université de Reims Champagne Ardenne, AFSSA, JE 2533-USC "VECPAR", Reims, France

2. Laboratoire associé au Centre national de référence des arbovirus, Unité de virologie tropicale, Institut de médecine tropicale du Service de santé des armées, Marseille, France

3. Centre national de référence des arbovirus, Institut Pasteur, Paris, France

4. Cellule d'Intervention Biologique d'Urgence (CIBU), Institut Pasteur, Paris, France

5. Unité de virologie, Département de Biologie des Agents transmissibles, Centre de Recherche du Service de Santé des Armées, Grenoble, France

Citation style for this article:

Citation style for this article: Depaquit J, Grandadam M, Fouque F, Andry P, Peyrefitte C. Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review. Euro Surveill. 2010;15(10):pii=19507. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19507>

This article has been published on 11 March 2010

Phlebotomine sandflies are known to transmit leishmaniases, bacteria and viruses that affect humans and animals in many countries worldwide. These sandfly-borne viruses are mainly the *Phlebovirus*, the *Vesiculovirus* and the *Orbivirus*. Some of these viruses are associated with outbreaks or human cases in the Mediterranean Europe. In this paper, the viruses transmitted by Phlebotomine sandflies in Europe (Toscana virus, Sicilian virus, sandfly fever Naples virus) are reviewed and their medical importance, geographical distribution, epidemiology and potential spreading discussed. Data on vertebrate reservoirs is sparse for sandfly fever viruses. The factor currently known to limit the spread of diseases is mainly the distribution areas of potential vectors. The distribution areas of the disease may not be restricted to the areas where they have been recorded but could be as wide as those of their vectors, that is to say *Larroussius* and *P. papatasi* mainly but not exclusively. Consequently, field work in form of viral isolation from sandflies and possible reservoirs as well as laboratory work to establish vectorial competence of colonised sandflies need to be encouraged in a near future, and epidemiological surveillance should be undertaken throughout the European Union.

Introduction

During the last decade, several cases of infections due to Toscana virus have been recorded in Europe (Italy, France, Spain, and Portugal). A few studies focusing on the viruses transmitted by Phlebotomine sandflies have been carried out. This review summarises the data related to arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe.

Phlebotomine sandflies are the vectors of the *Leishmania*, pathogens that cause diseases called leishmaniases in more than 80 countries in the Old and New World. Sandflies are also vectors of other human pathogens such as Bartonella and viruses belonging

to three different genera: (i) the *Phlebovirus* (family Bunyaviridae) including sandfly fever Sicilian virus, sandfly fever Naples virus, Toscana virus and Punta Toro virus; (ii) the *Vesiculovirus* (family Rhabdoviridae) including Chandipura virus [2-3] and (iii) the *Orbivirus* (family Reoviridae) including Changuinola virus [1]. The latter viruses have been associated with several outbreaks in humans. Further less important viruses have also been found in Europe. Chios virus was isolated from a human case of severe encephalitis (Papa and Pavlidou, personal communication) in Greece and additionally three other viruses were isolated from Phlebotomine sandflies: Corfou virus from *Phlebotomus (Larroussius) neglectus*, in Greece [4], Massilia virus from *P. (L.) perniciosus*, in France [5] and Arbia virus from *P. (L.) perniciosus* and from *P. (L.) perfiliewi* in Italy. However, so far there are no reports of human disease from these viruses.

Little is known about the viruses transmitted by Phlebotomine sandflies and they can be, to our opinion, considered as neglected pathogens. However, the Toscana virus, sandfly fever Naples and Sicilian viruses are endemic in the Mediterranean region and could spread to more temperate areas in Europe where vectors are abundant. Moreover, other viruses transmitted by sandflies and circulating in India may be imported into Europe by introduction of viremic patients emphasising the need to consider these viruses relevant from a European public health perspective.

Clinical picture and geographical distribution

Sandfly fever Sicilian and Naples virus infections

Sandfly fever Sicilian and Naples virus and other related viruses cause the "three-day fever" or "pappatacci fever". Patients present with influenza-like symptoms including fever, retro-orbital pain, myalgia and malaise and usually recover fully within a week.

However, infections with sandfly fever Naples and Sicilian viruses, even when mild, have shown to be highly incapacitating for the time patients are affected.

The human cases and some virus isolation from sandflies were reported around the Mediterranean Sea (Figure 1) in Algeria [6], Cyprus [7, 8], Egypt [9], Iran [9-11], Israel [12], Italy [13], Jordan [14] and Portugal [15]. An earlier review based on serological data, without virus isolation and characterisation, indicated that sandfly fever Sicilian or Naples viruses have been recorded in Bangladesh, Djibouti, Ethiopia, Iraq, Morocco, Saudi Arabia, Somalia, Sudan, Tunisia, southern and central Asian republics of the former Soviet Union, and the former Republic of Yugoslavia [16]. The same study showed the absence of neutralising antibodies in humans in Algeria, central Africa and eastern Asia [16].

Sandfly fevers were first described in Italy, in 1943-1944 during outbreaks of influenza-like illness among United States (US) soldiers due to Sicilian and Naples viruses [16]. Human cases are often found in people visiting Mediterranean countries. A total of 37 cases of sandfly fever Sicilian virus infections and one case of sandfly fever Naples virus infection were recorded in Swedish tourists returning from Cyprus between 1986 and 1989. In 1985, the incidence was low (0.3%) among members of Swedish troops stationed in Cyprus [17]. More recently, a 2002 outbreak affecting 256 among 581 Greek soldiers stationed in Cyprus showed increasing incidence (44%) for infections with sandfly fever Sicilian virus [8].

Toscana virus infections

Many infections with the Toscana virus are asymptomatic. Reported clinical cases mostly present with influenza-like symptoms, but the virus displays a strong neurotropism. Outbreaks of acute meningitis or meningo-encephalitis due to infections with Toscana virus have been reported in several European countries bordering the Mediterranean Sea: (Italy, France [18-25], Spain [26-30] and Portugal [31]).

Seroprevalence studies in Italy, show large variations ranging from 3% in northern Italy (Torino) [32], to 16% in Umbria [33] and 22% in central Italy. The virus is widespread in several regions including Tuscany, Piedmont, the Marches, Umbria and Emilia-Romagna.

In Spain, the seroprevalence rate is higher and ranges from 5% [27] to 26% [26]. However, the large difference in prevalence observed between the two surveys might be related to the fact that the authors did not use the same serological tests [21]. In France, the seroprevalence observed recently was 12% in a survey using blood from donors in south-eastern France [21]. In Turkey [34], a pilot study reports also positive serologies for sandfly fever Toscana, Naples and Sicilian viruses.

In Italy, from May to October, Toscana virus is a major cause of meningitis and meningo-encephalitis with a peak of incidence in August. During this period it causes 80% of cases in children and 50% of cases in adults [32, 33]. Toscana virus is among the three most prevalent viruses associated with meningitis during the warm season. Therefore, Toscana virus must be considered as an emerging pathogen in the Mediterranean basin [19] and significant public health issue in Europe.

Chandipura virus infections

Outside of Europe, epidemics of acute encephalitis characterised by rapid onset of fever and central nervous system involvement with high case fatality rate were reported in Asia [35, 36]. These outbreaks were caused by the highly pathogenic Chandipura virus, a *Vesiculovirus* of the *Rhabdoviridae* family originally isolated in India from a patient [2]. To date, no human cases have been reported in Europe and Africa, although Chandipura virus has been isolated in Nigeria from hedgehogs (*Atelerix spiculus*) [37]. The fact that no human cases have been reported from there so far may reflect a lack of specific testing for Chandipura virus.

FIGURE 1

Distribution of (a) Toscana, (b) Sicilian, and (c) Naples viruses in the European Union and neighbouring countries around the Mediterranean Sea up to 2009

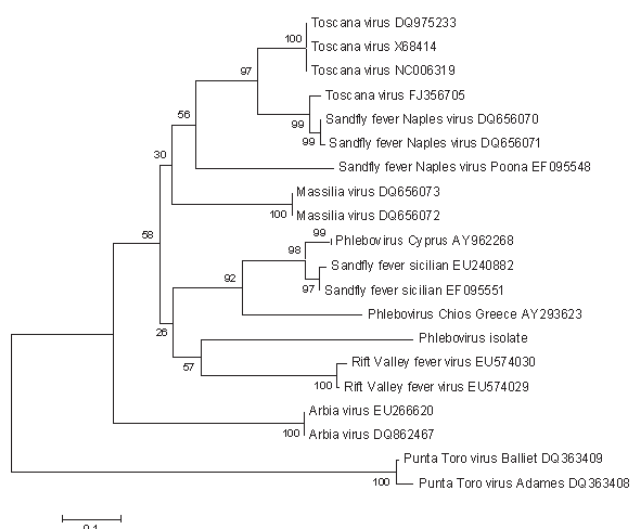


Countries with confirmed cases are depicted in mid grey, the estimated distribution limits are depicted with a dark grey line. Source: V-borne project; reproduced with permission from the European Centre for Disease Prevention and Control.

Among the nine species of the genus *Vesiculovirus*, Chandipura virus should be considered of great public health importance. Conducting surveys on Chandipura virus in the south of Europe and along the south-eastern European borders is necessary to anticipate an introduction of the virus into Europe from Asia and/or Africa.

FIGURE 2

Neighbour-joining tree based on nucleotide sequences of the large segment encoding the viral polymerase with bootstrap values (%) calculated with 500 replicates.



Transmission

Genus Phlebovirus

This genus contains the majority of known sandfly-borne viruses. Many serotypes have been characterised in the Americas from sandflies belonging to the genera *Lutzomyia sensu lato*, and in Africa, Europe and Central Asia mainly from *Phlebotomus* and also from *Sergentomyia*.

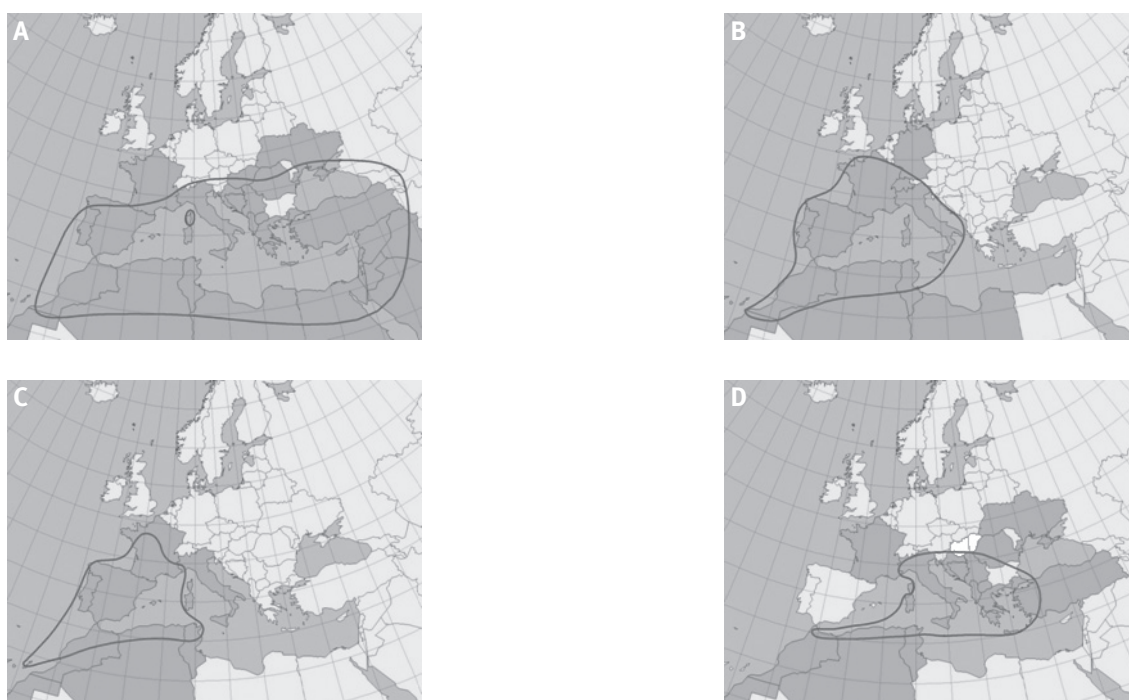
According to the eighth Report of the International Committee on Taxonomy of Viruses [37], the genus *Phlebovirus* can be divided into nine antigenic complexes and includes 37 classified viruses. Further 16 virus serotypes are unclassified and are considered to be tentative members of the genus. Current knowledge suggests that many of the phleboviruses are maintained in their arthropod vectors by vertical (transovarial) transmission and that vertebrate hosts play little or no role in the basic maintenance cycle of these agents [1]. This maintenance mechanism has important ecological implications for the phleboviruses, as it allows them to persist during periods when adult vectors are absent or when susceptible vertebrate hosts are not available.

Sandfly fever Sicilian virus

This virus has been isolated in natura [39] and in vitro [40, 41] from *P. papatasi* captured from the Mediterranean basin to Central Asia. It has also recently been isolated in natural conditions from *P. (L.) ariasi* in Algeria [6]. In some parts of its distribution area such as Cyprus where a local strain, Cyprus virus, has been isolated from Swedish troop members [7],

FIGURE 3

Distribution of main vectors in the European Union and neighbouring countries around the Mediterranean Sea up to 2009



From left to right and from top to bottom: (a) *Phlebotomus papatasi*, (b) *P. perniciosus*, (c) *P. ariasi*, and (d) *P. perfiliewi s. st.* Countries with confirmed presence are depicted in mid grey, estimated distribution limits are depicted in dark grey.

Source: V-borne project; reproduced with permission from the European Centre for Disease Prevention and Control.

P. papatasi is an abundant species [42] and could be a suspected vector. However, in Italy *P. papatasi* is now scarce whereas it was abundant before DDT was used in the 1940s and cannot be a candidate for the transmission of sandfly fever Sicilian virus. Autochthonous *Phlebotomus* belonging to the subgenus *Larroussius* (*P. perniciosus*, *P. perfiliewi* and *P. neglectus*) seem to be better candidates for its transmission. In Greece, a closely related virus called Corfou virus has been isolated from *P. (Larroussius) neglectus* [4].

Different vertebrate species including rodents (*Apodemus* spp., *Mus musculus*, *Rattus rattus*, *Clethrionomys glareolus*, *Meriones libycus*, *Gerbillus aureus*), insectivora (Soricidae and Talpidae) and carnivora (*Mustela nivalis*) may participate to the maintenance of Sandfly Sicilian virus life cycle [43-46].

The virus is endemic in Europe and currently there are no known reasons why it would not extend over the entire distribution range of the vectors. Its further spread could follow a wider distribution of the vector and/or the reservoirs taking into account the unknown potential impact of climatic shifts on the development of the virus in the vector. Future studies will have to determine (i) the distribution and prevalence of the disease according to serological studies in the European Mediterranean region, (ii) the vector competences and capacities of local sandfly species, (iii) the temperatures required for viral replication in infected sandflies in order to evaluate the risk of development in local vectors, and field work will have to be performed in foci where human cases, infected animal reservoirs and infected sandflies occur.

Sandfly fever Naples virus

The virus has been isolated in Italy from *P. perniciosus* [47], in Serbia from *P. perfiliewi* [48] and in Egypt from *P. papatasi* [49]. The area in which a stable focus is recorded has been delimited to Serbia [50]. Reservoirs for Sandfly fever Naples virus are unknown. An important seroprevalence rate of 30% has been recorded in Jordan [14]. Because the identity of the virus cannot be assessed with certainty, the virus could circulate in Turkey [51]. Future investigations similar to those developed for Toscana virus need to be carried out to gain better understanding of the potential spread of the virus.

Toscana virus

The distribution of Toscana virus includes Spain, France, Italy, Greece, Cyprus [19], Portugal [30], and Turkey [38] and it has been isolated several times from *P. perniciosus* and *P. perfiliewi* belonging to the subgenus *Larroussius*. Transovarial transmission has been demonstrated in laboratory conditions and by viral isolation from male *Phlebotomus* spp. Venereal transmission from infected males to uninfected females has also been demonstrated [52]. It is suggested that the reservoir of Toscana virus is most likely the vector itself. However, a progressive decline of vector infected rates

from generation to generation, suggests that this virus cannot be maintained indefinitely by vertical transmission [53-55]. Consequently, the existence of reservoirs has to be considered. Serological data have shown no evidence of infection among domestic or wild animals. However, a Toscana virus strain was isolated from the brain of the bat *Pipistrellus kuhli* [56]. The viral genome detection of Toscana virus in *Sergentomyia minuta* [57], a species considered as feeding exclusively on lizards and geckos, points towards the possible existence of unknown reservoirs. The short duration of viraemia, and the lack of evidence for a persistent infection in humans, compromises the participation of humans in the maintenance of the virus.

The geographical extension potential of Toscana virus is high in Europe. At this time numbers of endemic foci of the virus have been identified in different neighbouring countries (Spain, France, Italy) and potential vectors are widely dispersed. However, a rapid spreading of the virus is unlikely due to the lack of evidence of animal reservoir. Humans may favour viral transportation but the shortness of viraemia may limit an efficient transmission to naïve vectors. Moreover, the potential impact of climatic shifts on the vector competence is unknown. Similarly as for the two viruses mentioned above, future studies - both field work and experimental - will have to determine the distribution and prevalence of the disease caused by Toscana virus based on serological investigations around the European Mediterranean region, the vector competences and capacities of local sandfly species (in particular *P. ariasi* and *P. perniciosus*), the temperatures required for viral replication in infected sandflies and the possible impact of climate change on the potential spread in Europe.

Massilia virus, phlebovirus isolate and Punta Toro virus

The recent isolation of Massilia virus - a new *Phlebovirus* - from *P. (L.) perniciosus* in south-eastern France [5], emphasised the necessity of performing field studies to anticipate the possible eruption in humans of this new virus. Furthermore, the isolation of a probable new *Phlebovirus* from a sandfly (Figure 2) in southern France during the summer 2007 increases the number of phleboviruses and the potential pathogens for humans. This highlights the need to carry out new investigations in Europe taking into account the variability of phleboviruses [58].

The distribution area of Punta Toro virus is limited to Central America where it is transmitted by *Lutzomyia* (*Nyssomyia*) *trapidoi* and *L. (Ny.) ylephiletor*. The taxonomic status of these vectors has to be clarified in the light of an entomological revision. Even if the subgenus *Nyssomyia* has never been recorded in the West Indies, some species belonging to it have been recorded in French Guyana such as *L. anduzei*, *L. flaviscutellata*, *L. umbratilis*, *L. yuilli pajoti* [59]. These sandfly species could be considered as possible candidates for

native transmission in the overseas territories of the European Union (EU) which are important leisure destinations during local dry seasons. Whereas importation of Punta Toro virus in European countries is unlikely, the possible emergence of the virus will highlight the importance to have the capacity to diagnose etiologically any imported febrile syndromes in tourists returning from these areas.

The main natural *Phlebotomus* vectors seem to belong to the subgenus *Larroussius*. The vectors of the main phleboviruses in the eastern part of the Mediterranean basin are not known: in Turkey, *P. perniciosus* and *P. ariasi* are not recorded (figure 3). However, it appears difficult to assess a co-evolution between viruses and sandflies within the subgenus *Larroussius*: the isolation of viruses (or viral RNA) in *P. papatasi* or in *Sergentomyia* spp. strongly suggests the capture of the viruses by Phlebotomine sandflies.

Genus Vesiculovirus Chandipura virus

Under laboratory conditions, *P. papatasi* is an efficient reservoir for the virus, showing growth, and venereal and transovarial transmission [60, 61]. The experimental transmission of Chandipura virus by *P. (Euphebotomus) argentipes* has been recently demonstrated [62]. In natural conditions, it has been isolated from a pool of 253 unidentified Phlebotomine sandflies (*Phlebotomus* spp.) in the Maharashtra State of India [63] and from unidentified *Sergentomyia* in the Karimnagar district in Andhra Pradesh, India [64]. Four strains have also been isolated from batches of sandflies from Senegal, belonging probably to the genus *Sergentomyia* [65, 66]. These data show a wide distribution of the virus and the capacity of two genera of sandflies namely *Phlebotomus* (subgenera *Phlebotomus* and *Euphebotomus*) and *Sergentomyia* to transmit the virus.

Chandipura virus is currently endemic only in India and its introduction to Europe by an infected Phlebotomine sandfly is unlikely to occur, due to the fact that no settlement of Phlebotomine Chandipura virus vector has been documented yet. However, the importation of Chandipura virus through an infected individual with or without clinical symptoms cannot be excluded. This could be the main risk of introduction in European areas where *P. papatasi* is an abundant species. To assess the transmission risk, it is necessary to carry out studies on the duration of the viraemia in infected humans and the vector competence of autochthonous Phlebotomine species in European countries where *P. papatasi* is scarce or not recorded. The recent introduction in Cyprus of *Leishmania donovani*, an Asiatic and African parasite transmitted by local Phlebotomine sandflies highlights the risk of introduction diseases potentially transmitted by European Phlebotomine sandflies [67-69].

The virus Isfahan has been isolated only in Iran in *P. papatasi*, rodents and patients [70]. The Jug Bogdanovac virus has been isolated in *P. (L.) perfiliewi* in Serbia [71].

Genus Orbivirus

Orbiviruses transmitted by sandfly bites are restricted to the 12 species from the Americas belonging to the Changuinola virus group. Human infection caused by this group is not well documented and until now has presented with mild influenza-like symptoms and does not show major clinical importance [72].

Laboratory diagnosis

Direct viral diagnosis, such as isolation, RT-PCR, in blood or cerebrospinal fluid is only possible in early stages of infection i.e. the first two days after symptom onset and before the IgM sero-conversion. In most cases the diagnosis is based on serological investigation of acute and early convalescent sera. In-house enzyme-linked immunosorbent assay (ELISA) methods (MAC-ELISA and IgG sandwich) are developed in reference laboratories. To date, only one commercial kit is registered in Italy for Toscana virus diagnosis. Serological cross reactions exist within the sandfly fever Naples virus and sandfly fever Sicilian virus antigenic complex. Seroneutralisation assays using early convalescent sera remain the reference method to specifically identify the viruses or to assess the antibody response specificity. Reference tools, reagents and quality control are not widely available. However, the collaborative working group of the European Network for the diagnosis of imported viral diseases (ENIVD www.enivd.de/index.htm) is able to provide some of these reagents.

Treatment and prevention

The treatment of phlebovirus infections is symptomatic. Treatment with hepatotoxic medication as well as aspirin and other NSAIDs such as ibuprofen and ketoprofen are not recommended.

No human vaccine against *Phlebotomus*-borne virus is available. The prevention of phlebovirus infection relies on the control of vector proliferation in limited areas where people are highly exposed. Individual protective measures such as insect repellents and insecticide impregnated mosquito bednets are recommended in these areas.

In most cases, phlebovirus infections are self-resolving pathologies. Only two complicated forms of Toscana virus infections have been reported in the literature. If a vaccine were available, the implementation of mass vaccination programmes would not seem to be relevant for the prevention for sandfly fever Naples and sandfly fever Sicilian viruses.

Risk for the future

Data on vertebrate reservoirs is sparse for sandfly fever viruses. The factor currently known to limit the

spread of diseases is the distribution areas of potential vectors. The distribution areas of the disease may not be restricted to the areas where they have been recorded but could be as wide as those of their vectors, that is to say *Larroussius* and *P. papatasi* mainly but not exclusively (figure 3). Consequently, field work in form of viral isolation from sandflies and possible reservoirs as well as laboratory work to establish vectorial competence of colonised sandflies need to be encouraged in a near future for three main reasons: (i) phleboviruses already endemic in the southern part of Europe have a potential to spread to other areas where their vectors are circulating, (ii) new phleboviruses of unknown pathogenicity such as the Massilia virus, that circulate among Phlebotomine sandflies may emerge in humans, (iii) the highly pathogenic Chandipura virus is paradigmatic of arthropod-borne viruses transmitted by Phlebotomine sandflies that may be introduced to Europe. At the present time, Rift Valley Fever virus has not been isolated from Phlebotomine sandflies under natural conditions. However, sandfly infections have been demonstrated under laboratory conditions for *P. (P.) papatasi*, *P. (P.) duboscqi*, *P. (Paraphlebotomus) sergenti*, *Sergentomyia schwetzi* and *Lutzomyia longipalpis* [73-75]. A vector competence has been demonstrated after oral infection for *P. papatasi* and *P. duboscqi* whereas *P. sergenti*, *S. schwetzi* and *L. longipalpis* do not seem to be able to transmit Rift Valley Fever virus after oral infection [73-75]. The lack of isolates of Rift Valley Fever virus from field-collected Phlebotomine sandflies may could be a consequence of the low rates of capture of sandflies in arthropod field collections for virus isolation assays. Their geographical range coinciding with that of Rift Valley Fever virus in sub-Saharan Africa, and nearly all known phleboviruses seem primarily associated with sandflies. Thus, additional studies are needed to evaluate the role of sandflies as maintenance and epizootic vectors for Rift Valley Fever virus [75]. An epidemiological surveillance is also required in the EU.

Phleboviruses as a potential means of biological warfare

Efficient arboviruses transmission mainly depends on vectors. Except for RVFV, no other way of *Phlebovirus* transmission has been reported. Breeding of Phlebotomine species and artificial infection difficulties are limiting factors for the use of phleboviruses as efficient biological weapons. Moreover, most phleboviruses are associated with asymptomatic or mild self-resolving infections in humans. Direct inter-human transmission has never been demonstrated. These criteria make phleboviruses bad candidates for the development of biological weapons. Phleboviruses are characterised by their tripartite RNA genome. Genetic exchanges between phleboviruses are possible with unpredictable effects. Compared to RVFV, only less pathogenic phleboviruses have been identified so far. The possible genesis of a new, highly virulent *Phlebovirus* by this genetic-exchange mechanism seems unlikely.

Acknowledgements

This review is based on an extensive literature study conducted as part of the European Centre for Disease Prevention and Control funded V-borne project "Assessment of the magnitude and impact of vector-borne diseases in Europe", tender n° OJ/2007/04/13-PROC/2007/003.

References

1. Tesh RB. The genus Phlebovirus and its vectors. *Annu Rev Entomol.* 1988;33:169-81.
2. Bhatt PN, Rodrigues FM. Chandipura virus: a new arbovirus isolated in India from patient with febrile illness. *Indian J Med Res.* 1967;55:1295-305.
3. Dhanda V, Rodrigues FM, Ghosh SN. Isolation of Chandipura virus from sandflies in Aurangabad. *Indian J Med Res.* 1970;58(2):179-80.
4. Rodhain F, Madulo-Leblond G, Hannoun C, Tesh RB. Le virus Corfou. Un nouveau Phlebovirus virus isolé de Phlébotomes en Grèce. *Ann. Inst. Pasteur/Virol.* 126E 161-166, 1985. [French].
5. Charrel RN, Moureau G, Temmam S, Izri A, Marty P, Parola P, et al. Massilia virus, a novel Phlebovirus (Bunyaviridae) isolated from sandflies in the Mediterranean. *Vector Borne Zoonotic Dis.* 2009;9(5):519-30.
6. Izri A, Temmam S, Moureau G, Hamrioui B, de Lamballerie X, Charrel RN. Sandfly fever Sicilian virus, Algeria. *Emerg Infect Dis.* 2008;14(5):795-7.
7. Papa A, Konstantinou G, Pavlidou V, Antoniadis A. Sandfly fever virus outbreak in Cyprus. *Clin Microbiol Infect* 2006;12(2):192-4.
8. Konstantinou GN, Papa A, Antoniadis A. Sandfly-fever outbreak in Cyprus: are phleboviruses still a health problem? *Travel Med Infect Dis.* 2007;5(4):239-42.
9. Darwish MA, Feinsod FM, Scott RM, Ksiazek TG, Botros BA, Farrag IH, et al. Arboviral causes of non-specific fever and myalgia in a fever hospital patient population in Cairo, Egypt. *Trans R Soc Trop Med Hyg.* 1987;81(6):1001-3.
10. Saidi S, Tesh R, Javadian E, Sahabi Z, Nadim A. Studies on the epidemiology of sandfly fever in Iran. II. The prevalence of human and animal infection with five Phlebotomus fever virus serotypes in Isfahan province. *Am J Trop Med Hyg.* 1977;26(2):288-93.
11. Mehrabi Tavana A. The seroepidemiological studies of sandfly fever in Iran during imposed war Iran. *J Public Health.* 2001;30(3-4):145-6.
12. Cohen D, Zaide Y, Karasenty E, Schwarz M, LeDuc JW, Slepon R, et al. Prevalence of antibodies to West Nile fever, sandfly fever Sicilian, and sandfly fever Naples viruses in healthy adults in Israel. *Public Health Rev.* 1999;27(1-3):217-30.
13. Dionisio D, Esperti F, Vivarelli A, Valassina M. Epidemiological, clinical and laboratory aspects of sandfly fever. *Curr Opin Infect Dis.* 2003;16(5):383-8.
14. Batieha A, Saliba EK, Graham R, Mohareb E, Hijazi Y, Wijeyaratne P. Seroprevalence of West Nile, Rift Valley, and sandfly arboviruses in Hashimiah, Jordan. *Emerg Infect Dis.* 2000;6(4):358-62.
15. Filipe AR. Serological survey for antibodies to arboviruses in the human population of Portugal. *Trans R Soc Trop Med Hyg.* 1974;68(4):311-5.
16. Tesh RB, Saidi S, Gajdamovic SJ, Rodhain F, Vesenjakk-Hirjan J. Serological studies on the epidemiology of sandfly fever in the Old World. *Bull World Health Organ* 1976;54(6):663-74.
17. Eitrem R, Vene S, Niklasson B. Incidence of sandfly fever among Swedish United Nations soldiers on Cyprus during 1985. *Am J Trop Med Hyg* 1990;43(2):207-11.
18. Charrel RN, Izri A, Temmam S, Delaunay P, Toga I, Dumon H, et al. Cocirculation of 2 genotypes of Toscana virus, southeastern France. *Emerg Infect Dis.* 2007;13(3):465-8.
19. Charrel RN, Gallian P, Navarro-Mari JM, Nicoletti L, Papa A, Sánchez-Seco MP, et al. Emergence of Toscana virus in Europe. *Emerg Infect Dis.* 2005;11(11):1657-63.
20. Peyrefitte CN, Devetakov I, Pastorino B, Villeneuve L, Bessaud M, Stolidi P, et al. Toscana virus and acute meningitis, France. *Emerg Infect Dis.* 2005;11(5):778-80.
21. De Lamballerie X, Tolou H, Durand JP, Charrel RN. Prevalence of Toscana virus antibodies in volunteer blood donors and patients with central nervous system infections in southeastern France. *Vector Borne Zoonotic Dis.* 2007;7(2):275-7.

22. Hemmersbach-Miller M, Parola P, Charrel RN, Paul Durand J, Brouqui P. Sandfly fever due to Toscana virus: an emerging infection in southern France. *Eur J Int Med.* 2004;15(5):316-7.
23. Dionisio D, Valassina M, Cuifolini MG, Vivarelli A, Esperti F, Cusi MG, et al. Encephalitis without meningitis due to sandfly fever virus serotype Toscana. *Clin Infect Dis.* 2001;32(8):1241-3.
24. Di Nicuolo G, Pagliano P, Battisti S, Starace M, Mininni V, Attanasio, et al. Toscana virus central system infection in southern Italy. *J Clin Microbiol.* 2005;43(12):6186-8.
25. Valassina M, Meacci F, Valensin PE, Cusi MG. Detection of neurotropic viruses circulating in Tuscany: the incisive role of Toscana virus. *J Med Virol.* 2003;60(1):86-90.
26. Mendoza-Montero J, Gámez-Rueda MI, Navarro-Marí JM, de la Rosa-Fraile M, Oyonarte-Gómez S. Infections due to sandfly fever virus serotype Toscana in Spain. *Clin Infect Dis.* 1998;27(3):434-6.
27. Echevarria JM, de Ory F, Guisasaola ME, Sanchez-Seco MP, Tenorio A, Lozano A, et al. Acute meningitis due to Toscana virus infection among patients from both the Spanish Mediterranean region and the region of Madrid. *J Clin Virol.* 2003;26(1):79-84.
28. Navarro JM, Fernández-Roldán C, Pérez-Ruiz M, Sanbonmatsu S, de la Rosa M, Sánchez-Seco MP. [Meningitis by Toscana virus in Spain: clinical description of 17 cases]. *Med Clin (Barc).* 2004;122(11):420-2. [Spanish]
29. Sanbonmatsu-Gámez S, Pérez-Ruiz M, Palop-Borrás B, Navarro-Marí JM. Unusual manifestation of Toscana virus infection, Spain. *Emerg Infect Dis.* 2009;15(2):347-8.
30. Sanbonmatsu-Gámez S, Pérez-Ruiz M, Collao X, Sánchez-Seco MP, Morillas-Márquez F, de la Rosa-Fraile M, et al. Toscana virus in Spain. *Emerg Infect Dis.* 2005;11(11):1701-7.
31. Santos L, Simões J, Costa R, Martins S, Lecour H. Toscana virus meningitis in Portugal, 2002-2005. *Euro Surveill.* 2007;12(6). pii=715. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=715>
32. Pugliese A, Beltramo T, Torre D. Seroprevalence study of Tick-borne encephalitis, Borrelia burgdorferi, Dengue and Toscana virus in Turin Province. *Cell Biochem Funct.* 2007;25(2):185-8.
33. Francisci D, Papili R, Camanni G, Morosi S, Ferracchiato N, Valente M, et al. Evidence of Toscana virus circulation in Umbria: first report. *Eur J Epidemiol.* 2003;18(5):457-9.
34. Ozbel Y, Ertabaklar H, Ciufolini MG, Marchi A, Fiorentino C, Erensoy S, et al. A neglected vector-borne disease: papatasi fever and its vectors in Turkey. ISOPS V 5th International Symposium on Phlebotomine Sandflies Tunis, 17-21 April, 2005. *Arch Inst Pasteur Tunis.* 2005;82: 55-56.
35. Tandale BV, Tikute SS, Arankalle VA, Sathe PS, Joshi MV, Ranadive SN, et al. Chandipura virus: a major cause of acute encephalitis in children in North Telangana, Andhra Pradesh, India. *J Med Virol.* 2008;80(1):118-24.
36. Rao BL, Basu A, Wairagkar NS, Gore MM, Arankalle VA, Thakare JP, et al. A large outbreak of acute encephalitis with high fatality rate in children in Andhra Pradesh, India, in 2003, associated with Chandipura virus. *Lancet.* 2004;364(9437):869-74.
37. Kemp GE. Viruses other than arenaviruses from West African wild mammals. *Bull World Health Organ.* 1975;52(4-6):615-20.
38. Nichol ST, Beaty BJ, Elliott RM, Goldbach R, Plyusnin A, Schmaljohn CS, et al. Genus Phlebovirus. Fauguet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. In *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 709-711. San Diego, CA: Elsevier Academic Press, 2005.
39. Tesh R, Saidi S, Javadian E, Nadim A. Studies on the epidemiology of sandfly fever in Iran. I. Virus isolates obtained from Phlebotomus. *Am J Trop Med Hyg.* 1977;26(2):282-7.
40. Jennings M, Boorman J. Laboratory infection of the sandfly Phlebotomus papatasi Scopoli (Diptera, Psychodidae) with three Phleboviruses. *Trans R Soc Trop Med Hyg.* 1983;77(1):62-4.
41. Watts DM, MacDonald C, Bailey CL, Meegan JM, Peters CJ, McKee KT Jr. Experimental infection of Phlebotomus papatasi with sand fly fever Sicilian virus. *Am J Trop Med Hyg.* 1988;39(6):611-6.
42. Depaquit J, Léger N, Ferté H, Rioux JA, Gantier JC, Michaelides A, Economides P. [Phlebotomines of the Island of Cyprus. III. Species inventory]. *Parasite.* 2001;8(1):11-20. [French].
43. Chastel C, Bach-Hamba D, Launay H, Le Lay G, Hellal H, Beaucournu JC. Arbovirus infections in Tunisia: new serological survey of small wild mammals]. *Bull Soc Path Exot Filiales.* 1983;76(1):21-33. [French].
44. Chastel C, Launay H, Bailly-Choumara H, Le Lay G, Beaucournu JC. [Arbovirus infections in Morocco: serosurvey in small wild mammals in the northern part of the country]. *Bull. Soc Path Exot Filiales.* 1982;75(5):466-75. [French].
45. Chastel C, Launay H, Rogues G, Beaucournu JC. [Arbovirus infections in Spain: serological survey on small mammals]. *Bull Soc Path Exot Filiales.* 1980;73(4):384-90. [French].
46. Le Lay Roguès G, Valle M, Chastel C, Beaucournu JC. [Small wild mammals and arboviruses in Italy]. *Bull Soc Path Exot Filiales.* 1983;76(4):333-45. [French].
47. Verani P, Lopes MC, Nicoletti L, Balducci M. Studies on Phlebotomus-transmitted viruses in Italy: I. Isolation and characterization of a Sandfly fever Naples-like virus. Arboviruses in the Mediterranean Countries, Zbl. Bakt. Suppl. 9, Gustav Fischer Verlag. Stuttgart-New York, 195-201, 1980.
48. Gligic A, Miscevic Z, Tesh RB, Travassos da Rosa A, Zivkovic V. First isolation of Naples sandfly fever in Yugoslavia. *Acta Biol Jug Mikrobiol.* 1982;19:167-75.
49. Schmidt JR, Schmidt ML, Said MI. Phlebotomus fever in Egypt. Isolation of Phlebotomus fever viruses from Phlebotomus papatasi. *Am J Trop Med Hyg.* 1971;20(3):483-90.
50. Vesenjaj-Hirjan J, Punda-Polić V, Dobe M. Geographical distribution of arboviruses in Yugoslavia. *J Hyg Epidemiol Microbiol Immunol.* 1991;35(2):129-40.
51. Becker M, Zielen S, Schwarz TF, Linde R, Hofmann D. [Pappataci fever]. *Klin Padiatr* 1997;209(6):377-9. [German].
52. Tesh RB, Lubroth J, Guzman H. Simulation of arbovirus overwintering: survival of Toscana virus (Bunyaviridae: Phlebovirus) in its natural sand fly vector Phlebotomus perniciosus. *Am J Trop Med Hyg.* 1992;47(5):574-81.
53. Tesh RB, Modi GB. Maintenance of Toscana virus in Phlebotomus perniciosus by vertical transmission. *Am J Trop Med Hyg* 1987;36(1):189-93.
54. Ciufolini MG, Maroli M, Guandalini E, Marchi A, Verani P. Experimental studies on the maintenance of Toscana and Arbia viruses (Bunyaviridae:Phlebovirus). *Am J Trop Med Hyg.* 1989;40(6):669-75.
55. Ciufolini MG, Maroli M, Verani P. Growth of two phleboviruses after experimental infection of their suspected sand fly vector, Phlebotomus perniciosus (Diptera:Psychodidae). *Am J Trop Med Hyg.* 1985;34(1):174-9.
56. Verani P, Ciufolini MG, Cacioli S, Renzi A, Nicoletti L, Sabatinelli G, et al. Ecology of viruses isolated from sand flies in Italy and characterization of a new Phlebovirus (Arbia virus). *Am J Trop Med Hyg.* 1988;38(2):433-9.
57. Charrel RN, Izri A, Temmam S, de Lamballerie X, Parola P. Toscana virus RNA in Sergentomyia minuta flies. *Emerg Infect Dis.* 2006;12(8):1299-300.
58. Valentini M, Valassina M, Savellini GG, Cusi MG. Nucleotide variability of Toscana virus M segment in strains isolated from clinical cases. *Virus Res.* 2008;135(1):187-90.
59. Fouque F, Gaborit P, Issaly J, Carinci R, Gantier JC, Ravel C, et al. Phlebotomine sand flies (Diptera: Psychodidae) associated with changing patterns in the transmission of the human cutaneous leishmaniasis in French Guiana. *Mem Inst Oswaldo Cruz.* 2007;102(1):35-40.
60. Tesh RB, Modi GB. Growth and transovarial transmission of Chandipura virus (Rhabdoviridae: Vesiculovirus) in Phlebotomus papatasi. *Am J Trop Med Hyg.* 1983;32(3):621-3.
61. Mavale MS, Fulmali PV, Geevarghese G, Arankalle VA, Ghodke YS, Kanojia PC, et al. Venereal transmission of Chandipura virus by Phlebotomus papatasi (Scopoli). *Am J Trop Med Hyg.* 2006;75(6):1151-2.
62. Mavale MS, Fulmali PV, Ghodke YS, Mishra AC, Kanojia P, Geevarghese G. Experimental transmission of Chandipura virus by Phlebotomus argentipes (Diptera:Psychodidae). *Am J Trop Med Hyg.* 2007;76(2):307-9.
63. Dhanda V, Rodrigues FM, Ghosh SN. Isolation of Chandipura virus from sandflies in Aurangabad. *Indian J Med Res.* 1970;58(2):179-80.
64. Geevarghese G, Arankalle VA, Jadi R, Kanojia PC, Joshi MV, Mishra AC. Detection of chandipura virus from sand flies in the genus Sergentomyia (Diptera: Phlebotomidae) at Karimnagar District, Andhra Pradesh, Indian J Med Entomol. 2005;42(3):495-6.
65. Ba Y, Trouillet J, Thonnon J, Fontenille D. [Phlebotomus of Senegal: survey of the fauna in the region of Kedougou. Isolation of arbovirus]. *Bull Soc Pathol Exot.* 1999;92(2):131-5. [French].
66. Fontenille D, Traore-Lamizana M, Trouillet J, Leclerc A, Mondo M, Ba Y, et al. First isolations of arboviruses from Phlebotomine sand flies in West Africa. *Am J Trop Med Hyg.* 1974;50(5):570-4.

67. Antoniou M, Haralambous C, Mazeris A, Pratlong F, Dedet JP, Soteriadou K. *Leishmania donovani* leishmaniasis in Cyprus. *Lancet Infect Dis.* 2008;8(1):6-7.
68. Antoniou M, Haralambous C, Mazeris A, Pratlong F, Dedet JP, Soteriadou K. *Leishmania donovani* leishmaniasis in Cyprus. *Lancet Infect Dis.* 2009;9(2):76-7.
69. Léger N, Depaquit J. *Leishmania donovani* leishmaniasis in Cyprus. *Lancet Infect Dis.* 2008;8(7):402.
70. Tesh R, Saidi S, Javadian E, Loh P, Nadim A. Isfahan virus, a new Vesiculovirus infecting humans, gerbils, and sandflies in Iran. *Am J Trop Med Hyg.* 1977;26(2):299-306.
71. Gligic A, Tesh RB, Miscevic Z, Travassos da Rosa A, Zivkovic V. Jug Bogdanovac virus – A new of the vesicular virus serogroup (Rhabdoviridae: Vesiculovirus) isolated from Phlebotomine sandflies in Yugoslavia. *Acta Biol Jug Mikrobiol.* 1983;20:97-105.
72. Polly R. Orbiviruses. In: *Fields Virology*, 5th Edn. 2007 (D.M. Knipe and P. Howley, eds), pp 1975-1997. Lippincott, Williams and Wilkins, Philadelphia.
73. Dohm DJ, Rowton E, Lawyer PG, O'Guinn M, Turell MJ. Laboratory Transmission of Rift Valley Fever Virus by *Phlebotomus duboscqi*, *Phlebotomus papatasi*, *Phlebotomus sergenti*, and *Sergentomyia schwetzi* (Diptera: Psychodidae). *J Med Entomol.* 2000;37(3):435-8.
74. Hoch AL, Turell MJ, Bailey CL. Replication of Rift Valley fever virus in the sand fly *Lutzomyia longipalpis*. *Am J Trop Med Hyg.* 1984;33(2):295-9.
75. Turell MJ, Perkins PV. Transmission of Rift Valley fever virus by the sand fly *Phlebotomus duboscqi* (Diptera: Psychodidae). *Am J Trop Med Hyg.* 1990;42(2):185-8.

Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness

H C Maltezou (helen-maltezou@ath.forthnet.gr)¹, L Andonova², R Andraghetti³, M Bouloy⁴, O Ergonul⁵, F Jongejan⁶, N Kalvatchev⁷, S Nichol⁸, M Niedrig⁹, A Platonov¹⁰, G Thomson¹¹, K Leitmeyer¹², H Zeller¹²

1. Hellenic Center for Diseases Control and Prevention, Athens, Greece

2. Medical University, Sofia, Bulgaria

3. World Health Organization, Copenhagen, Denmark

4. Institut Pasteur, Paris, France

5. Marmara University, Istanbul, Turkey

6. Utrecht Centre for Tick-borne Diseases, Utrecht University, Utrecht, the Netherlands

7. National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria

8. Centers of Disease Control and Prevention, Atlanta, United States

9. Robert Koch Institute, Berlin, Germany

10. Central Research Institute of Epidemiology, Moscow, Russian Federation

11. Health Protection Agency, London, United Kingdom

12. European Centre for Disease Control and Prevention, Stockholm, Sweden

Citation style for this article:

Citation style for this article: Maltezou HC, Andonova L, Andraghetti R, Bouloy M, Ergonul O, Jongejan F, Kalvatchev N, Nichol S, Niedrig M, Platonov A, Thomson G, Leitmeyer K, Zeller H. Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness. *Euro Surveill*. 2010;15(10):pii=19504. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19504>

This article has been published on 11 March 2010

During the last decade Crimean-Congo hemorrhagic fever (CCHF) emerged and/or re-emerged in several Balkan countries, Turkey, southwestern regions of the Russian Federation, and the Ukraine, with considerable high fatality rates. Reasons for re-emergence of CCHF include climate and anthropogenic factors such as changes in land use, agricultural practices or hunting activities, movement of livestock that may influence host-tick-virus dynamics. In order to be able to design prevention and control measures targeted at the disease, mapping of endemic areas and risk assessment for CCHF in Europe should be completed. Furthermore, areas at risk for further CCHF expansion should be identified and human, vector and animal surveillance be strengthened.

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is an acute, highly-contagious viral zoonosis transmitted to humans mainly by ticks of the genus *Hyalomma*, but also through direct contact with blood or tissues of viraemic hosts. In humans CCHF typically presents with high fever of sudden onset, malaise, severe headache and gastrointestinal symptoms. Prominent hemorrhages may occur in late stages of the disease with published fatality rates ranging from 10% to 50% [1,2]. The disease is endemic in parts of Africa, Asia, the Middle East and eastern Europe. Main animal hosts include a number of domestic animals such as cattle, sheep, goats, and hares. CCHF has the potential to cause community and nosocomial outbreaks. Due to the high case fatality rates and difficulties in treatment, prevention, and control, CCHF is a disease which should be notified immediately to public health authorities in the European Union (EU). CCHF virus is also in

the list of agents for which the Revised International Health Regulations of 2005 call for implementation of the decision algorithm for risk assessment and possible notification to the World Health Organization (WHO) [3].

In Europe, CCHF is currently only endemic in Bulgaria, however during the last decade an increased number of CCHF cases and outbreaks have been recorded in other countries in the region such as Albania, Kosovo, Turkey, and the Ukraine as well as south-western regions of the Russian Federation [4-9]. In June 2008, the first case was registered in Greece [10]. In response to this situation, the European Centre for Disease Prevention and Control (ECDC) invited a group of CCHF experts to review the situation of CCHF in Europe and to consult on interventions necessary to strengthen preparedness and response at the European level [11]. This article provides an update on the current situation of CCHF in Europe, and emphasises existing prevention and control capacities within the EU. Aspects relevant to strengthen preparedness for CCHF are also discussed.

CCHF situation in Europe

CCHF is endemic in Bulgaria since the 1950's, when a large outbreak occurred from 1954 to 1955 with 487 notified cases mainly in the Shumen area in north-east Bulgaria. In total, 1,568 CCHF cases were notified in Bulgaria from 1953 to 2008, with an overall case fatality rate of 17% [4]. Endemic areas are confined to the vicinity of Shumen, Razgrad, Veliko Tarnovo, Plovdiv, Pazardjik, Haskovo, Kardjali, and Bourgas, however in April 2008 a cluster of six probable cases occurred in Gotse Delchev in the south-western province

Blagoevgrad near the border with Greece, an area considered of low CCHF endemicity until recently [5]. During the last decade, CCHF outbreaks have also been noted in Albania in 2001 and 2003, and in Kosovo in 2001 [6,7].

In Turkey, the first symptomatic human CCHF cases were noted in 2002, however, serologic evidence of enzootic CCHF virus circulation as well as limited evidence of CCHF infections among humans (2.4% among 1,100 tested humans) has been found since the 1970's [4]. Starting in 2003, Turkey has experienced an expanding outbreak with increasing numbers of notified cases and associated fatalities (2002: 17/0; 2003: 133/6; 2004: 249/13; 2005: 266/13; 2006: 438/27; 2007: 713/33; 2008: 1315/63; 2009: 1300/62) [4,12]. Overall, there are more than 4,400 recorded laboratory confirmed CCHF cases in this country, mainly among residents in rural areas in north-central and north-east Anatolia [4,8,12]. Within the CCHF endemic areas, there are hyperendemic areas where one out of every five residents and one out of every two residents with a history of tick bite has antibodies against CCHF virus [13]. A predictive map model using satellite-based climate data and high-resolution vegetation images from Turkey from 2003 to 2006 revealed that areas with higher CCHF reporting were significantly associated with zones of high climate suitability for *Hyalomma* ticks and high rate of fragmentation of agricultural land [13].

In Greece, a serosurvey conducted between 1981 and 1988 among 3,388 rural residents from across the country showed 1% seroprevalence rate against CCHF virus [4]. More than 400 cases with a CCHF compatible clinical syndrome have tested negative for CCHF virus in this country since 1982, therefore, the seroprevalence rate of 1% was attributed to the non-pathogenic AP-92 strain and not to the pathogenic Balkan CCHF virus strain. A number of the cases tested for CCHF were finally diagnosed as hemorrhagic fever with renal syndrome (HFRS), leptospirosis and rickettsial infections. Other diagnoses were meningococcal meningitis and unspecified bacterial sepsis. The first CCHF case was recorded in June 2008 in a woman with a tick bite working in agriculture near the city of Komotini in north-eastern Greece [10]. This town is situated within a few kilometres distance from there where the Bulgarian cluster occurred in 2008 [5]. A seroepidemiological study for CCHF virus among local population and animals are underway in northern Greece.

After nearly 27 years without any human cases, CCHF re-emerged in the south-western regions of the Russian Federation in 1999. Outbreaks have been reported in Astrakhan, Rostov and Volgograd Provinces, Krasnodar and Stavropol Territories, Kalmykia, Dagestan and Ingushetia Republics. Between 2000 and 2009 more than 1,300 clinical cases were diagnosed in the Russian Federation with an overall fatality rate of 3.2% for the period from 2002-2007 [4]. Most cases occurred

among residents of rural areas in the Southern Federal District. The largest number of cases was registered in Stavropol Territory, Kalmykia Republic and Rostov Province, where the mean annual CCHF incidence rate was 1.7, 10.1, and 0.7 cases per 100,000 population, respectively. During 2008 alone, the incidence in Stavropol Territory increased by 1.3 times, and was the highest recorded in this region during the last decade [4,9,14]. In 2009, CCHF cases were also reported from Georgia, Kazakhstan, Tajikistan, Iran, and Pakistan [15].

CCHF emergence and/or re-emergence in south-eastern Europe and neighboring countries is attributed to climate and ecologic changes and anthropogenic factors such as changes in land use, agricultural practices, hunting activities, and movement of livestock, that may have an impact on ticks and hosts and accordingly on CCHF epidemiology [1,2]. The geographic distribution of CCHF coincides with that of *Hyalomma* ticks. *H. marginatum*, the main CCHF virus vector in Europe, is found in Albania, Bulgaria, Cyprus, France, Greece, Italy, Kosovo, Moldavia, Portugal, Romania, Russia, Serbia, Spain, Turkey, and the Ukraine. In 2006 it was detected for the first time in the Netherlands and in southern Germany [16,17]. Given the wide distribution of its vector, the numerous animals that can serve as hosts, and the favorable climate and ecologic conditions in several European countries bordering the Mediterranean Sea, it is possible that the occurrence of CCHF will expand in the future. A model that studied various climate scenarios on the habitat areas of different ticks, showed that a rise in temperature and a decrease in rainfall in the Mediterranean region will result in a sharp increase in the suitable habitat areas for *H. marginatum* and its expansion towards the north, with the highest impact noted at the margins of its current geographic range [18].

Current prevention and control in Europe

Several elements relating to laboratory diagnosis, surveillance and therapy of CCHF should be addressed in order to increase preparedness capacity in Europe and to design appropriate prevention and control measures.

Laboratory diagnosis

In 2008 there were 20 laboratories with diagnostic capacities for CCHF virus in Europe: 14 in EU Member States, eight in the endemic regions of the Russian Federation, and one in Turkey. Most of them used immunofluorescence assays (IFA), ELISA, and/or molecular methods to diagnose CCHF whereas eight among them were also able to isolate CCHF virus [11], a BSL-4 containment agent. Limitations for diagnosing CCHF concern both the limited diagnostic capacities in several endemic areas as well as difficulties in the international transfer of samples for logistic and economic reasons. However, rapid and easy tests are needed to guide initial therapeutic decisions for the patient.

Surveillance

Currently, there are no standardised case definitions for CCHF notification and contact tracing within European countries [19]. Recent cases of nosocomial acquisition of CCHF in health care workers were well documented [6,8,20]. These cases underline the need for educating health-care workers about the modes of getting infected with CCHF virus and for strict implementation of infection control measures within health-care facilities, and the importance of providing adequate resources to do so [1,2].

Therapy

The World Health Organization (WHO) recommends ribavirin for the treatment of CCHF cases [21,22]. Ribavirin appears to be more effective when introduced early in the course of illness [23]. Evidence of its efficacy is based on in vitro data and on limited observations in humans [24-26]. Randomised controlled trials have not been conducted so far, and ethical issues concerning the use of a control group remain a major obstacle for this [27]. Severity of infection, duration of illness prior to initiation of therapy, and route of administration may impact the clinical outcome of CCHF cases. On individual country level, recommendations for treatment of CCHF cases with ribavirin existed in 2008 in Turkey, Russia, Bulgaria, and Greece. In Bulgaria, in addition, specific hyperimmune globulin collected from convalescent CCHF cases is used for prophylaxis and treatment and an inactivated suckling mouse brain vaccine is in use since the 1970's for high-risk groups living in CCHF endemic regions [28]. There is no vaccine against CCHF licensed in any other EU Member State.

Conclusions

CCHF is a disease with a high fatality rate and the potential to cause outbreaks. The vector for CCHF, the *Hyalomma* tick is present in southeastern and southern Europe. Climate factors may contribute to a further spread of the vector and to a consecutive extension of the geographic range of CCHF, which may further expand to European countries bordering the Mediterranean Sea, with the highest risk in neighbouring areas with already established endemicity. This highlights the need for strengthening human, vector, and veterinary surveillance, especially in areas where CCHF is expected to occur in the future. Together with the implementation of standardised case definitions for CCHF this will allow an estimate of the CCHF burden and of epidemiologic trends in various areas and countries. Guidance for contact tracing and the establishment of early detection and response systems will allow prompt interventions at patient, community, and hospital level. To enable early detection, laboratory capacities are crucial to rapidly confirm the suspected clinical diagnosis and besides being available, tests need to be reliable and affordable. Overall, laboratory capacities for CCHF should increase. Considering the high case fatality rate of CCHF, development of a vaccine and new drugs against CCHF are of major importance. Ribavirin efficacy should be assessed through well-designed clinical protocols and in endemic areas

general public and health-care workers should be aware about modes of CCHF transmission and prophylactic measures. Climate and environmental factors and human behavior that may influence CCHF epidemiology and spread should be further studied. Mapping of endemic areas and risk assessment for CCHF in Europe should be completed and areas at risk for CCHF expansion should be identified and finally, appropriate tick-control strategies including public education should be implemented. All these measures should be undertaken as part of a multidisciplinary collaboration at interregional and international level and link with initiatives such as *the International network for capacity building for the control of emerging viral vector-borne zoonotic diseases: ARBO-ZOONET* [29].

In accordance with an ECDC-initiated assessment on the importance of vector-borne diseases in 2008, CCHF has been identified as a priority disease for the EU [12]. In order to strengthen preparedness and response for CCHF and build capacity for its prevention and control, it is necessary to identify relevant gaps and work in an integrated fashion.

References

1. Vorou R, Pierrotsakos IN, Maltezos HC. Crimean-Congo hemorrhagic fever. *Curr Opin Infect Dis.* 2007;20(5):495-500.
2. Ergönül O. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis.* 2006;6(4):203-14.
3. World Health Organization (WHO). [Internet]. International Health Regulations (2005). 2nd ed. Geneva, WHO 2008. Available from: http://whqlibdoc.who.int/publications/2008/9789241580410_eng.pdf
4. World Health Organization Regional Office for Europe (WHO). Epidemiology for Crimean-Congo haemorrhagic fever virus: Turkey, Russian Federation, Bulgaria, Greece, Albania, Kosovo. Available from: www.euro.who.int/surveillance/outbreaks/20080806_1
5. Kunchev A, Kojouharova M. Probable cases of Crimean-Congo haemorrhagic fever in Bulgaria: a preliminary report. *Euro Surveill.* 2008;13(17). pii=18845. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18845>
6. Papa A, Bino S, Llagami A, Brahina B, Papadimitriou E, Pavlidou V, et al. Crimean-Congo hemorrhagic fever in Albania, 2001. *Eur J Clin Microbiol Infect Dis.* 2002;21(8):603-6.
7. Papa A, Bozovi B, Pavlidou V, Papadimitriou E, Pelemis M, Antoniadis A. Genetic detection and isolation of crimean-congo hemorrhagic fever virus, Kosovo, Yugoslavia. *Emerg Infect Dis.* 2002;8(8):852-4.
8. Yilmaz GR, Buzgan T, Irmak H, Safran A, Uzun R, Cevik MA, et al. The epidemiology of Crimean-Congo hemorrhagic fever in Turkey, 2002-2007. *Int J Infect Dis.* 2009;13(3):380-6.
9. Federal Service for Surveillance on Consumer Rights Protection and Wellbeing, the Russian Federation. [Internet] On improvement preventive measures against Crimean-Congo haemorrhagic fever in Southern Federal District. Letter of 11.03.2009. Available from: <http://www.rosпотребнадзор.ru/documents/letters/2410/> [Russian].
10. Papa A, Maltezos HC, Tsioudas S, Dalla VG, Papadimitriou T, Pierrotsakos IN, et al. A case of Crimean-Congo haemorrhagic fever in Greece, June 2008. *Euro Surveill.* 2008;13(33). pii=18952. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18952>
11. European Centre for Disease Prevention and Control (ECDC). Meeting report: Consultation on Crimean-Congo haemorrhagic fever prevention and control. Stockholm, September 2008. Available from: http://ecdc.europa.eu/en/publications/Publications/o809_MER_Crimean_Congo_Haemorrhagic_Fever_Prevention_and_Control.pdf
12. Turkish Ministry of Health. [Internet]. Tarihli Aciklama, September 25, 2009. Available from: <http://www.saglik.gov.tr/KKKA/BelgeGoster.aspx?F6E10f8892433CFFA91171E62FoFF1532A030B47EF2AC66C> [Turkish].

13. Estrada-Peña A, Zatansever Z, Gargili A, Aktas M, Uzun R, Ergonul O, et al. Modeling the spatial distribution of Crimean-congo hemorrhagic fever outbreaks in Turkey. *Vector Borne Zoonotic Dis.* 2007;7(4):667-78.
14. Maletskaia OV, Beyer AP, Agapitov DS, Kharchenko TV, Taran AV, Taran TV, et al. Epidemic situation on Kongo-Crimean hemorrhagic fever in South Federal District of Russia. *Zh Mikrobiol Epidemiol Immunobiol* 2009;(6):51-4 [In Russian].
15. World Organisation for Animal Health (OIE), World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO). [Internet]. Global Early Warning and Response System for Major Animal Diseases, including Zoonoses. November 26, 2009. Available from: http://www.glews.net/index.php?option=com_content&view=article&id=85:crimean-congo-hemorrhagic-fever-cCHF&catid=64:disease-priority-list
16. Nijhof AM, Bodaan C, Postigo M, Nieuwenhuijs H, Opsteegh M, Franssen L, et al. Ticks and associated pathogens collected from domestic animals in the Netherlands. *Vector Borne Zoonotic Dis.* 2007;7(4):585-95.
17. Kampen H, Poltz W, Hartelt K, Wolfel R, Faulde M. Detection of a questing *Hyalomma marginatum marginatum* adult female (Acari, Ixodidae) in southern Germany. *Exp Appl Acarol.* 2007;43(3):227-31.
18. Estrada-Peña A, Venzal JM. Climate niches of tick species in the Mediterranean region: modeling of occurrence data, distributional constraints, and impact of climate change. *J Med Entomol.* 2007;44(6):1130-8.
19. Maltezos HC, Papa A, Tsiodras S, Dalla V, Maltezos E, Antoniadis A. Crimean-Congo hemorrhagic fever in Greece: a public health perspective. *Int J Infect Dis.* 2009;13(6):713-6.
20. Harxhi A, Pilaca A, Delia Z, Pano K, Rezza G. Crimean-Congo hemorrhagic fever: a case of nosocomial transmission. *Infection.* 2005;33(4):295-6.
21. World Health Organization (WHO). WHO Model List of Essential Drugs, 2007. Available from: http://www.who.int/entity/medicines/publications/o8_ENGLISH_indexFINAL_EML15.pdf
22. World Health Organization (WHO). WHO Model Formulary 2008. Available from: http://www.who.int/entity/selection_medicines/list/WMF2008.pdf
23. Tasdelen Fisgin N, Ergonul O, Doganci L, Tulek N. The role of ribavirin in the therapy of Crimean-Congo hemorrhagic fever: early use is promising. *Eur J Clin Microbiol Infect Dis.* 2009;28(8):929-33.
24. Paragas J, Whitehouse CA, Endy TP, Bray M. A simple assay for determining antiviral activity against Crimean-Congo hemorrhagic fever virus. *Antiviral Res.* 2004;62(1):21-5.
25. Watts DM, Ussery MA, Nash D, Peters CJ. Inhibition of Crimean-Congo hemorrhagic fever viral infectivity yields in vitro by ribavirin. *Am J Trop Med Hyg.* 1989;41(5):581-5.
26. Mardani M, Jahromi MK, Naeni KH, Zeinali M. The efficacy of oral ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Iran. *Clin Infect Dis.* 2003;36(12):1613-8.
27. Ergonul O. Treatment of Crimean-Congo hemorrhagic fever. *Antivir Res.* 2008;78(1):125-31.
28. Papa A, Christova I, Papadimitriou E, Antoniadis A. Crimean-Congo hemorrhagic fever in Bulgaria. *Emerg Infect Dis.* 2004;10(8):1465-7.
29. Ahmed J, Bouloy M, Ergonul O, Fooks A, Paweska J, Chevalier V, et al. International network for capacity building for the control of emerging viral vector-borne zoonotic diseases: ARBO-ZOONET. *Euro Surveill.* 2009;14(12). pii: 19160. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19160>