Almost a third of the recorded events related to emerging infectious diseases in Europe were due to vector-borne diseases during the decade 1990-2000.

In this special edition Eurosurveillance presents a series of review articles with a particular focus on arthropod-borne diseases transmitted by mosquitoes and phlebotomine sandflies and reports on recent outbreaks caused by vector-borne diseases in Europe.
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A head on view of a Culex pipiens mosquito, this species is an important vector in West Nile Virus.
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Retrospective screening of solid organ donors in Italy, 2009, reveals unpredicted circulation of West Nile virus
by M R Capobianchi, V Sambri, C Castilletti, A M Pierro, G Rossini, P Gaibani, F Cavrini, M Selleri, S Meschi, D Lapa, A Di Caro, P Grossi, C De Cillia, S Venettoni, M P Landini, G Ippolito, A Nanni Costa, on behalf of the Italian Transplant Network

Case report: West-Nile virus infection in two Dutch travellers returning from Israel
by N Aboutaleb, M FC Beersma, H F Wunderink, A CTM Vossen, L G Visser

Introduction and control of three invasive mosquito species in the Netherlands, July-October 2010
by E J Scholte, W Den Hartog, M Dik, B Schoelitsz, M Brooks, F Schaffner, R Foussadier, M Braks, J Beeuwkes
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 Maltezou 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


West Nile virus: the need to strengthen preparedness in Europe

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The ongoing outbreak of West Nile virus (WNV) infections in humans in Greece described in this issue of Eurosurveillance is a timely reminder that WNV is a re-emerging pathogen in Europe [1]. So far, WNV has been documented in animals and humans in several countries across Europe, mainly in central Europe and in the Mediterranean region. Over the last 15 years, outbreaks in horses and/or humans were reported from Romania, Hungary and Portugal, Spain, France, Italy and Greece [2].

In 2010, a single probable human case was reported in July in Portugal. Outside the European Union (EU), WNV circulation has been documented in horses in Morocco and human cases have occurred in Russia (Volgograd Oblast) and in Israel. All these regions are located along the main routes of migratory birds. The current outbreak in humans in northern Greece, is the first recognised WN fever outbreak in humans in this country. However, studies suggest that WNV has probably been circulating in humans in the region of central Macedonia in northern Greece for many years [3,4].

West Nile fever is a viral disease transmitted by mosquitoes and is distributed worldwide. The primary cycle of WNV involves ornithophilic mosquitoes and birds; some mosquito species mostly from the Culex genus can bite infectious birds and subsequently transmit the virus to humans and/or horses during another blood meal. Humans and horses are considered as dead-end hosts. The vast majority of human cases remain asymptomatic after infection and severe neuroinvasive illness is reported in less than 1% of the patients. The main risk factor for severe clinical presentation is to be an elderly person. In this age group, reported case fatality rates may reach 10% [5]. In addition the high number of non-symptomatic cases may increase the risk of WNV transmission through blood donation or organ transplants [6].

Each WNV outbreak is unique in that there is a complex interaction of different factors in space and time that contribute to the transmission of the virus to humans. These factors range from the introduction of infected migratory birds into native local bird populations, to climatic factors that increase the abundance of competent mosquito vectors and bridge vectors, to changes in human behaviour that favour exposure to infected mosquitoes. It is this complexity that makes each WNV outbreak particular and that make development and implementation of preparedness plans for the prevention of cases in humans so difficult.

The recently reported probable and confirmed cases of WNV infection in Portugal and Greece, respectively reconfirm that this virus is actively circulating in several countries in the EU and that transmission to humans can be expected on a regular basis during the mosquito season. Also, reports of sporadic cases from several regions in Hungary during previous years indicate that WNV activity is widely distributed throughout this country and not limited to a single focus [7]. A recent study in Italy linked to infected organ donors [8] draws the same conclusion, that the virus is being transmitted in areas previously thought to not be at risk or affected. Furthermore, the case report in this issue of a Dutch traveller returning from Israel with WN infection highlights the need for awareness among physicians and laboratory staff to consider WNV infections as a differential diagnosis in cases where patients return from areas where they may have been exposed to the virus [9].

The events described above strengthen the need for integrated multidisciplinary surveillance systems and response plans. This includes raising the awareness of clinicians and veterinarians of the clinical presentation of WNV disease in humans and horses (particularly during the mosquito season from June to October), primarily in areas considered as at major risk surrounding irrigated areas and river deltas. Furthermore, strengthening the understanding of suitable habitats for birds that would increase the bird-mosquito-human interface would be of value. In terms of entomology, a thorough understanding of competent vector species, their breeding ecology, their abundance and geographic range is of significant importance in establishing limits around WNV affected areas and in the identification of potential new at-risk areas.
In addition, there is a need to have a better and more precise picture of WNV risk areas in Europe and neighbouring countries in order to implement appropriate control measures, especially guidelines for blood donation and organ transplants. Also, studies in Europe are required to better understand the cycle of transmission and the maintenance of WNV in the environment over the years to provide appropriate indicators for risk assessment.

References

West Nile virus circulation in Emilia-Romagna, Italy: the integrated surveillance system 2009

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Following a large West Nile virus (WNV) epidemic in north-eastern Italy in 2008, human and animal surveillance activities were implemented in Emilia Romagna. Human surveillance was performed by serology or genome detection on blood and cerebrospinal fluid for all suspected cases suffering from acute meningoencephalitis in the regional territory. Animal surveillance consisted of passive and active surveillance of horses and active surveillance of wild birds and mosquitoes. Between 15 June and 31 October 2009, nine of 78 possible cases of West Nile neuroinvasive disease were confirmed (three fatal). From May to October, 26 cases of neurological West Nile disease were confirmed among 46 horses. The overall incidence of seroconversion among horses in 2009 was 13%. In 2009, 44 of 1,218 wild birds yielded positive PCR results for WNV infection. The planned veterinary and entomological surveillance actions detected WNV activity from the end of July 2009, about 2-3 weeks before the onset of the first human neurological case. Passive surveillance of horses seems to be an early and suitable tool for the detection of WNV activity, but it will be less sensitive in the future, because an intensive programme of horse vaccination started in June 2009.

Regional integrated surveillance system
In Italy a national veterinary plan for the surveillance of West Nile virus (WNV) circulation was set up in 2001 under the coordination of the National Reference Centre for Exotic Diseases of animals (CEntro Studi Malattie Eositeche; CESME). During the late summer of 2008 a large epidemic of WNV infection occurred in north-eastern Italy over an area exceeding 7,000 km², in three regions, including Emilia-Romagna. Twenty-three horse cases and three human cases of the neuroinvasive form of West Nile disease (WND) were confirmed by laboratory tests [1,2]. After the first evidence of WNV circulation in horses was found, additional surveillance on horses, birds and mosquitoes was activated.

In Emilia-Romagna the WNV surveillance plan 2009 adopted locally the surveillance measures indicated by the national plan. In particular, among the surveillance activities, the choice was to monitor wild non-migratory birds, such as corvids (the crow family), considered the most sensitive indicators among birds, which can be captured easily. As regards equine surveillance, the regional plan emphasised the education of veterinary practitioners, focusing on the inclusion of WNV in differential diagnosis and the achievement of rapid reporting. A major feature of this plan was to establish an extremely sensitive system of passive surveillance. In addition to passive surveillance, active monitoring of horses was implemented in the area involved in the 2008 outbreak, including Ferrara and the neighbouring provinces [3].

Evidence of WNV circulation in 2008 was found in animals [1,4], humans [5], and mosquitoes. This highlighted the need to implement an integrated surveillance system which would describe the phenomenon comprehensively. Such a system facilitates the collection of data to evaluate spatial distribution and time trends of viral circulation and shares information.

For this reason the 2009 Regional Surveillance Plan implemented activities beyond those of the National Plan, revised the surveillance system of human cases, activated intensive entomological monitoring, and enlarged the surveillance area to involve all the provinces along the Po River.

Human surveillance
The aim of the human surveillance system was the early detection of infection in humans and the estimation of its diffusion through the systematic analysis of newly emerging clinical cases, in order to manage specific interventions.
The surveillance was performed throughout the regional territory, from 15 June to 31 October 2009, corresponding to the period of vector activity in Emilia-Romagna and adjusted locally according to weather conditions and vector activity reports. In 2009, the 2008 case definition [6] was extended to include cases of all ages and not only those over 15 years of age.

The human surveillance activity was performed by serology or genome detection on blood and cerebrospinal fluid for all suspected cases suffering from acute meningoencephalitis in the regional territory. Active surveillance of people who live or work in areas of documented viral circulation was also performed. In addition blood and cerebrospinal fluid samples from subjects living or staying for at least one night in the regional area were sent to the Regional Reference Centre for Microbiological Emergencies (Centro di Riferimento Regionale per le Emergenze Microbiologiche; CRREM). In selected cases, positive specimens were confirmed by the National Health Institute (Istituto Superiore di Sanità; ISS) and by the National Institute for Infectious Diseases (Istituto Nazionale Malattie Infettive; INMI).

From October 2008 to April 2009 a serosurvey performed on 9,177 healthy blood donors living in the province of Ferrara detected a total of 62 IgG positive subjects, corresponding to a seroprevalence of 0.68%.

Animal surveillance

The regional veterinary WNV surveillance system was activated from May to October, performing passive and active surveillance on horses and on non-migratory wild birds.

Horse passive surveillance

In Italy all suspected signs of WND in horses must be notified to the official veterinary services. Suspected cases were confirmed if resulted positive by reverse transcription – polymerase chain reaction (RT-PCR) performed on central nervous system [7] or to a WNV virus cerebrospinal fluid for all suspected cases suffering from acute meningoencephalitis in the regional territory.

Note: The figure does not include a magpie (found in early May) or a jay (found in early November).
neutralisation (VN) test (cut-off titre 1:10) in microtitre plates and to IgM enzyme linked immunosorbent assay (ELISA) [8,9].

**Horse active surveillance**

In the provinces of Ferrara, Bologna, Modena, Ravenna, and Reggio Emilia, every 1,600 km², 28 seronegative unvaccinated equine sentinels, sufficient to detect an incidence above 10% (CI 95%), were selected in the spring of 2009. They were serologically tested twice after the selection, at the beginning of August and the beginning of September. Samples collected were screened by a home-made competitive ELISA [10]. Positive samples were confirmed by VN and IgM ELISA at the CESME in Teramo. A seroconversion was confirmed if VN titre was at least 1:10 and there was evidence of IgM antibodies.

**Wild bird surveillance**

Monitoring was carried out in all the provinces along the Po River, in the plain area of Emilia-Romagna. Every 1,600 km², a monthly sample of about 40 wild birds caught or shot within specific wildlife population control programmes was collected. Samples of organs (brain, heart, and kidney) of each bird were pooled and examined by RT-PCR [7].

**Entomological surveillance**

The surveillance system was based on the weekly to monthly (frequency depends on local resources) collection of mosquitoes in fixed stations and in the sites where birds, humans, or horses signalled WNV activity. Mosquito collections for WNV screening were conducted in six provinces: Ferrara, Ravenna, Bologna, Modena, Reggio Emilia, and Parma, using 92 CO₂ baited traps positioned in fixed stations. Moreover, mosquito collections were performed promptly using CO₂ and gravid traps in sites where positive horses and human cases had been detected.

The surveillance system was activated in the period 15 April to 10 October. Collected mosquitoes were pooled (maximum 200) by species, date, and site of collection and examined by RT-PCR [7]. In addition, overwintered mosquito females were collected during the period 3 March to 8 April by manual aspirator in rural buildings in the area where WNV was active in 2008.

**Virological analysis**

**Human samples**

The detection of WNV genome in human plasma, cerebrospinal fluid, and serum samples obtained from patient suffering from clinical symptoms of meningocéphalitis was performed by an RT-PCR assay based on specific TaqMan probes [7].

**Animal samples**

In horses RNA was extracted starting from 200 µl of serum or whole blood with EDTA as anticoagulant. In birds RNA was extracted from 200 µl of phosphate buffer saline homogenate (about 20% tissue g / buffer ml) of pooled brain, heart and kidney of each analysed bird. In mosquitoes RNA was extracted from 200 µl of a maximum of 200 pooled mosquitoes manual grinded by using copper stained beads, in 500-800 µl of PBS.

cDNA was submitted to RT-PCR according to the method of Tang and colleagues [7]. Positive samples were confirmed by sequencing of partial nucleocapsin and pre-membrane protein M amplified according to [11]. Finally from each RT-PCR positive sample WNV was isolated on Vero and RK13 cell lines.

**Results**

Results of the integrated surveillance system are mapped in figure 1, with the sequence of events summarised in figure 2 (July-October), and discussed below.

**Human cases**

As of 31 October 2009, nine out of 78 possible cases of West Nile neuroinvasive disease (WNND) notified in Emilia-Romagna have been confirmed (8/9 males; median age 72 years, range: 62-78). Three patients

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**Table 1**

<table>
<thead>
<tr>
<th>Species</th>
<th>Birds tested</th>
<th>WNV RT-PCR positive</th>
<th>% WNV positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>European magpie (Pica pica)</td>
<td>607</td>
<td>27</td>
<td>4.4</td>
</tr>
<tr>
<td>Carrion crow (Corvus corone cornix)</td>
<td>350</td>
<td>5</td>
<td>1.4</td>
</tr>
<tr>
<td>European starling (Sturnus vulgaris)</td>
<td>98</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Eurasian jay (Garrulus glandarius)</td>
<td>96</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Common blackbird (Turdus merula)</td>
<td>30</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Strigiformes</td>
<td>11</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Charadriiformes</td>
<td>8</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>Other Passeriformes</td>
<td>7</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Other bird orders</td>
<td>5</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Piciformes</td>
<td>4</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Columbiformes</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>1,218</td>
<td>44</td>
<td>3.6</td>
</tr>
</tbody>
</table>

RT-PCR: reverse transcription-polymerase chain reaction; WNV: West Nile virus
died, two living in Ferrara Province and one in Modena. In addition, not reported in figures, the local health units of Parma and Modena notified two other confirmed cases, both 72 year-old women resident in Mantua province (Lombardy region), treated in hospital in Emilia-Romagna. Another case of infection was that of a 78 year-old female liver donor. Before her death, she had spent two weeks visiting relatives in the WNV affected area (Reggio Emilia).

Horse cases

Passive surveillance

From May to October, 26 cases of neurological WND were confirmed among 46 horses in which it was suspected. Four of the eight provinces involved in the regional surveillance system had WND cases in horses. The first symptoms in horses were detected in the second half of July in the provinces of Ferrara and Reggio Emilia, but the most cases were notified between mid-August and mid-September (figure 2).

Active surveillance

Seroconversions in sentinel horses were detected in three provinces (Ferrara, Modena, and Reggio Emilia). Early seroconversions were registered among the controls examined at the beginning of August. Serological controls around the stables with WND cases also confirmed recent WNV infections in the province of Parma. The overall incidence of seroconversion among horses in 2009 was 13% (95% CI: 10% to 16%), but in Ferrara it was 28% (95% CI: 19% to 39%).

Wild birds

Six of the eight provinces that took part in the regional surveillance system reported positive birds. In 2009, 44 wild birds out of 1,218 (tested by PCR) yielded positive results for WNV infection. With the exception of a magpie caught in May, positive wild birds were detected from the end of July (figure 2). Most of the infected wild birds were corvids (Pica pica, Corvus corone cornix, Garrulus glandarius), collected within population control programmes in August and September, but WNV was detected also in other species, mainly found dead in wildlife recovery centres (table 1).

| Table 1. Species distribution of wild birds tested for West Nile virus (n=1,218), Emilia-Romagna, Italy, May-October 2009 |
| Province | Species | Bombolilus | Ae. albopictus | Ae. caspius | Ae. detritus | Ae. dorsalis | Ae. geniculatus | Ae. vexans | An. maculipennis | An. plumbeus | Cx. molestus | Cx. pipiens | Total |
| Bologna | 169 | 13 | 392 | 31 | 227 | 107 | 11 | 58 | 38 | 2 | 2 | 2 | 86,120 |
| Ferrara | 1,773 | 54 | 14,514 | 1,142 | 1,601,571 | 121 | 2 | 12 | 2 | 1 | 1 | 1 | 91 | 714 |
| Modena | 1,273 | 14 | 9,953 | 144 | 16,015 | 121 | 2 | 12 | 2 | 1 | 1 | 1 | 1 | 1 | 10 |
| Parma | 13 | 1 | 4,090 | 41 | 6 | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Piacenza | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ravenna | 12 | 2 | 606 | 11 | 68 | 9 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Reggio Emilia | 248 | 6 | 6,926 | 50 | 68 | 9 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Total | 86,120 | 714 | 9,075 | 557 | 5,064,605 | 285 | 7 | 7,530 | 62 | 1 | 1,477 | 21 | 0 | 3,139 | 95 |

Mosquitoes

About 190,000 mosquitoes were collected, pooled and tested using PCR (1,789 pools of ≤200 individuals/pool). Culex pipiens were the most abundant species (81.4%) followed by Aedes caspius (15.4%) and Aedes vexans (2.4%). Other collected species are shown in table 2.

Twenty-seven pools, all consisting of Culex pipiens, yielded positive results for WNV. Early positive pools were collected in the province of Reggio Emilia at the end of July. Minimum infection rates (MIR: (no. of
positive pools/no. of mosquitoes tested) x 1,000) [12] were calculated, with higher MIR values recorded in August in the provinces of Reggio Emilia (3.08) and Modena (1.44).

Referring to the collection of overwintering mosquitoes, three mosquito species were collected: *Culex pipiens* (516 females, 52%), *Anopheles maculipennis* s.l. (475 females, 48%), *Culiseta annulata* (four females, 0%): all specimens were tested and yielded negative results.

**Conclusions**

The planned veterinary and entomological surveillance actions detected WNV activity from the end of July 2009, about 2-3 weeks before the onset of the first human neurological case. Figure 2 shows that mosquitoes and birds were the first indicators of WNV circulation. The same figure makes it clear that human cases occurred later in the season, as reported elsewhere [6]. Passive surveillance of horses also seems to be an early and suitable tool for the detection of WNV activity, but it will be less sensitive in the future, because an intensive programme of horse vaccination started in June 2009.

More human cases of WND occurred in 2009 than in 2008, and three were fatal. It is important to note that in 2008 the epidemic became evident in the late summer (beginning of September). In 2009 the first human cases were detected earlier than 2008. It is likely that increased attention of clinicians to this emerging disease improved the surveillance system sensitivity in 2009.

The circulation of WNV in a large area of the Po plain in two consecutive years shows that this territory is becoming suitable to support WNV establishment and possible endemicity. This indicates a need to organise standard surveillance measures to detect WNV activity early and assess risk to public health.

The results of the entomological surveillance confirm that the CO2 trap is a reliable and valuable tool for early detection of WNV. *Culex pipiens*, the most abundant mosquito species in the region, is the only vector species incriminated, since no other species collected in the field were found to be infected.

The quick and intensive spread of WNV in the past two years suggests that the whole Po plain may be affected in the future. In forthcoming years, surveillance of wild birds and insects will be used to forecast the extension and spread of WNV. The information gathered will be used to direct or optimise actions intended to prevent virus transmission, such as vector monitoring and control, information campaigns to improve personal protection, and deploy screening tests on blood, tissue, and organs for transplant.

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**References**

Crimean-Congo haemorrhagic fever (CCHF) is a viral zoonotic disease with a high mortality rate in humans. The CCHF virus is transmitted to humans through the bite of Ixodid ticks or contact with blood or tissues of CCHF patients or infected livestock. In December 2008, a re-emerging outbreak of CCHF occurred in the southern part of Iran. Five people were hospitalised with sudden fever and haemorrhaging, and CCHF was confirmed by RT-PCR and serological assays. One of the cases had a fulminant course and died. Livestock was identified as the source of infection, and all animals in the incriminated herd were serologically analysed and more than half of them were positive for CCHFV. We demonstrated that two routes of transmission played a role in this outbreak: contact with tissue and blood of infected livestock, and nosocomial transmission. Phylogenetic analyses helped to identify the origin of this transmission. This outbreak should be considered as a warning for the national CCHF surveillance system to avoid further outbreaks through robust prevention and control programmes.

Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a viral zoonotic haemorrhagic fever with up to 13-50% mortality rate in humans. Infected animals are unsymptomatic. The disease is caused by Crimean-Congo haemorrhagic fever virus (CCHFV) that belongs to the family Bunyaviridae, genus Nairovirus. The of negative single-stranded RNA genome consists of three segments, large (L), medium (M) and small (S), coding for the viral polymerase (L), the envelope glycoproteins (M) and the viral nucleoprotein (S) [1-5]. The typical course of CCHF progresses through four distinct phases: incubation, pre-haemorrhagic phase, haemorrhagic phase and convalescence [6-8]. After a incubation period of one to three days, the patient has sudden onset of fever, myalgia, nausea and severe headache. Within three to six days of the onset of illness, a petechial rash and haemorrhagic symptoms such as epistaxis, haematemesis, and melaena may occur. The most severely ill patients develop multiorgan failure characterised by shock, haemorrhaging and coma [9-11]. The virus is transmitted to humans through the bite of Ixodid ticks or by contact with blood or tissues from infected livestock [12-14]. In addition to zoonotic transmission, CCHFV can be spread from person to person and is one of the rare haemorrhagic fever viruses able to cause nosocomial outbreaks in hospitals [15-20].

In the period from 1 January 2000 to 12 September 2010, 738 confirmed cases of CCHF and 108 associated fatalities were notified in Iran [15,21]. The province reporting most infections was Sistan-va-Baluchistan, Isfahan and Fars (Figure 1).
**Outbreak description**

Here, we report a CCHF outbreak in Fars province, Iran, caused by contact of humans with blood or tissues of infected livestock, with additional nosocomial transmission. In total, five patients (A-E) were admitted to the regional hospital with similar presentations of a haemorrhagic condition in the period from 18 December to 21 December 2008. This period coincides with the Muslim ceremony Eid-al-Adha (the ceremony of sacrificing livestock) which is celebrated in Islamic countries on 9 December.

Patients A and D (who are part of the same family) bought a calf from a butchery run by two brothers, patients B and C, and hired them to sacrifice the animal. On the morning of 18 December 2008, Patient A, the index case of this outbreak, was admitted to hospital and died after a fulminant course of CCHF. In the evening of the same day, Patients B and C were admitted to the same hospital with fever and chill, severe headache, dizziness, photophobia. Patient D developed similar clinical signs on 21 December and was hospitalised. Nine days after the index case, the nurse caring of these four patients was also hospitalised with haemorrhagic symptoms (Patient E).

**Materials and methods**

**Case definition**

The case definition for probable cases included patients admitted between 18 December and 27 December 2008 in the regional hospital and presenting with a clinical picture compatible with CCHF, or contact with tissues or blood from a possibly infected animal, or a healthcare worker with a history of contact with a CCHF case. Probable cases with positive IgM serology and/or positive RT-PCR were considered as CCHF confirmed cases.

**Laboratory analysis**

Human and animal sera were analysed by ELISA for anti-CCHFV IgM and IgG as described [15,22]. Viral RNA was extracted from patient’s serum using QIAamp RNA Mini kit (QIAGen GmbH, Hilden, Germany) and analysed by gel-based and real-time RT-PCR with a one-step RT-PCR kit (QIAGen GmbH, Hilden, Germany). A 536 bp fragment of the S segment of the CCHFV genome was amplified [4,8,12] and sequenced.

Phylogenetic analysis was performed with the neighbour-joining method based on Kimura two-parameter distances by using Mega 4 software. Bootstrap confidence limits were based on 500 replicates. Evolutionary divergence, distance matrix and subsequently sets of phylogenetic trees were calculated by the software [23].

**Results and discussion**

As summarised in the Table, five probable CCHF cases in this outbreak were confirmed by serological and molecular methods. It is worth mentioning that no immunological response was detected in the fatal case that had a fulminant course, Patient A, and CCHF.
in this case was only confirmed by a strongly positive RT-PCR. There is evidence of other fatal cases lacking an immune response to CCHFV [6,24]. Patients C and E were positive both for viral RNA and antibodies against CCHFV, while Patients B and D were negative in the PCR and only confirmed by serological assay. Notably, ribavirin was administered to the patients in hospital.

At the same time, serum samples were collected from 50 animals in the herd from which the calf had been bought. In 30 of these samples antibodies to CCHFV were detected. Although CCHF is an asymptomatic disease in livestock that does not kill the animals, seroepidemiological surveys of animal populations in endemic areas and high risk regions could be useful in that they may complement the national surveillance system and serve as an early warning of CCHF in the area.

In this outbreak, it was demonstrated that the main transmission route of CCHF was through handling blood or tissues of infected livestock (for patients A, B, C and D), while patient E had had no contact to livestock and was infected nosocomially. It is unclear why Patient A had such a fulminant course of disease and died. Patients B, C and D were infected through the same route, by direct contact with tissue and blood of the same animal, but had a milder course of disease and recovered. It is important in infectious disease outbreaks to investigate what factors determine the severity of the disease in different individuals [6]. There are published reports on the influence of cytokine levels on the immune response to CCHFV in different patients [24,25]. It has been shown that patients infected with a higher dose of virus develop more severe disease symptoms and outcomes [26-28]. Although we did not use quantitative RT-PCR, the band density of the PCR product obtained from patient A was much higher than that of the other patients. On the other hand, it seems likely that patients B and C presented a mild form of the disease because they may already have had antibodies against CCHFV due to their professional exposure. Moreover, no anti-CCHFV IgG antibody response was detected for patient B, whereas a normal serological and molecular pattern was seen in patient C, which

**Figure 2**
Phylogenetic comparison of Crimean-Congo haemorrhagic fever virus isolates from Iran with isolates form patients in the recent outbreak in Fars province, Iran, December 2008

**A**

**B**

<table>
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A. The phylogeny tree of nucleotide sequences spanning described regions of the S-segment of CCHF virus genome which is detected in the outbreak. B. Nucleotide identity and divergence of CCHF virus genomes isolated from patients of the outbreak.
might be interpreted to indicate that patient B was infected with a very low viral dose. Recent investigations have concluded a relationship linking the severity and outcome of CCHFV infections with the strength of the host immune response and the initial viral load [24,25,27].

Phylogenetic analysis of alignments of three partial genomic sequences (536 bp) of the CCHFV S segment indicates that the viruses isolated from patients A, C and E can be differentiated into two distinct branches, with a slightly lower identity between patients A and E. As illustrated in Figure 2, the sequences obtained in this outbreak are not clustered with other CCHFV sequences isolated in Iran (about 97.5% identity). It is possible that a new strain occurred in the outbreak region, and further phylogenetic analyses are required to identify the precise origin of this genetic variant. However, comparison of the isolates from our patients with isolates from other areas may give some indications as to the origin of this outbreak [12].

One of the factors that contributed to the control of this outbreak was the well-coordinated and efficient surveillance system for CCHF and other viral haemorrhagic fevers that is in place in Iran. The system is not only responsible for continuous monitoring of these diseases but also deals with outbreaks. Rapid and precise laboratory diagnosis of CCHF allowed controlling this outbreak. Nevertheless, a higher level of training and precautionary measures for healthcare workers (such as use of isolation chambers in hospital wards, mask and other medical shields during contact to CCHF patients) and other high risk professions could help to decrease the outbreak rate in the endemic areas. In conclusion, with Iran being an endemic country for CCHF in the Middle East and neighbouring Turkey an endemic country in Europe, efficient surveillance and control programmes on CCHF in Iran could prove beneficial also for the European region.

Acknowledgements

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

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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 toptype 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%). Coquillettidia 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 state, 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.

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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 carcases 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 Bosporus 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 nº 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 GOC-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


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]

* Aeles 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 *Haemogogus* 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]. 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.

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.

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>
<thead>
<tr>
<th>Year</th>
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<tbody>
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</tr>
<tr>
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<tr>
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<td>Virginia</td>
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<td>1741</td>
<td>Virginia, Philadelphia, New York</td>
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<td>Virginia, New York</td>
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<td>1802</td>
<td>Philadelphia</td>
</tr>
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</table>

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

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

www.eurosurveillance.org
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...
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 Colombia, 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 [39]. 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

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References


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 imagos.

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

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<th>Mortality 10-70%</th>
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Reproduced from Lefèvre et al. [5] with permission from the publisher (Lavoisier, France)

**Figure 1**

Epidemiological cycle of Rift Valley fever

- Vectorial transmission
- Direct transmission
- Vertical transmission
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<td>dalzieli</td>
<td>Senegal (1974, 1983)</td>
</tr>
<tr>
<td></td>
<td>dentatus</td>
<td>Zimbabwe (1969)</td>
</tr>
<tr>
<td></td>
<td>durbanensis</td>
<td>Kenya (1937)</td>
</tr>
<tr>
<td></td>
<td>ochraceus</td>
<td>Senegal (1993)</td>
</tr>
<tr>
<td></td>
<td>tarsalis</td>
<td>Uganda (1944)</td>
</tr>
<tr>
<td></td>
<td>vexans arabiensis</td>
<td>Senegal (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saudi Arabia (2000)</td>
</tr>
<tr>
<td>Aedes (Neomelaniconion)</td>
<td>circumluteolus</td>
<td>Uganda (1955)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>South Africa (1955, 1981)</td>
</tr>
<tr>
<td></td>
<td>mcintoshi</td>
<td>Zimbabwe (1969)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>South Africa (1974-1975)</td>
</tr>
<tr>
<td></td>
<td>palpalis</td>
<td>Central African Republic (1969)</td>
</tr>
<tr>
<td>Ochlerotatus (Ochlerotatus)</td>
<td>caballus</td>
<td>South Africa (1953)</td>
</tr>
<tr>
<td></td>
<td>caspius</td>
<td>Suspected, Egypt (1993)</td>
</tr>
<tr>
<td></td>
<td>juppi</td>
<td>South Africa (1974-1975)</td>
</tr>
<tr>
<td>Aedes (Stegomya)</td>
<td>africurus</td>
<td>Uganda (1956)</td>
</tr>
<tr>
<td></td>
<td>demeilloni</td>
<td>Uganda (1944)</td>
</tr>
<tr>
<td>Aedes (Diceromya)</td>
<td>furcifer group</td>
<td>Burkina Faso (1983)</td>
</tr>
<tr>
<td>Anopheles (Anopheles)</td>
<td>coustani</td>
<td>Zimbabwe (1969)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Madagascar (1979)</td>
</tr>
<tr>
<td></td>
<td>fuscicolor</td>
<td>Madagascar (1979)</td>
</tr>
<tr>
<td>Anopheles (Cellia)</td>
<td>chrityi</td>
<td>Kenya (1981-1984)</td>
</tr>
<tr>
<td></td>
<td>cinereus</td>
<td>South Africa (1974-1975)</td>
</tr>
<tr>
<td></td>
<td>pauliani</td>
<td>Madagascar (1979)</td>
</tr>
<tr>
<td>Culex (Culex)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>antennatus</td>
<td>Nigeria (1967-1970)</td>
</tr>
<tr>
<td></td>
<td>neavi</td>
<td>South Africa (1981)</td>
</tr>
<tr>
<td></td>
<td>pipiens</td>
<td>Egypt (1977)</td>
</tr>
<tr>
<td></td>
<td>theleri</td>
<td>South Africa (1970)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zimbabwe (1969)</td>
</tr>
<tr>
<td></td>
<td>tritaeniorhynchus</td>
<td>Saudi Arabia (2000)</td>
</tr>
<tr>
<td></td>
<td>zombaensis</td>
<td>South Africa (1981)</td>
</tr>
<tr>
<td>Culex (Eumelanomya)</td>
<td>rubinotus</td>
<td>Kenya (1981-1984)</td>
</tr>
<tr>
<td>Eretmapodites</td>
<td>chrysoaster</td>
<td>Uganda (1944)</td>
</tr>
<tr>
<td></td>
<td>guinequevittatus</td>
<td>South Africa (1971)</td>
</tr>
<tr>
<td>Coquillettidia</td>
<td>fuscopennata</td>
<td>Uganda (1959)</td>
</tr>
<tr>
<td></td>
<td>grandidieri</td>
<td>Madagascar (1979)</td>
</tr>
<tr>
<td>Mansonia (Mansoniodes)</td>
<td>africana</td>
<td>Uganda (1959, 1968)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Central African Republic (1969)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kenya (1989)</td>
</tr>
<tr>
<td></td>
<td>uniformis</td>
<td>Uganda (1959)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Madagascar (1979)</td>
</tr>
<tr>
<td>Other diptera</td>
<td>Culicoides spp.</td>
<td>Nigeria (1967)</td>
</tr>
</tbody>
</table>

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**

Larvicidal 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 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

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Ecosystem</th>
<th>Vector</th>
<th>Hosts</th>
<th>Triggering factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976</td>
<td>Sudan</td>
<td>Irrigated area</td>
<td>?</td>
<td>Small ruminants</td>
<td>Irrigation (?)</td>
</tr>
<tr>
<td>1977</td>
<td>Egypt</td>
<td>Irrigated area</td>
<td>Culex pipiens</td>
<td>Small ruminants, camels, humans</td>
<td>Irrigation, cattle trade</td>
</tr>
<tr>
<td>1987</td>
<td>Mauritania, Senegal</td>
<td>Irrigated area</td>
<td>Culex pipiens</td>
<td>Small ruminants, cattle, camels, humans</td>
<td>?</td>
</tr>
<tr>
<td>1993</td>
<td>Egypt</td>
<td>Irrigated area</td>
<td>?</td>
<td>Small ruminants, humans</td>
<td>Irrigation</td>
</tr>
<tr>
<td>1997</td>
<td>Egypt</td>
<td>Irrigated area</td>
<td>?</td>
<td>Small ruminants, humans</td>
<td>Irrigation</td>
</tr>
<tr>
<td>1997-1998</td>
<td>Kenya</td>
<td>Dambos</td>
<td>Aedes spp., Culex zombaensis</td>
<td>Small ruminants</td>
<td>Rainfall</td>
</tr>
<tr>
<td>2000</td>
<td>Yemen, Saudi Arabia</td>
<td>Wadi</td>
<td>Aedes vexans, Culex tritaeniorhynchus</td>
<td>Small ruminants, cattle, camels, humans</td>
<td>Rainfall and virus introduction</td>
</tr>
<tr>
<td>2007</td>
<td>Sudan</td>
<td>Irrigated area</td>
<td>?</td>
<td>Small ruminants, cattle, humans</td>
<td>?</td>
</tr>
<tr>
<td>2007-2008</td>
<td>Mayotte</td>
<td>Island</td>
<td>?</td>
<td>Small ruminants, cattle, humans</td>
<td>Virus introduction</td>
</tr>
<tr>
<td>2008</td>
<td>Madagascar</td>
<td>Rice field in highlands</td>
<td>Culex? Anopheles?</td>
<td>Small ruminants, cattle, humans</td>
<td>?</td>
</tr>
</tbody>
</table>
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 cannot 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, 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.

### Table 4

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

<table>
<thead>
<tr>
<th>Country</th>
<th><em>Aedes vexans vexans</em></th>
<th><em>Ochlerotatus caspius</em></th>
<th><em>Culex theileri</em></th>
<th><em>Culex pipiens</em></th>
<th><em>Culex perexiguus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>X</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Belgium</td>
<td>X</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Croatia¹</td>
<td>X</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Cyprus</td>
<td>?</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>X</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Denmark</td>
<td>X</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Estonia</td>
<td>X</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Finland</td>
<td>X</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>France (mainland)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>France (Corsica)</td>
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<td>?</td>
<td>X</td>
<td>?</td>
</tr>
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<td>Germany</td>
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<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
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<tr>
<td>Greece</td>
<td>X</td>
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<td>X</td>
<td>X</td>
<td>X</td>
</tr>
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<td>Hungary</td>
<td>X</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Ireland</td>
<td>?</td>
<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Italy (mainland)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Italy (Sardinia)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>?</td>
</tr>
<tr>
<td>Italy (Sicily)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
<td>?</td>
<td>X</td>
<td>?</td>
</tr>
</tbody>
</table>

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

¹EU candidate country.

Adapted from [1].
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 [5].

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 Sicilia 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

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* Erratum: This reference was corrected on 18 March.

References


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 anthropo- notic, 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 [35].

**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 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 glycan 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 antileishmanial 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 tryaredoxin 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

<table>
<thead>
<tr>
<th>Human disease</th>
<th>(Diffuse and muco-) cutaneous leishmaniasis</th>
<th>Cutaneous leishmaniasis</th>
<th>Cutaneous leishmaniasis</th>
<th>Visceral leishmaniasis</th>
<th>Visceral leishmaniasis</th>
<th>Cutaneous leishmaniasis</th>
<th>Cutaneous leishmaniasis</th>
<th>Muco-cutaneous leishmaniasis</th>
<th>Diffuse cutaneous leishmaniasis</th>
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<tbody>
<tr>
<td>Leishmania species</td>
<td>L. tropica species complex</td>
<td>L. major</td>
<td>L. infantum (= L. chagasi in Neotropics)</td>
<td>L. infantum (= L. chagasi in Neotropics)</td>
<td>Other members of L. donovani species complex</td>
<td>L. braziliensis species complex; L. mexicana species complex</td>
<td>L. braziliensis</td>
<td>L. mexicana species complex</td>
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</tr>
<tr>
<td>Reservoir hosts (zoonosis) or anthroponosis</td>
<td>Often anthroponotic</td>
<td>Domestic dogs, wild canids</td>
<td>Domestic dogs, wild canids</td>
<td>Anthroponotic</td>
<td>Edentates, primates, rodents, marsupials</td>
<td>Rodents, marsupials</td>
<td>Rodents, marsupials</td>
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<td></td>
</tr>
<tr>
<td>EU biome</td>
<td>Mediterranean forests</td>
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<td>Mediterranean forests, temperate broadleaf forest</td>
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<td>Present</td>
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<td>Present</td>
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<tr>
<td>EU: France</td>
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<td>Absent</td>
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<tr>
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<td>Absent</td>
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<td>Present</td>
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<td>Formerly sporadic? (untyped parasites)</td>
<td>Absent</td>
<td>Formerly sporadic? (untyped parasites)</td>
<td>Formerly present</td>
<td>Absent</td>
<td>Absent</td>
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<td>Absent</td>
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<td>EU candidate: Former Yugoslav Republic of Macedonia</td>
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<td>Absent</td>
<td>Formerly sporadic? (confirmed in Croatia)</td>
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<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
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<tr>
<td>EU candidate: European Turkey (Asiatic Turkey)</td>
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<td>Absent (absent)</td>
<td>Absent (present)</td>
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<td>Absent (present)</td>
<td>Absent (present)</td>
<td>Absent (present)</td>
<td>Absent (present)</td>
<td></td>
</tr>
<tr>
<td>Other Europe: Albania</td>
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<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Other Europe: Switzerland</td>
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<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
</tbody>
</table>

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

---

**Figure 1**

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

*L. infantum*  
*L. tropica*

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

Phlebotomus ariasi

P. perniciosus

P. sergenti

P. perfiliew

P. neglectus

P. tobbi

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.
abundant human-biting species [3, 57, 68], from which it is concluded that the principal vectors of L. infantum in the Mediterranean region are members of the subgenus Larroussius (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

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

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 (R0) 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 anthropo- nontic, 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 anthropontic 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 leishmaniases 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 Ro 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.

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References


**Review articles**

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

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Phlebotomine sandflies are known to transmit leishmaniasis, 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 leishmaniasis 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.) 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 “papatacci 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 Toscanca, 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.

**Figure 3**
Distribution of main vectors in the European Union and neighbouring countries around the Mediterranean Sea up to 2009

**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], *P. papatasi* is an abundant species [42] and could be a

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.
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 [59], 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 competence 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 Guiana 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 Vesciculovirus
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. (Euphlebotomus) argenteipes 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 Euphlebotomus) 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 schwetzii and Lutzomyia longipalpis [73-75]. A vector competence has been demonstrated after oral infection for P. papatasi and P. duboscqi whereas P. sergenti, S. schwetzii 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.

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References


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...
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: 1,315/63; 2009: 1,300/62) [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, 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

www.eurosurveillance.org


Chikungunya infection in a French traveller returning from the Maldives, October, 2009

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In the last years, cases of chikungunya fever have been reported in international travellers returning from the Indian Ocean region. The cases have been linked to the re-emergence of chikungunya fever on Indian Ocean islands in 2006. We describe the first case of chikungunya fever in a French traveller returning from Malé, an island of the Maldives islands, confirming the permanence of virus circulation by the end of 2009.

Introduction
Chikungunya virus is a mosquito-borne alphavirus found in the tropical regions of Africa and Asia where it causes endemic and epidemic chikungunya fever, an acute self-limiting febrile algo-eruptive illness [1]. Chikungunya fever has been increasingly reported in international travellers following its re-emergence on Indian Ocean islands and its spread to southern Asia thereafter [2-4]. Moreover, some African and southeast Asian countries show an endemic circulation of the virus [5] which may contribute to occurrence of the disease among travellers. The illness was suspected to have emerged in the Maldives archipelago in 2007 [6], following the sweeping succession of outbreaks that occurred in the Indian Ocean region where it first affected Kenya in 2004, Réunion Island in 2005 and southern India in 2006 [1,7]. Here we report a confirmed case of chikungunya fever in a French traveller returning from Malé island, the Maldives, where an outbreak of chikungunya fever was reported starting in January 2009.

Case report
A French male in his thirties presented at the post-travel clinic of the Department of Internal Medicine and Tropical Diseases of the University Hospital Centre, Bordeaux, France in October 2009 with symptoms of recurrent high-grade fever (up to 40°C), headache, generalised muscle aches and severe joint pain mainly affecting fingers, wrists, knees and ankles, and an itching skin rash, since three days. Two days before, he had returned directly from a holiday trip to the Maldives where he had stayed for 14 days exclusively in the northern part of Malé island.

In our centre, the patient presented with a slight macular skin rash on the trunk and limbs, a slightly swollen right knee and small joints of hands and feet. Laboratory tests at the time of presentation showed a leucocyte cell count of 4,600 white blood cells (WBC)/µL, a thrombocyte count of 178,000 platelets/µL and an elevated C-reactive protein level (27 mg/L; normal ≤5 mg/L). Alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase were within normal limits. Blood smears for malaria and blood cultures were negative.

Chikungunya virus serology testing was conducted for specific immunoglobulin (Ig) G and M using IgM-capture and IgG-sandwich enzyme-linked immunosorbent assay (ELISA) with inactivated cell-culture-ground chikungunya virus and mouse anti-chikungunya hyperimmune ascitic fluid at the National Reference Centre for Arboviruses, Institut Pasteur, Paris, France. Serology for chikungunya virus revealed positive results for both specific IgM (optic density (OD)=1.633; serum control OD=0.073) and IgG (OD=0.475; serum control OD=0.096).

Paired serology for specific IgG and M by ELISA against dengue virus and Japanese B encephalitis were negative, as well as tests for leptospirosis, rickettsiosis, Q fever, West Nile virus and cytomegalovirus. A real-time PCR test was negative for dengue viruses and chikungunya virus RNA [8]. Fever decreased the day following the consultation, but severe joint pain persisted over six weeks until the end of December despite symptomatic treatment.

Discussion
Over the last couple of years and following successive waves of outbreaks in the Indian Ocean area since 2006, chikungunya has increasingly been reported in travellers returning from vacation in the region and in expatriates or immigrants back from visits to their home countries [2-4,9].

The new case described provides definite evidence of ongoing chikungunya virus transmission in the
Maldives. To the best of our knowledge, this case is reportedly the third confirmed chikungunya fever case imported from the Maldives since the first documented outbreak of chikungunya in Malé and other islands of the Maldives that lasted from December 2006 to April 2007 [6], followed by a suspected cluster on the Laamu Atoll from December 2008 to January 2009 [10] and the report of two confirmed cases in German travellers, a father and son returning from a 10-day visit to the Maldives mid-September 2009 [11].

The region is probably one of the most popular travel destinations in the Indian Ocean area. This may result in an increase of symptomatic travellers returning from this area and seeking medical advice at travel or primary care clinics. Hence, chikungunya together with dengue fever should be considered as an important differential diagnosis in those patients, assuming that both diseases are endemic in certain regions of India and the Indian Ocean area and may present with similar symptoms.

For more than 10 years, dengue fever was the only vector-borne viral disease reported in the Maldives. Aedes aegypti and A. albopictus, the dengue virus vectors which can also transmit the chikungunya virus, have been identified in the Maldives, with A. aegypti identified as the predominant vector in Malé [6]. The first chikungunya fever outbreak occurred from December 2006 to April 2007 with abrupt onset and high attack rates due to the lack of herd immunity. Epidemics may occur following an interval of 20-30 years of the virus not circulating as has been the case in western Africa and Malaysia [5, 6]. Confirmed imported cases among travellers support the assumption of endemic circulation of the virus which is consistent with the prevailing chikungunya epidemic in the Indian Ocean region.

This report highlights the need for surveillance in countries where emerging infections may be introduced by returning travellers as in the case with the Italian chikungunya fever epidemic which occurred in the province of Ravenna in 2007 [12]. It illustrates how travellers can serve as sentinel population providing information regarding the emergence or re-emergence of an infectious pathogen in a source region. Travellers can thus act as carriers who inadvertently ferry pathogens that can be used to map the location, dynamics and movement of pathogenic strains [9]. Thus, with the increase in intercontinental travel, travellers can provide insights into the level of the risk of transmission of infections in other geographical regions.

Conclusion
We report a case of dengue-like illness diagnosed as chikungunya in a tourist returning from the Maldives, a popular tourist spot. Despite the clinical similarity with dengue fever, chikungunya should be recognised early in returning travellers because of its specific protracted morbidity and its potential for causing local outbreaks in European countries, where local transmission is possible through the presence of the receptive vector in southern European countries.

References
This report describes the first isolation and molecular characterisation of a chikungunya virus from two German tourists who became ill after a visit to the Maldives in September 2009. The virus contained the E1 A226V mutation, shown to be responsible for an adaptation to the Asian tiger mosquito *Aedes albopictus*. The E1 coding sequence was identical to chikungunya virus isolates from Sri Lanka and showed three nt-mismatches to the only available E1 nt sequence from the Maldives.

### Introduction

Since the start of the current chikungunya fever pandemic on the east coast of Africa in 2005, many cases have been reported in countries in Asia and south-east Asia [1,2]. These cases were attributed to a particular chikungunya virus (CHIKV) strain that has adapted to very efficient transmission to humans via the Asian tiger mosquito (*Aedes albopictus*) due to a A226V mutation in the E1 envelope protein [3,4]. The Maldives were first hit by the chikungunya virus pandemic in late 2006 after the wet season which usually lasts until September. Based on almost 12,000 suspected cases of chikungunya fever the disease was reported on 121 of the 197 inhabited islands with incidence rates between 82 and 722 per 1,000 population [5]. A small set of blood samples from febrile patients with symptoms meeting the chikungunya fever case definition at that time confirmed CHIKV as causative agent in 64 of 67 cases by reverse-transcription PCR (RT-PCR) [8,9]. Chikungunya virus was isolated in Vero B4 cells from both sera and the entire nucleotide sequence of the isolate from the father was determined. The viral genome was 11,811 nucleotides in length and showed high levels of identity with the pandemic CHIKV that is circulating in many parts of the Indian subcontinent and other parts of Asia since 2006. Most interestingly the CHIKV isolate from the Maldives contained the A226V change in the E1 glycoprotein which has been shown to be responsible for shorter extrinsic incubation periods in *Aedes albopictus* mosquitoes [4]. While the son made an uneventful recovery after one week of symptoms, the father developed persisting arthralgias with limited mobility in the affected extremities and still requires analgesic treatment (Table).

### Case report and laboratory findings

Between 1 and 10 September 2009, a German couple visited the Dhiffushi Holiday Island resort at the southern tip of the Ari Atoll, the Maldives (Figure 1), together with their seven year-old son. They flew directly from Munich to Malé with a stopover in Dubai, United Arab Emirates.

Two and three days respectively after the family had returned to Munich, the son and the 35 year-old father developed symptoms compatible with either dengue or chikungunya fever (Table) while the wife stayed healthy. A test for dengue virus showed neither virus RNA nor anti-dengue virus (DENV) IgM for both patients, but the father had IgG antibodies reactive against DENV indicating an earlier anamnestic dengue fever or a cross-reaction with an earlier flavivirus vaccination. CHIKV-specific real-time RT-PCR yielded ct-values of 23 (son) and 22.5 (father) in the respective acute serum samples obtained on 14 September, indicating high-level viremia [8,9]. Chikungunya virus was isolated in Vero B4 cells from both sera and the entire nucleotide sequence of the isolate from the father was determined. The viral genome was 11,811 nucleotides in length and showed high levels of identity with the pandemic CHIKV that is circulating in many parts of the Indian subcontinent and other parts of Asia since 2006. Most interestingly the CHIKV isolate from the Maldives contained the A226V change in the E1 glycoprotein which has been shown to be responsible for shorter extrinsic incubation periods in *Aedes albopictus* mosquitoes [4]. While the son made an uneventful recovery after one week of symptoms, the father developed persisting arthralgias with limited mobility in the affected extremities and still requires analgesic treatment (Table).

### Discussion

Together with a very recent report on chikungunya fever in a French traveller returning from the northern part of Malé Island, Maldives, in October 2009 [10], our findings suggest a continuous circulation of CHIKV also in other parts of the Maldives. The family stayed...
on Dhiffushi Holiday Island throughout their holidays with a daytrip to the neighboring Sun Island. Malé with its international airport was only visited for the inter-continental flight connection, leaving not much time to become exposed to mosquito bites. We cannot rule out that both infections were acquired while waiting at the airport, because this would fit well with both the incubation period of the disease and with the previous case report of the French traveller, who became infected while staying at the Malé Atoll. However, given the presence of Aedes aegypti in Malé, the source of infection might be the airport.

**Figure 1**
Location of Holiday and Sun Islands on the southernmost rim of the south Ari Atoll, about 100 km away from Male International Airport

**Table**
Clinical and laboratory data of patients diagnosed with chikungunya fever, Germany, September 2009

<table>
<thead>
<tr>
<th>Patient</th>
<th>Son (7 years)</th>
<th>Father (35 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Travel schedule</td>
<td>Munich-Dubai-Male and back on 1-10 September, 2009</td>
<td>Munich-Dubai-Male and back on 1-10 September, 2009</td>
</tr>
<tr>
<td>Onset of disease</td>
<td>12 September 2009</td>
<td>13 September 2009</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td>Fever 39.5°C</td>
<td>Fever 39.0°C</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
<td>Ague, retrobulbar pressure and pain, arthritis of both wrists and ankles</td>
</tr>
<tr>
<td></td>
<td>Macular and partially confluent exanthema (mainly on face and torso)</td>
<td>Erythema, macular and partially confluent exanthema (mainly on torso and arms)</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td>Leucocytes 2,700/µl CRP 2.5 mg/dl</td>
<td>Leucocytes 5,300/µl CRP 13 mg/dl Creatinine 1.4 mg/dl</td>
</tr>
<tr>
<td></td>
<td>CHIKV RT-PCR positive</td>
<td>CHIKV RT-PCR positive</td>
</tr>
<tr>
<td></td>
<td>DENV RT-PCR negative</td>
<td>DENV-PCR negative; anti-DENV IgG 15E</td>
</tr>
<tr>
<td>Therapy</td>
<td>Paracetamol, Ibuprofen</td>
<td>Paracetamol, Ibuprofen</td>
</tr>
<tr>
<td>Further course</td>
<td>Since 16 September fever-free, exanthema gone on 17 September, no further complications since then</td>
<td>Since 16 September fever-free and creatinin back to normal (1.1 mg/dl), exanthema gone, but arthralgias of ankles, wrists, and digital joints persist for more than six months including limited mobility and requiring NSAID treatment</td>
</tr>
</tbody>
</table>

CRP: C-reactive protein; CHIKV: Chikungunya virus; DENV: Dengue virus; RT-PCR: reverse transcription-polymerase chain reaction; NSAID: non steroidal anti-inflammatory drugs
the high incidence rates of 65.2 per 1,000 population previously reported for the Ari Atoll [5], both infections could likewise have been acquired on Holiday Island. Further, a considerable number of people travel constantly between India and Sri Lanka and the tourist resorts on the Atolls’ islands of the Maldives where they are employed. This frequent exchange may argue for a repeated and renewed introduction of CHIKV from India or Sri Lanka via viraemic workers or tourists and limited local transmission through aedine mosquitoes at the respective islands. Analyses of the E1 gene revealed three nt-mismatches when compared to the 2007 case that was analysed in Singapore [6], but identical nt sequences to a series of CHIKV strains from Sri Lanka (Figure 2) [6,11].

**Figure 2**
Phylogenetic relationship generated by Maximum Parsimony method as implemented in MEGA4 based on the complete E1 protein coding sequence (1314 nt) of a set of CHIKV of different geographic origin

Sequence data are provided with their accession number, country and year. The term Cluster A and B for sequence data from Sri Lanka is adapted from [10]. Please note that the only available CHIKV E1 sequence from the Maldives from 2007 clusters together with CHIKV from India from 2006, while the CHIKV reported here is part of the Cluster B from Sri Lanka in 2008. Sri = Sri Lanka, Mal = Maldives, Ita = Italy (imported from India in 2007), Ind = India, Reu = Reunion, Sey = Seychelles, Com = Comores, Mau = Mauritius, My = Malaysia, Indo = Indonesia. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches. The branch lengths are informative (bar length corresponds to 1 nt-differences)

It will be seen in the near future whether more cases of chikungunya fever will be reported for the Maldives, but we feel that this is already an issue in travel medicine although the German Robert Koch Institute reported only three chikungunya fever cases in returning travellers from the Maldives in 2009 (two of which we describe here). A crucial question concerning the current global situation on chikungunya fever is the adaptation of the pandemic CHIKV strain to *Ae. albopictus*. *Aedes aegypti* has been long known to occur on several islands of the Maldives and seems to be the predominant vector on Malé itself while *Ae. albopictus* has established foci on other islands where it seems to be the main mosquito vector species [5]. We do not know which *Aedes* species has infected the German tourists, but we do know that the A226V mutation is suggestive for *Ae. albopictus* as the vector. This particular mosquito is present in many areas around the Mediterranean Sea and was responsible for a CHIKV outbreak in Italy in 2007 resulting in more than 300 cases [12,13]. With a continuing circulation of CHIKV in major tourist destinations in Asia and Africa, imported cases of chikungunya fever will also be seen in Europe and North America. In countries were *Ae. albopictus* is abundant, returning viraemic tourists could cause smaller outbreaks.

Acknowledgements

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References


In October 2009, a traveller returning from Africa to Italy was hospitalised with symptoms suggestive of a haemorrhagic fever of unknown origin. The patient was immediately placed in a special biocontainment unit until laboratory investigations confirmed the infection to be caused by a dengue serotype 3 virus. This case reasserts the importance of returning travellers as sentinels of unknown outbreaks occurring in other countries, and highlights how the initial symptoms of dengue fever resemble those of other haemorrhagic fevers, hence the importance of prompt isolation of patients until a final diagnosis is reached.

Case description
In October 2009, a Senegalese man in his forties, who had been living in Italy for 20 years, presented to Hospital A in the northern Italian city of Turin three days after returning (via Madrid) from a four-month visit to his home village in Senegal. During his stay, he had never left the village and had not visited any healthcare centres. The initial symptoms of disease, a persistent fever (≥38 °C) accompanied by an unrelenting headache, started during the flight from Madrid to Turin worsened over the previous two days despite treatment with paracetamol.

On the day after hospitalisation, when laboratory test results showed a plateleto count of 5,000 cells/mm³ as well as evidence of altered liver function (aspartate aminotransferases (AST): 3,539 U/L; alanine aminotransferases (ALT): 815 U/L; lactate dehydrogenase (LDH) 3,609 U/L; gamma-glutamyl transferases (γ- GT): 112 U/L), the patient was first transferred to an infectious diseases hospital in Turin (Hospital B), and two days later to the National Institute for Infectious Diseases in Rome (Hospital C), which is the Italian national reference centre for emerging infections and bioterrorism, on suspicion of a viral haemorrhagic fever. The patient was admitted to a special biocontainment unit during the same night. Over the following days, the patient’s clinical condition improved and he was discharged nine days after admission.

Laboratory investigations
After the patient’s admission, series of tests were run in parallel on samples taken at Hospital C and in Turin, before the transfer to Rome. The tests included in the differential diagnosis ruled out malaria (which had already been excluded in Turin), as well as infections with the major agents of viral hepatitis, herpes simplex virus and viral haemorrhagic fever viruses. Serological investigation using an in-house immunofluorescence assay (slides prepared with a mix of uninfected and dengue type 2-infected Vero cells) revealed a high IgG titre against dengue virus soon after the onset of symptoms (in a sample taken on 10 October), with the presence of IgM antibodies at a low titre, and reverse-transcription polymerase chain reaction (RT-PCR) followed by sequencing of the NS5 region confirmed the infectious agent to be a serotype 3 dengue virus. In addition, a 224 bp fragment of the E gene was amplified and sequenced. The phylogenetic analysis of this fragment is shown in the Figure. Our patient was infected with a strain belonging to dengue virus serotype 3, genotype III closely related to other strains found in Africa, but never reported from Senegal before the case described here [1]. The tests carried out the day before the patient left the hospital showed that his IgG titre was still increasing. Overall, the immune response was consistent with a re-infection with a dengue serotype 3, genotype III virus which can lead to a severe form of the disease.

Case management
At the time the patient reported to the first hospital in Turin, there was no information on any outbreaks of dengue virus taking place in Senegal [1]. Therefore, the fact that the patient developed symptoms suggestive of a haemorrhagic disease led the clinicians to suspect also other, more dangerous viral infections, namely...
Lassa virus or Crimean-Congo haemorrhagic fever virus, both categorised as biosafety level 4 (BSL4) agents [2-3]. As these infections could not be ruled out on clinical and epidemiological criteria, it was decided to transfer the patient to a high-security isolation facility.

When contacted by Hospital B in Turin, the National Institute for Infectious Diseases immediately requested the shipping of clinical samples, but because of logistical difficulties the transport could not be organised. It was then decided by the Italian Ministry of Health, in consultation with the institute, to make use of the procedure already in place in Italy for the transport of highly infectious patients under high isolation conditions, which included the use of military aircraft and equipment of the Italian Air Force and Hospital C (stretcher isolators and a high containment ambulance). On arrival, the patient was immediately transferred to the biocontainment unit and was attended to by specially trained, dedicated staff employing all necessary biosafety precautions.

Conclusions

We have described a case of dengue virus infection imported into Italy from Africa, which to date has been a rare occurrence as most cases seen in European travel clinics have been imported either from Asia or the Americas. Dengue virus has been known to circulate in parts of Africa for decades, and Senegal in particular has experienced several outbreaks, mainly of dengue serotype 2 virus [1]. Following our report [4], which was the first for a dengue serotype 3 virus in Senegal, the country’s health authorities were alerted to a possible epidemic and since then, over 50 cases have been registered in the country [5-6], confirming the importance of returning travellers as sentinels of as yet unreported outbreaks occurring abroad [7].

From a clinical point of view, the patient did not meet the World Health Organization’s (WHO) criteria for severe dengue fever [8], but presented a set of warning signs indicative of haemorrhagic fever such as liver enlargement (>2 cm), altered liver enzymes (20-fold) and a rapid decrease in platelet count. Therefore, and because the information available at the time did not allow excluding the involvement of a BSL4 agent, the decision to treat the case as a potential viral haemorrhagic fever was made early in the management process. The recent report of a case of Marburg haemorrhagic fever imported from Uganda into the United States [9] that was diagnosed retrospectively in a serum sample archived six months earlier after it had tested negative for agents of viral haemorrhagic fever, should teach us that in similar cases, patients should immediately be placed in an adequate containment facility until a final diagnosis is reached. Our recommendation is to perform all diagnostic procedures safely at the appropriate biosafety level, and as a general rule any activities involving patient samples at a level lower than BSL3 should be kept to a minimum.

It remains a problem that the transport of samples is still an unsolved issue in Italy as well as in Europe and worldwide. The timely shipping of biological samples in advance of the patient’s arrival would have shortened the time to reach a diagnosis and avoided the extra costs of unnecessary biosafety measures.

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Sequence identification is as follows: strain name, country and year of isolation.

The scale bar indicates nucleotide substitutions per site. Multiple alignment of our sequence and other dengue serotype 3 virus sequences available in GenBank was generated with ClustalW1.7 software included in the Bioedit package. The phylogenetic tree was constructed by using nucleotide alignment, the Kimura-2-parameters algorithm, and the neighbour-joining method implemented in the MEGA 4.1 software. The robustness of branching patterns was tested by 1,000 bootstrap pseudoreplications.

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**Figure**

Phylogenetic relationship of the strain isolated in October 2009 in Rome (identified by the black dot) with other dengue serotype 3 virus strains described elsewhere [10]
References


Due to non-existing or limited surveillance in Africa, little is known about the epidemiology of dengue illness in the continent. Serological and virological data obtained from returning European travellers is a key complement to this often flawed information. In the past years, dengue 3 virus has emerged in West Africa and has been detected in travellers returning to Europe. The first dengue epidemic in Cape Verde with more than 17,000 cases from September to December 2009 demonstrated that dengue virus is still expanding worldwide to new territories.

**Introduction**

Dengue virus is widely distributed in tropical and subtropical countries and is transmitted by day-biting mosquitoes of the genus Aedes. It often goes unrecognised in African countries, where the lack of surveillance systems, or their poor implementation, is the cause of missing information on dengue virus activity [1].

Laboratory-based surveillance of dengue virus infection in febrile travellers could provide useful information about the different dengue virus serotypes circulating worldwide and in particular those circulating in areas where limited surveillance is available. To this end, the European Network for Imported Viral Disease - Collaborative Laboratory Response Network (ENIVD-CLRN network) provides outbreak support, in particular related to laboratory diagnostics, to assist European Union (EU) Member States, candidate countries and members of the European Economic Area and European Free Trade Association (EEA/EFTA) in detecting, investigating and responding to outbreak-prone diseases, imported, rare or unknown infectious agents, or outbreaks related to the intentional release of pathogens.

A large outbreak of dengue illness with more than 17,000 cases occurred in the Cape Verde archipelago at the end of 2009 [2]. It was the first time that dengue virus was detected in the archipelago. Concomitant detection of dengue virus in Senegal and identification of several imported cases among travellers returning from West Africa were reported. This article provides a brief review of historic reports of dengue virus in Africa focused on West Africa and summarises the recent outbreaks and the links to imported cases of dengue virus infection in Europe.

**Dengue virus in West Africa**

The burden of dengue virus infection in Africa has not been estimated yet. Outbreaks of dengue fever and dengue haemorrhagic fever are poorly documented, however, we cannot conclude that mild and severe dengue infection is infrequent in African countries. The circulation of different dengue virus serotypes is also poorly documented. Nevertheless some information is provided in publications on outbreaks and serosurvey studies in Africa and reports involving dengue virus infection in travellers.

A retrospective serological study in 1956 [3] suggests that dengue virus caused an epidemic in Durban, South Africa in 1927. This report is the first documented dengue virus epidemic in Africa. It was not until the end of the 1960s, however, that the virus responsible for dengue fever outbreaks in Africa could be isolated. The first dengue virus (DENV) isolate was DENV-1, detected in Nigeria in 1964 [4]. Since then DENV-1, 2 and 4 have been circulating in West Africa although the main serotype reported has been DENV-2 [1]. Viral isolates have been predominantly detected in wild-caught mosquitoes (Aedes luteocephalus, Ae. taylori and/or Ae. furcifer) involved in sylvatic transmission cycles in Senegal and Nigeria, and from a few cases in humans who were in contact with forest cycles [5,6]. DENV-2 from sylvatic cycles have also been isolated in Côte d’Ivoire, Burkina Faso and Guinea [5,7,8]. More recently in 2005, DENV-2 was identified in a traveller returning from Ghana [9]. The last detection of DENV-4 in West Africa was in the 1980s in two inhabitants of Dakar, Senegal [10].
The first description of DENV-3 activity in Africa was related to outbreaks detected during 1984 and 1985 in Pemba, Mozambique, with two deaths due to dengue haemorrhagic fever [11]. DENV-3 was then detected in 1993 in Somalia and areas around the Persian Gulf [12]. Phylogenetic studies suggested that these outbreaks were caused by a virus imported from the Indian sub-continent [13]. DENV-3 circulation in West Africa was first identified in a traveller returning to Spain from Cameroon in 2006 and subsequently in a traveller returning to Spain from Senegal in 2007 (C. Domingo et al., unpublished results). However the first article on DENV-3 in West Africa was published in 2008, when DENV-3 was detected co-circulating with yellow fever in Côte d’Ivoire [14].

Dengue virus importation from Africa into Europe

Reports on the importation of dengue virus to Europe have been increasing since the 1990s. Some of these are publications or reports from single countries or networks and show that the frequency of travel-acquired dengue virus infections in Africa is low compared to south-east Asia and the Americas [15,16]. This distribution is due to two main factors: worldwide dengue virus activity and the popularity of certain countries as tourist destinations. In a study by the European Network on Imported Infectious Disease Surveillance (TropNetEurop) covering 481 European travellers between 1999 and 2002, 8% of dengue fever cases were imported from Africa [17], a proportion similar to that found in other European studies. In France, between 2002 and 2005, 14% of imported dengue fever cases originated in Africa [18]. Ten per cent of the cases in Austrian and Finnish travellers were also acquired in Africa, as analysed over a 15-year (1990-2005) and 10-year (1999-2009) period, respectively [19,20]. In 2008, dengue virus cases imported from Africa reported by TropNetEurop dropped to 4% [21].

Dengue virus importation from West Africa (2006-2008)

In recent years, dengue fever has been documented in travellers returning from several West African countries, caused in particular by DENV-3 which was recently identified in that region (Figure 1).

From January 2006 to August 2008, 19 imported cases of dengue virus infection were reported in travellers from West Africa in France: 11 cases from Côte d’Ivoire (one in 2006, three in 2007 and seven in 2008), four cases in Burkina Faso (one in 2006 and three in 2007), two cases in Benin in 2006, one case in Senegal in 2007 and one case in Mali in 2008 [22]. Dengue serotypes detected during this period were DENV-1 in Burkina Faso [22], DENV-2 in Côte d’Ivoire [22] and DENV-2 in a simultaneous outbreak of chikungunya and dengue viruses in Gabon [23], all in 2007. In 2008, DENV-2 serotype was identified in Mali [14] and in Senegal [24]. Moreover, DENV-3 was detected in a Japanese tourist and in a French expatriate returning from Côte d’Ivoire between May and July 2008 [25]. DENV-3 activity was also detected in East Africa in 2008 in a Finnish traveller returning from Eritrea (O. Vapalahti, personal communication).

Recent DENV-3 activity in West Africa

In the beginning of October 2009, a case of dengue fever was reported in a Senegalese returning to Italy after a holiday in his home country. DENV-3 infection was diagnosed at the National Institute for Infectious Diseases in Rome [26]. At the same time France reported DENV-3 in travellers returning from Senegal (C. Renaudat, personal communication). Also, ProMED posted several archives describing dengue virus outbreaks in Senegal: in the Kedoungo region [27] and Dakar [28]. The Pasteur Institute in Dakar identified DENV-3 in febrile patients (A. Sall, personal communication).

Meanwhile, an unprecedented outbreak has been detected in the Cape Verde archipelago in the beginning of September 2009 (week 40) [29]. This is the first...
The highest number of cases were reported during week 45 (5,512 cases), decreasing in week 47 to 1,447 cases and finally five cases in week 53. A total of 17,224 cases including six deaths were reported from 18 of the 22 municipalities in Cape Verde by the end of 2009. The municipality of Praia on Santiago island, notified the highest number of cases (13,000 cases) followed by Sao Felipe in Fogo Island (3,000 cases) [2]. The first samples tested at the Pasteur Institute in Dakar confirmed DENV-3 circulation [29].

Recently imported DENV-3 cases in Europe and the identified outbreaks in West Africa suggest that this serotype is spreading in the region.

We used a phylogenetic approach in order to determine genotype association among the recent DENV-3 circulating in the region, using sequences provided by ENIVD-CLRN laboratories. DENV-3 viruses are divided into four geographically different genotypes (I, II, III, and IV) [13]. The emergence of a virulent lineage of genotype III in Sri Lanka at the end of 1980s was largely associated with a high incidence of the disease and the emergence of Dengue haemorrhagic fever in Asia and the Americas. All DENV-3 detected in East Africa from 1984 to 1993 [13] belonged to this lineage of genotype III (Figure 2).

Also, isolates from geographically distant outbreaks, strains ENIVD Spain ex Cameroon 2006, Japan ex Ivory Coast 2008 and Saudi Arabia 2004 (Figure 2), and from Eritrea in 2008 (not shown in Figure 2; E. Huhtamo et al, unpublished results) belong to DENV-3 genotype III. The DENV-3 strain that circulated in Senegal in 2009 and was isolated from a traveller in Italy [26], also belonged to genotype III and was closely related to the DENV-3 strain circulating in Côte d’Ivoire in 2008. Therefore, the DENV-3 that has emerged in the Cape Verde archipelago is likely to have been introduced from a West African country due to the geographical proximity with strong trade and travel activities.

Future outlook

The recent DENV-3 expansion in West Africa was first detected in European travellers returning from this area, which triggered the alert for active surveillance in the exporting countries.

**Figure 2**

Phylogenetic tree of DENV-3 sequences

This tree is based on a 139 nt fragment of the E gene. Sequence identification is as follows: country of origin, year of identification. The sequences from imported cases are labelled as ENIVD/country of detection/country of exportation/year of detection. Phylogenetic analysis was conducting using MEGA 4.0 (Tamura, Dudley, Nei, and Kumar 2007). Genetic distance was calculated with the Tamura Nei algorithm. Phylogenetic tree was constructed using Neighbor-Joining model and the resultant tree was tested by Bootstrap (1,000 Replicates). Only bootstrap probabilities over 60% are shown.
However, although most of the African countries are prepared for surveillance of yellow fever and human immunodeficiency virus infections, most of them lack specific methods for dengue virus diagnostics and require new diagnostic tools. As a recent example of such technology transfer, the Pasteur Institute laboratories in Paris and Dakar have implemented differential diagnostics for dengue at the Pasteur Institute in Abidjan [14]. This model of cooperation is required also in other African countries.

As long as active dengue virus surveillance is poorly implemented in Africa, the study of febrile travellers returning to Europe could help to detect viral activity on the African continent.

As part of the ENIVD-CLRN, a collaborative study on imported chikungunya and dengue virus infections in European travellers will start in 2010. The aim of the study is to complete the global map of chikungunya and dengue virus circulation, including the global distribution of viral genotypes of those viruses. It will permit clinicians to compare the clinical symptoms, signs and analytical data of imported cases in Europe. The surveillance of travellers returning to Europe will continue to improve our knowledge about dengue virus distribution in Africa.

References


In late February–early April 2010, five cases of dengue fever were diagnosed in returning travellers in Europe in EurotravNet sites in Sweden and France in patients with travel history to the Comoros and/or Zanzibar, Tanzania. Four cases were non-complicated dengue fever and one case dengue hemorrhagic fever. Three patients were viraemic at the time of diagnosis and infected with Dengue type 3 virus.

An estimated 100 million cases of dengue fever and 250,000 cases of dengue haemorrhagic fever occur annually worldwide [1]. The past 20 years have seen a dramatic geographic expansion of epidemic dengue fever from Southeast Asia to the South Pacific Islands, the Caribbean, and the Americans. An increasing number of reports of dengue fever and associated illness among travellers to dengue virus–infected areas paralleled the changing epidemiology of dengue in local populations [1].

In 2010 (until 14 April), five cases of dengue fever including one case of dengue haemorrhagic fever, have been reported from EurotravNet sites in France and Sweden, in four travellers returning from the Comoros and one traveller returning from Zanzibar, Tanzania. EurotravNet, the Network for travel medicine and tropical diseases of the European Centre for Disease Control consists of 14 core sites in nine European countries and participants monitor travel related infectious diseases in Europe (www.eurotravnet.eu).

Case reports

Cases were diagnosed in Paris (one case) and Marseille (three cases), France and Stockholm, Sweden (one case). The age of cases ranged from 41 to 69 years, three were females, two males. All travellers to the Comoros had visited friends and relatives where they had stayed between 15 and 93 days in the period from December to March. The case returning from Zanzibar who had travelled as a tourist, had stayed for two days in Stone Town and seven days in Nungw. All cases were non-complicated dengue except for one case of dengue hemorrhagic fever. Detailed clinical presentations and onset of symptoms after return and laboratory findings are displayed in Table 1.

Cases were confirmed by serology and four were positive for IgM and IgG and once case positive for IgM only. Three cases were confirmed as dengue type 3 virus (DENV-3) by PCR.

Discussion and conclusion

Six autochthonous cases of dengue fever were recently identified in the Comoros (March 2010). Additional cases were identified in individuals with travel history from the Comoros, in Madagascar (1 case), Mayotte (3 cases) and Reunion Island (1 case) [1,2]. In addition, two cases were potentially imported from Tanzania to Japan [2,3]. DENV-3 was identified in the cases in Madagascar and Japan. These results indicate that DENV-3 is currently circulating in the Comoros and Zanzibar, and given that the last outbreak in the Comoros took place in 1993 and involved DENV-1, we may face a situation with the possibility for the emergence of a new outbreak including possible severe cases, similarly to what was recently observed in Sri Lanka, East Africa and Latin America [5].

In order to protect themselves, travellers to areas where vector-borne diseases such as dengue fever and malaria are present should be advised to adopt some protective measures to avoid mosquito bites. Moreover, physicians should be prepared to diagnose and manage imported cases of dengue fever in travellers returning from the Comoros and East Africa early. Viraemic patients may spread the infection to regions where competent vectors are present, including the Mediterranean area and the south of Europe.
In metropolitan France, dengue fever is a mandatory notifiable disease since *Aedes albopictus* has become established in the Mediterranean French littoral in 2004 and in Corsica in 2006 [6].

*A. albopictus* were found in August 2009, in the centre of Marseille [7]. Given the intensity of population flows between the Comoros and Marseille, especially during summer, early detection of viraemic travellers and entomological surveillance are critical. The establishment of *A. albopictus*, the vector for dengue and chikungunya viruses, in the south of Europe and the presence of viraemic imported cases of dengue fever in these regions could lead to autochthonous transmission [8]. In 2007, a viraemic patient infected with chikungunya virus was the source of an outbreak of chikungunya in Emilia-Romagna, Italy, with 205 cases occurring between 4 July and 27 September 2007 [9]. So far no sustained outbreaks from imported dengue fever or chikungunya have occurred but vigilance is needed.

Our report confirms that returning travelers may serve as sentinels for local outbreaks of dengue fever in endemic areas [10]. Finally, the case presenting exclusively with fever and without additional symptoms commonly associated with dengue fever, illustrates that dengue fever should be included early in the differential diagnosis in febrile travellers particularly when returning from areas with potential transmission of the disease [11].

![Image](pe_dengue_250310.pdf)

**References**


**Table**

Clinical characteristics dengue fever in travellers returning from the Comoros and Zanzibar (Tanzania), February-April 2010 (n=5)

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever, shivering, myalgias</td>
<td>Fever, arthralgias, myalgias, diarrhoea, headache, seizures, bleeding (haematemesis, ulcer-ha, metrorrhagia)</td>
<td>Fever, shivering, arthralgias, myalgias, headaches, diffused non-petechial rash</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Onset after return (in days)</strong></td>
<td>7</td>
<td>4 (before return)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Leucocyte count/µL</td>
<td>5,280</td>
<td>2,300</td>
<td>3,200</td>
<td>4,900</td>
</tr>
<tr>
<td>Platelet count/µL</td>
<td>83,000</td>
<td>34,000</td>
<td>15,500</td>
<td>13,000</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>214 (norm 4.6)</td>
<td>257 (norm 6.5)</td>
<td>Not available</td>
<td>506 (norm 10.1)</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>125 (norm 1.9)</td>
<td>183 (norm 4.5)</td>
<td>Not available</td>
<td>191 (norm 3.2)</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>54 (norm 1.5)</td>
<td>70 (norm 2.3)</td>
<td>Not available</td>
<td>278 (norm 4.6)</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>785 (norm 1.6)</td>
<td>1,221 (norm 3.2)</td>
<td>Not available</td>
<td>2,199 (norm 3.5)</td>
</tr>
<tr>
<td>Serology</td>
<td>IgM + IgG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IgM + IgG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>IgM + IgG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>IgM&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCR</td>
<td>Negative</td>
<td>DENV-3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Not available</td>
<td>DENV-3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Notes:**

- **DENV-3:** Dengue virus type 3; **GGT:** Gamma-glutamyl transpeptidase; **LDH:** Lactate dehydrogenase; norm: normal upper value; **SGOT:** Serum glutamic oxaloacetic transaminase; **SGPT:** Serum glutamic pyruvic transaminase.
- **a** Detected by in house enzyme-linked immunosorbent assay (Associated National Reference Center for arboviruses IRBA-IMTSSA, Marseille).
- **b** Panbio ELISA IgM and IgG (rapid test dengue duo Ig M and Ig G Panbio negative); EIA Biotrin Ig M (1.7; N 1.5) and Ig G (3.6; N 1.5).
- **c** Dengue IgM specific for dengue virus was detected (54 PBU; ≥11 PBU positive) by ELISA (Dengue IgM PanBio) and high levels of dengue IgG was detected with IFI.
- **d** Detected by indirect immunofluorescence (IFI) test (Standard Diagnostics Dengue Duo) and confirmed by ELISA (Panbio Dengue DUO Test).
- **e** DENV-3 RNA was demonstrated in serum using 4 real time reverse-transcription-PCR (Taqman RT-PCR)-based assays [2].


Between 2008 and 2010, eight cases of viraemic dengue fever in travellers were diagnosed in Norway. They had returned from Eritrea, Thailand and Indonesia. All cases were primary dengue infections, seven non-complicated dengue fever and one dengue shock syndrome with a fatal outcome. Four patients were infected with dengue virus serotype 1, one with type 2 and three with type 3. Two cases from Thailand, the fatal case and the two imported from Eritrea were infected with type 1.

Introduction
Global incidence of dengue fever has increased strongly in recent decades, and dengue infections are now endemic in more than 120 countries throughout the world [1-3]. South-east Asia is the most important region of origin for the import of dengue fever into Europe [4]. In recent years, dengue virus has become a more prevalent cause of imported fever in Norwegian patients than malaria. Due to this increase, the Norwegian Institute of Public Health (NIPH) has recently proposed to the health authorities to make dengue fever a notifiable disease. Most cases diagnosed in Norway have been mild, but there have also been several cases with complicated dengue infections, including one fatal case in 2005 [5].

Of all dengue cases confirmed at the NIPH, we describe here the eight viraemic cases imported to Norway between 2008 and 2010.

Case descriptions
The eight viraemic cases reported in Norway between 2008 (n=1), 2009 (n=1) and 2010 (n=6), were imported from Eritrea (n=2), Thailand (n=4) and Indonesia (n=2). The patient’s ages ranged from 19 to 65 years, five females and three males. None of the cases had evidence of previous dengue virus infection based on their medical history and serological evidence. Seven of the cases had non-complicated dengue fever, but one patient suffered from dengue shock syndrome with a fatal outcome.

The fatal case first presented to the local health centre with a febrile viral influenza-like illness four days after returning from Thailand [6]. Nine days after returning, the patient visited the emergency centre as no relief was obtained from using paracetamol and ibuprofen, but returned home to continue ibuprofen treatment. Twelve hours later the patient was admitted to the intensive care unit, but was then suffering from circulatory collapse and died within a few hours. During the resuscitation attempts there was abnormal bleeding from the endotracheal tube and needle injection sites. Laboratory results showed a fall in haemoglobin from 15 to 7 g/dL and thrombocytopenia.

Another patient returning with dengue fever from Bali was examined for airway infections due to hoarseness and nasal congestion. *Mycoplasma pneumoniae* was detected by PCR in nasopharyngeal secretions and erythromycin tablets were prescribed. Clinical characteristics of all patients and their laboratory results are displayed in the Table.

Laboratory methods
Acute phase sera were obtained from the eight patients. Cases with no previous history of dengue virus infection and acute serum negative for anti-dengue IgG were defined as primary infections. Convalescent sera were available from only three patients and were taken 18-22 days after the acute sera. The acute samples were initially tested at the local laboratory and the positive samples were then referred to the virology laboratory at the NIPH for confirmation, except for one sample which was analysed directly at the NIPH. Infection with dengue virus was initially diagnosed in seven of the travellers by Panbio Dengue Duo IgM and IgG Rapid Strip Test (Inverness Medical Innovations, Australia) or SD Bioline Dengue NS1 Antigen and IgG/IgM tests (Standard Diagnostics, South Korea). All
### Characteristics of the viraemic cases of dengue fever in Norway, 2008–2010 (n=8)

<table>
<thead>
<tr>
<th>Case 1 2008</th>
<th>Case 2 2009</th>
<th>Case 3 2010</th>
<th>Case 4 2010</th>
<th>Case 5 2010</th>
<th>Case 6 2010</th>
<th>Case 7 2010</th>
<th>Case 8 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical symptoms</strong></td>
<td>Fever, headache, vomiting, nuchal rigidity, dyspnoea, cyanosis, hypothermia, confusion, hypotension, cardiac arrest</td>
<td>Fever, headache, fatigue, muscle aches, non-petechial rash</td>
<td>Fever, shivering, hoarseness, nasal congestion, epistaxis, non-petechial rash</td>
<td>Fever, headache</td>
<td>Fever, cough, headache</td>
<td>Fever, shivering, headache, arthralgias</td>
<td>Fever, headache, muscle aches, arthralgias</td>
</tr>
<tr>
<td><strong>Onset of symptoms after return (days)</strong></td>
<td>4</td>
<td>Symptoms started before return 1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Travel destination</strong></td>
<td>Thailand</td>
<td>Bali, Indonesia</td>
<td>Bali, Indonesia</td>
<td>Eritrea</td>
<td>Thailand</td>
<td>Eritrea</td>
<td>Thailand</td>
</tr>
<tr>
<td><strong>Initial laboratory findings</strong></td>
<td>DENV IgM+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Dengue IgM(+)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Mycoplasma pneumoniae PCR+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>DENV NS1 antigen&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DENV NS1 antigen&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DENV NS1 antigen&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DENV NS1 antigen+/IgM&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>DENV serology</strong></td>
<td>IgG- / IgM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>IgG- / IgM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Acute sample: IgG- / IgM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Convalescent sample: IgG+ / IgM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Acute sample: IgG- / IgM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Convalescent sample: IgG+ / IgM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>IgG- / IgM&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>DENV PCR+ (day of sampling after symptom debut)</strong></td>
<td>PCR+ (day 9)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>PCR+ (day of symptom debut unknown)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>PCR+ (day 3)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>PCR+ (day 4)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>PCR+ (day 5)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>PCR+ (day 4)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>PCR+ (day 6)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>DENV serotype</strong></td>
<td>DENV-1</td>
<td>DENV-2</td>
<td>DENV-3</td>
<td>DENV-1</td>
<td>DENV-3</td>
<td>DENV-1</td>
<td>DENV-3</td>
</tr>
</tbody>
</table>

DENV-1: dengue virus serotype 1; DENV-2: dengue virus serotype 2; DENV-3: dengue virus serotype 3

<sup>a</sup> Detected by Panbio Dengue Duo IgM and IgG rapid test

<sup>b</sup> Detected by SD Bioline Dengue NS1 Antigen, IgG and IgM test

<sup>c</sup> Detected by Euroimmun Indirect Immunofluorescens anti-dengue virus IgM and IgG test

<sup>d</sup> Detected by RT-PCR assays [7,8]
Discussion and conclusion
We have described eight cases of viraemic dengue virus primary infection imported from endemic areas to Norway in 2008 to 2010, one of them with a fatal outcome. All patients developed fever within four days after returning to Norway, and the serological analyses demonstrated that they suffered from primary dengue infections. Anti-dengue IgM antibodies were detected in acute samples from the fatal case, in addition to the dengue virus serotype 2-positive case from 2009 and two cases from 2010. During primary infection, IgM and IgG antibodies are usually detectable from respectively five and 14 days after onset of symptoms.

The tree is based on an approximately 300 nt fragment of the E glycoprotein gene. Sequence identification of selected dengue virus sequences is as follows: country of origin, year of isolation and NCBI GenBank accession number. The sequences determined in our study are identified by case number and country of origin. Phylogenetic analysis was conducted using MEGA 4 [12], and the tree was constructed using neighbour-joining method.
[3,9]. The fatal case highlights that fatal or severe dengue fever can also be caused by a primary infection. Severe or fatal dengue fever cases are more frequent in secondary than primary infections, but fatal primary dengue virus infection has been described in earlier reports [3,5,9].

For the reported cases, the rapid NS1 antigen tests were helpful for the initial diagnosis of dengue fever in the early phase of the disease. Our results show that dengue virus can be detected by NS1 antigen tests in patients who are negative for anti-dengue IgG and IgM.

This report shows the importance of performing dengue virus diagnostics in febrile patients returning from endemic areas even if other pathogens have been detected. Awareness of the different causes of travel-related infections and early inclusion of these in the differential diagnosis is particularly important in the context of destinations with a risk of such transmission.

Other studies have shown that most dengue virus infections diagnosed in European countries have been imported from Asia or the Americas, and in these regions all four dengue virus types have been shown to circulate [9,10]. Six of our study cases had returned from south-east Asia with dengue virus infection caused by virus serotype 1, 2 or 3. Import of dengue virus serotype 4 into Norway has so far not been reported.

Two of the cases in this study were imported from Eritrea, where only one dengue virus serotype 3 isolate has been reported earlier [10]. This country is not a specifically popular destination for Norwegian travellers and we are not aware of a concurrent outbreak in Eritrea. To date, there have been few reports of viraemic dengue fever cases imported into Europe from Africa. This may be due to underreporting in some African countries, as well as lack of adequate diagnostic tools [11]. Dengue surveillance is poorly implemented in Africa and surveillance of febrile travellers returning to Europe will add new knowledge on dengue virus distribution throughout Africa.

A comparison of sequences obtained in this study and from studies published elsewhere, are shown in the phylogenetic tree (Figure). In general, the sequence similarity between isolates of one dengue serovirus type was greater than 95%. The dengue virus serotype 1 isolates from Eritrea were closely related to dengue virus serotype 1 isolated in Kenya in 2004-5. Similarly, the dengue virus serotypes 1, 2 and 3 imported from south-east Asia in our study clustered together with the respective serotypes reported from this area earlier. To our knowledge, only few reports of dengue virus serotype 1 isolates from East Africa have been published [10], and this study provides evidence that this serotype 1 is circulating in this area.

Our report confirms that returning travellers may serve as sentinels for local outbreaks of dengue fever in endemic areas. The worldwide surveillance of dengue virus requires simple and accurate methods for the identification of virus types and is especially important since air travellers move quickly between endemic and non-endemic regions, allowing introductions of dengue virus to new areas that already are populated with Aedes mosquitoes.

References
First two autochthonous dengue virus infections in metropolitan France, September 2010

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In September 2010, two cases of autochthonous dengue fever were diagnosed in metropolitan France for the first time. The cases occurring in Nice, southeast France, where *Aedes albopictus* is established, are evidence of dengue virus circulation in this area. This local transmission of dengue calls for further enhanced surveillance, active case finding and vector control measures to reduce the spread of the virus and the risk of an epidemic.

Dengue fever is the most important mosquito-borne viral disease in the world and is endemic in Africa, Asia, Caribbean and Latin America. According to the World Health Organization, there are annually more than 50 million cases and 22,000 deaths [1]. Dengue fever is caused by viruses of the Flaviviridae family and transmitted by mosquito vectors of the *Aedes* genus, mainly *Ae. aegypti* and *Ae. albopictus* [2].

In Europe, the last dengue epidemic was reported from 1927 to 1928 in Greece with high mortality and *Ae. aegypti* was implicated as the vector [3]. Since the 1970s, mainly through global trade of car tyres, *Ae. albopictus* has become increasingly established in European Union Member States, including France, Greece, Italy, the Netherlands (though only in greenhouses), Slovenia and Spain [4]. This mosquito species is also established in neighbouring countries such as Albania, Bosnia and Herzegovina, Croatia, Monaco, Montenegro, San Marino, Switzerland and Vatican City [2,5]. Imported cases of dengue fever in travellers returning from countries where dengue is endemic or where dengue epidemics are taking place have been frequently reported in European countries in recent years [6-10].

In metropolitan France, sporadic *Ae. albopictus* mosquitoes were first detected in Normandy in 1999 [11], but the mosquito is known to have been established since 2004 in south-east France [12]. Since 2006, and the widespread epidemic of chikungunya in Réunion which had posed an increased risk of importation of cases, enhanced surveillance is implemented each year from May to November in the departments where *Ae. albopictus* is established, as part of the national plan against the spread of chikungunya and dengue viruses in metropolitan France [13]. Enhanced surveillance, compared with routine surveillance, allows the reporting and confirmation of suspected cases to be accelerated. The laboratory network surveillance system, the most sensitive routine system in France, detected around 350–400 imported dengue cases per year between 2006 and 2009 in metropolitan France [14,15]. During the same four-year period, enhanced surveillance reported a total of 33 imported dengue cases (including 11 cases in 2009). Between 1 May and 17 September 2010 (i.e. the first 4.5 months of surveillance), 120 imported cases of dengue have been reported by the enhanced surveillance system [16], which represents an 11-fold increase when compared with the entire 2009 season. This increase in imported cases is mostly related to the ongoing epidemics in the French West Indies, Martinique and Guadeloupe, since the beginning of 2010. Here we report on the two first cases of autochthonous dengue virus infection ever diagnosed in metropolitan France and the public health measures subsequently implemented.

Case 1
The first case was detected through the routine enhanced surveillance system. The patient was a man...
in his 60s, resident in Nice, Alpes-Maritimes department, who developed fever, myalgia and asthenia on 23 August 2010. He was hospitalised on 27 August 2010, but his clinical condition remained stable. A temporary thrombocytopenia with a minimal platelet count of 48,000/µL (norm: 150,000–400,000) on day five of the illness resolved without complications and he recovered within a few days after disease onset.

**Laboratory findings**

A panel of sera obtained during the acute and recovery phases on days five, seven, 11 and 25 of the illness was investigated by serological tests (in-house MAC-ELISA and direct IgG ELISA) and real-time RT-PCR. Moreover, a serum sample collected during a previous medical examination in May 2010 was tested retrospectively. Presence of IgM and IgG against dengue virus antigens was documented in all samples except for the serum sampled in May 2010. Antibody titration revealed sharp increases in IgM titres from 1:800 to 1:12,800 and in IgG titres from 1:32,000 to 1:1,128,000 over the 25 days follow-up. Anti-dengue virus IgA (Assure Dengue IgA rapid test, MP Biomedicals) were also detected on days five and seven. The dengue NS1 antigenic test (Dengue NS1 strip, Biorad) was positive on days five and seven. The dengue NS1 antigenic test was positive on day five and negative thereafter. Molecular typing identified a dengue virus serotype 1.

It is of interest to note that high levels of specific anti-dengue IgG were detected during the acute phase of disease. Our hypothesis is that these IgG might result from activation of memory B cells (original antigenic sin) related to an ancient primary infection with a heterologous serotype of dengue virus. Seroneutralisation tests will be informative on the immunological status of the patient regarding a possible previous infection with a dengue virus of another serotype. Virus isolation and sequencing are also ongoing. No serum cross-reactions were observed with tick-borne encephalitis and West Nile viruses and no markers of chikungunya virus infection were found (absence of IgM and IgG antibodies, negative RT-PCR). The patient had been vaccinated against yellow fever 28 years ago.

Friends from the French West Indies had stayed with him since April 2010. He had no recent history of international travel or blood transfusion. Consequently, the patient was considered a confirmed autochthonous case of dengue virus infection.

**Control measures**

This classification prompted an immediate reaction of public health authorities to reduce the risk of further spread of the virus. Various measures were undertaken by health authorities as laid out in the national plan against the spread of dengue in France (level 2 of the plan) [13]: (i) 200 metres perifocal vector control activities centred on the case’s residence, including spraying for adult mosquitoes and destruction of breeding sites; (ii) active case finding in the neighbourhood of the case’s residence and in other areas visited by the case; (iii) providing information about dengue virus to health professionals, including incitation for screening suspected dengue cases and information to the public. The active case finding conducted by physicians and laboratories will be continued on a weekly basis up to 45 days after the onset of symptoms of the last autochthonous case.

The routine laboratory network surveillance system noticed that six recently imported confirmed dengue cases, including four with a RT-PCR positive for dengue, had been detected in Nice between 24 July and 23 August 2010. One of them had returned from Martinique and lives about 200 metres from the autochthonous case. This imported case was reported too late to implement vector control measures which routinely follow imported viraemic dengue cases in those departments where the vector is present, and could therefore be a potential source of infection of local *Ae. albopictus*. As of 24 September 2010, the active case finding has detected nine new suspected autochthonous cases of dengue fever in the neighbourhood of the index case. In four of them, no markers of dengue virus infection were found (absence of IgM and IgG antibodies, negative RT-PCR), results from epidemiological and laboratory investigations for further four patients are still pending. One case was confirmed to be infected by dengue virus; the latter patient is the second autochthonous dengue fever case ever diagnosed in metropolitan France.

**Case 2**

This second case is an 18 year-old man who had no recent history of international travel. He lives approximately 70 metres from the first autochthonous case. He developed fever, myalgia, headache and asthenia on 11 September 2010. He was hospitalised briefly because of fever of unknown origin and thrombocytopenia with a mild clinical disease. The thrombocytopenia (platelet count 53,000/µL on day seven of the illness) was temporary and moderate, and he has recovered fully.

**Laboratory investigations**

Laboratory tests conducted on an early serum sample on day three of illness indicate negative serology for IgG and IgM antibodies but strongly positive RT-PCR for dengue virus. Molecular typing identified a dengue virus serotype 1. The strain appears to be quite similar to those which currently circulate in Martinique; more detailed analyses are ongoing.

**Discussion**

The identification of two autochthonous cases of dengue fever which are clustered in space and time is strongly suggestive that a local transmission of dengue virus is ongoing. Therefore level 3 of the national plan against the spread of dengue virus has been activated [13]. It entails additional measures to those taken
at level 2: (i) active case finding of autochthonous cases in hospital emergency wards, at present in Nice and surrounding towns, (ii) implementation of vector control measures in hospitals, together with protection of potential viraemic patients against mosquito bites using electric light traps, electric diffusers for insecticides, and repellents, and vector control measures around the port and the international airport of Nice including enhanced entomological surveillance, and (iii) toxicovigilance related to the wide use of insecticides.

Based on the currently available information, these are the first confirmed cases of autochthonous transmission of dengue fever in metropolitan France and Europe, since the epidemic in Greece in the late 1920s and apart from one nosocomial case of dengue infection reported from Germany in 2004 [17]. The event is not entirely unexpected, as reflected in a specific preparedness plan and taking into account the increase in imported cases from the French West Indies and other endemo-epidemic areas. It is known that France, as well as other countries in Europe, has competent vectors for transmitting this flavivirus. The chikungunya outbreak in Italy that occurred in 2007, with over 300 cases reported, has shown that non-endemic arboviruses can be efficiently transmitted in continental Europe [18].

Whether the transmission of dengue virus in France followed a bite from an infectious mosquito imported to the area via airplanes or boats, or one already present in the area after biting a viraemic person residing or visiting Nice, remains to be determined. However, with the second confirmed case, the latter scenario is the most likely one. Therefore, taking into consideration the longest possible incubation period for dengue fever, 15 days, it can be considered that the conditions for successful transmission of dengue virus to humans existed in Nice during August 2010. To date, only two autochthonous cases of dengue fever have been detected in Nice, but the identification of new dengue cases in the near future cannot be excluded. The enhanced surveillance and strict vector control measures are expected to limit the risk for further spread as much as possible.

At this stage, the risk for further spread to humans in Europe, as well as the possibility of the establishment of dengue virus transmission in Nice or in neighbouring areas in France, may appear limited but needs to be closely monitored. Recent evidence demonstrates that compared with *Ae. aegypti*, which has been implicated in the majority of large dengue outbreaks worldwide, *Ae. albopictus* is a less efficient vector of this virus [2]. Nevertheless, it was involved in outbreaks in Japan from 1942 to 1945 [19], the Seychelles in 1977 [20], Hawaii from 2001 to 2002 [21] and Réunion island in 2004 [22]. Vertical transmission of dengue virus from mosquitoes to their offspring does not seem very efficient, and therefore overwintering of the virus in continental European *Ae. albopictus* populations is unlikely [2] but cannot be excluded [23,24]. The public health consequences of the presence of *Ae. albopictus*, in this context, appear to be more important for the transmission of chikungunya for example, for which experimentally better competence has been demonstrated, although the competence of local *Ae. albopictus* for dengue virus is far from being negligible [25]. It should also be noted, that the currently affected area of France as well as other countries in Europe is faced with a high number of imported dengue cases every year. However, despite this and established mosquito populations being potentially able to transmit arboviral diseases, local transmission of the dengue virus with *Ae. albopictus* as the vector in mainland Europe has never been observed before this reported emergence in the south-east of metropolitan France. The high vector density in Nice and the increase in the number of imported cases in this area in 2010, mainly due to intense epidemics in the French West Indies, are two major factors to explain this emergence and highlight the need to maintain an appropriate active surveillance.

In terms of blood safety, reported dengue infection following blood transfusion in dengue endemic areas is rare [26-28] but is also difficult to detect as a large proportion of the population would already have antibodies against the virus. However, as dengue infection is mild or asymptomatic in 40–80% of infected persons, depending on the area and the epidemiological context [29-31], it does pose a risk to blood safety. The two identified cases in Nice are suggestive that other infected persons may have lived in the city during the same period of exposure, without showing any symptoms. Asymptomatic carriers of dengue virus could pose a potential risk to blood safety if they donate blood while being viraemic. It is possible however, that the duration of viraemia in mild or asymptomatic cases is shorter and the virus titre is lower than in symptomatic persons, but this hypothesis is far from proven. Moreover, the limited extend of current virus dissemination, as shown by the actual clustering of confirmed autochthonous cases, does not indicate that such asymptomatic infections could have been spread around the whole city of Nice. At present, it is difficult to quantify this risk, and only a retrospective survey of blood supplies from Nice between July and September 2010 would allow to estimate it better. In France, authorities in charge of blood routinely exclude all febrile donors from donation. No additional exclusion measures have been implemented after the two neighbouring cases as the risk for dengue transmission has been considered very low.

Further investigations to identify the likely source of exposure of the two cases, as well as extensive comparison of the dengue virus genotypes between the locally identified viruses and the strains currently circulating in the French West Indies, will hopefully allow a better understanding of this event. The reactive surveillance in addition to the routine enhanced surveillance...
is likely to identify new symptomatic cases in the area, determining also the potential geographic extension of the risk. Finally, better understanding is needed on how the vector abundance, activity and competence of Ae. albopictus for dengue transmission influence the risk for further transmission in the region [25,32].

Conclusion
The current clustering of cases of locally transmitted dengue fever in Nice is a significant public health event, but is not unexpected and more cases can be predicted. Such transmission was anticipated by the development of a national plan. Although this plan should be adjusted in the light of this experience, this event shows the advantage of such preparedness in order to implement rapid and proportionate measures of surveillance and control. Previous events, including a mosquito-borne arbovirus outbreak in Italy, the occurrence of vector-borne diseases around airports and other ports of entry and a previous risk assessment on dengue virus introduction in European Union countries [4] indicate that autochthonous transmission in continental Europe is possible, as confirmed by the present event. However, according to the available epidemiological information, the risk for establishment of dengue transmission in south-eastern France or further spread in Europe currently appears limited. Further data available in the near future will allow us to re-assess this likelihood.

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Dengue virus (DENV) is endemic in south-east Asia and Central to South America. In August 2010, a DENV infection was diagnosed in a German traveller returning from a trip to Croatia in south-east Europe. The patient presented with fever and other typical symptoms of DENV-infection. Virological investigation revealed the presence of DENV-specific IgM, a rise in DENV-specific IgG and the presence of DENV NS1 antigen in the patient’s blood.

Dengue virus (DENV) is an arthropod-borne RNA virus of the Flaviviridae family causing dengue fever in humans. Since 2001 dengue fever has been mandatory reported to the German public health authorities, in accordance with the Federal Protection against Infection Act [1]. According to German notification data, between 60 and 387 imported DENV infections are reported annually (Table).

The DENV infections in imported cases are mainly acquired in south-east Asia as well as South and Central America. Very recently, autochthonous DENV infections were reported in southern France, diagnosed for the first time ever in Europe [2]. Here we report on a case of DENV infection that was apparently acquired in Croatia and imported to Germany by a traveller.

**Case report**

A 72-year-old man from Germany visited Croatia in August 2010: he left on 1 August and returned on 15 August. He was accompanied by seven family members, including grandchildren. The family travelled by car from Germany via Austria and Slovenia to Croatia without overnight stops. The group stayed the entire time around Podobuce close to Orebić on the Peljesac peninsula and on the isle of Korčula in the south of Croatia. Podobuce and Korčula are located approximately 100 km north-west of the city of Dubrovnik, which was also visited. Temperatures were reported to be very high (approximately 30 °C at night). After returning to Germany, on 16 August, the patient developed a febrile illness with a temperature of up to 39 °C, chills, arthralgia, headache, and retro-orbital pain. Following a short period of improvement, his temperature rose again to 39 °C on 21 August, and he continued to have arthralgia, myalgia, weakness and dyspnoea. Among several other diseases, dengue fever was suspected by the general practitioner, because of the clinical picture.

**Laboratory results**

Serum samples were taken from the patient for virological investigation on 23 and 30 August and on 2 September. The sample from 23 August was positive for DENV-specific IgM, but negative for IgG in an enzyme-linked immunosorbent assay (ELISA). On 30 August, DENV-specific IgM and IgG was positive with a titre of 1:2,560 (cut-off 1:20) and 1:80 (cut-off 1:20), respectively, in an indirect immunofluorescence assay based on DENV-infected cells. In addition, the serum sample tested positive for DENV NS1 antigen (Dengue Early ELISA, Panbio). Real-time reverse transcription-polymerase chain reaction (RT-PCR) for DENV [3] was negative. The detection of DENV NS1 antigen and the simultaneous absence of DENV RNA during this
phase of dengue fever are in line with previous studies demonstrating an acute DENV infection [4]. The sample taken on 2 September showed an increase in the DENV-specific IgG titre (1:1,280), while the IgM titre remained unchanged and the ELISA for NS1 antigen was negative. In this sample, immunofluorescence assay titres for related flaviviruses were lower than for DENV: West Nile virus (IgM negative, IgG 1:160) and tick-borne encephalitis virus (IgM 1:80, IgG 1:80). The patient did not report vaccination against tick-borne encephalitis or yellow fever. A temporary thrombocytopenia with a minimal platelet count of 97,000/µl (norm: 150,000–440,000/µl) on the eighth day of the illness resolved without complications and the patient recovered within two weeks after disease onset.

Conclusions
The clinical suspicion of dengue fever was confirmed by the laboratory tests. As the incubation period for dengue fever ranges from three to 14 days, the infection was probably acquired in southern Croatia and not en route. The Croatian authorities were given all available information about the case, enabling them to investigate this further at local level. To our knowledge, this is the second report on an autochthonous DENV transmission in Europe after France. Antibodies against DENV have been previously detected in Croatian individuals in the context of international travel; however, the specificity of the assay is questionable [5]. The presence of *Aedes albopictus* as a potential DENV vector in Croatia [6] and the importation of confirmed dengue fever cases from endemic areas into Croatia [7,8] allow autochthonous DENV transmission within this country. The mosquito season in parts of the northern Mediterranean coast may last from May to November. Therefore, dengue fever should be considered in patients with fever of unknown origin and relevant clinical symptoms who stayed in areas in Europe where *Ae. albopictus* occurs.

Acknowledgements
We thank M. Reichmann, B. Noack, S. Kunstmann, P. Emmerich, B. Braunsdorf and B. Hiller for preparation of additional clinical, laboratory and epidemiological data related to this case.

References
We report on a cluster of relapsing vivax malaria among Eritrean refugees residing in Israel. Since the beginning of 2010, 15 cases have been identified. Five of the six patients who had complete medical and epidemiological histories, reported Sudan as the place of primary infection during their journey to Israel, and having had the first relapse in Israel, six months later (median). Suggested place of exposure is the region of the Eritrean refugee camps in Sudan.

Introduction
Malaria, once endemic in Israel, was eradicated almost 50 years ago, although its vectors, several malaria-transmitting species of Anopheles mosquitoes, still exist in various parts of the country [1,2]. Every year between 60 and 100 imported cases of malaria are reported to the Ministry of Health. Most of these cases are travellers returning from endemic countries to Israel and only few of them are immigrants from Sub-Saharan Africa [3].

Currently, there is a cluster of relapsing Plasmodium vivax malaria among Eritrean refugees in Israel. The epidemiological investigation, which is summarised here, was conducted by the local health office in Tel Aviv and is therefore limited to the Tel Aviv district.

Methods
On 7 June 2010, a cluster of five malaria cases was reported to the Tel Aviv District Health Office. These cases were Eritrean refugees under treatment in the same hospital during the first week of June. An epidemiological investigation was initiated. The case definition was laboratory-confirmed malaria excluding returning travellers.

The following investigation measures were taken.

- Species were identified by thick and thin smears with confirmation by real-time reverse-transcriptase PCR [4].
- Medical records of cases were obtained from hospitals.
- Oral interviews were conducted with four cases by a native speaker of Tigrinya (one of the two working languages in Eritrea). The interviews were based on a short epidemiological questionnaire that contained questions on demographics, the route of the journey to Israel, current and past malaria illness history and possible exposures (time, place, mosquito bites) in Israel or abroad.
- The local health office provided records on past epidemiological data regarding non-traveller cases of malaria in the district since 2006.
- An alert was issued to the hospitals and laboratories in the district, which were requested to report all cases of diagnosed malaria and to confirm them at the national reference laboratory.
- With the assistance of an expert malaria advisor, concise clinical guidelines for proper management [5,6] of cases were promptly issued to local infectious diseases units at all hospitals in the district.

Results
Five cases of non-traveller malaria were reported in the district from 2006 to 2009 (Figure). All of these cases had been imported, three of whom were refugees from Eritrea. Since the beginning of 2010, 15 cases, all of them Eritrean refugees, have been reported. Most of them (nine of 15) were diagnosed in the first two weeks of June 2010. No other cases of imported malaria in other migrants were documented during this period of 2010 in the district.

Twelve cases were male (80%) and the median age was 25 years (interquartile range (IQR): 21.5–29 years). All the cases had laboratory-confirmed P. vivax infection. Low parasitaemia, ranging from <0.1% to 1% at the time of diagnosis, was demonstrated for 12 patients for whom these data were known.

All 15 patients presented with similar clinical characteristics of intermittent fever which was usually accompanied by shivering and headaches. Eleven patients had mild to moderate anaemia, usually normocytic, and four of 15 patients had splenomegaly.

Thirteen of the 15 cases for whom the onset of illness and date of arrival to Israel were known had arrived in Israel during the six months before that date.
(median: 2.8 months, IQR: 0.7–4.4 months). Ten of the 15 cases had information on previous malaria attacks and reported having had one during the journey to Israel. For six of these 10 it was known that this previous attack occurred in Sudan, within the two-month period before arriving in Israel, of whom five recalled it was the primary infection. A further patient of these 10 reported a one-month period between the previous attack on the journey and arrival to Israel and another patient had the previous attack in Ethiopia, 12 months before arrival to Israel. An additional of the 15 patients, a 2.5-year-old female baby, had the first attack in Israel, approximately one month after her arrival through Sudan. No data on any previous attacks and their location were available for the last four of the 15 patients.

The median time interval between attacks was 6.1 months (IQR: 4.0–8.1 months, n=9). Five cases had received partial, inadequate or no treatment at the time of their previous attack, whereas no complete past medical history was available for the rest of the 15 patients.

The probable place of exposure of four patients who have been interviewed to date, was a complex with three refugee camps in Shagrab, operated by the United Nations’ Refugee Agency, in southeastern Sudan, near the Eritrean border. In addition, the reported duration of stay in these camps was between one and two months before continuing directly to the Israeli border, by organised smuggling [7], apparently without significant stations of stay in neighbouring Egypt.

**Discussion**

We describe a cluster of relapsing vivax malaria among Eritrean refugees who had recently arrived in Israel. The fact that this cluster is mostly limited to young men probably reflects the overall current composition of Eritrean refugee population arriving in Israel. During the last several years until the end of 2009, 9,517 Eritrean refugees, who constituted 48.6% of all asylum seekers, entered Israel. Since 2009, the Eritrean refugees have gradually become the predominant group of asylum seekers who enter Israel from the Sinai desert (Egypt). In the first four months of 2010, 3,793 Eritrean refugees entered the country and constitute 82% of all asylum seekers who entered in this period [8].

These figures represent a 40% increase in the Eritrean refugee population in Israel during the first four months of 2010 alone. In addition, a more than fourfold increase in the crude incidence rate of non-traveller malaria in Israel was observed in the same period: in 2009, the crude incidence rate was 0.77 cases per 1,000 migrants, while in the first half of 2010, it was 3.58 cases per 1,000 migrants*. These estimated calculations are based on data from the National Department of Epidemiology in the Israeli Ministry of Health and from an analysis report of the Knesset Research and Information Centre [8]. Consequently, an overall increase in malaria activity along the refugee route in Africa must have played a significant role in this cluster.

Therefore, it is reasonable to assume that a place on the journey is a recent common place of exposure. This place was most probably in Sudan rather than Eritrea as the lowland of Eritrea is mostly affected by *P. falciparum* and not by *P. vivax* [9]. Furthermore, a dramatic decline in the incidence rate of malaria was observed in the recent years in Eritrea, due to successful eradication programmes [10]. In fact, almost all cases who had complete medical and epidemiological history, namely a third of the total number of cases, specifically recalled having had the first malaria attack during their stay in Sudan, which was usually a period of two months before arriving to Israel.

Moreover, this cluster may actually reflect the expanding prevalence of a previously reported [11] small focus of vivax malaria in Sudan: 10 of 83 blood samples were PCR-positive for *P. vivax* (on the background of predominant *P. falciparum* in this area) in some of the Eritrean refugee camps in eastern Sudan, a frequently flooded low plain region in which malaria may have remained the leading cause of morbidity and mortality.

**Conclusions**

The evident ongoing rise of human reservoirs of malaria in the region may potentiate the risk for the re-emergence of locally acquired mosquito-transmitted malaria in Israel and neighbouring countries. This warrants tight national surveillance for new cases, proper clinical management and follow-up of current cases, and effective control measures of the local *Anopheles* vectors. In addition, it highlights the need for increased malaria surveillance in the refugee camps of eastern Sudan.

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* Number of cases until 15 June 2010. Two additional cases registered outside the district of Tel Aviv are not shown in the figure.
Acknowledgements

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*Authors' correction
The following correction was made on 5 July 2010 on request of the authors: in the Discussion section, the second sentence of the second paragraph: ‘in 2009, the crude incidence rate was 0.77 per 1,000 cases, while in the first half of 2010, it was 3.58 per 1,000 cases.’ was replaced with the following sentence: ‘in 2009, the crude incidence rate was 0.77 cases per 1,000 migrants, while in the first half of 2010, it was 3.58 cases per 1,000 migrants.’

References

First autochthonous malaria case due to *Plasmodium vivax* since eradication, Spain, October 2010

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In October 2010, one case of autochthonous malaria due to *Plasmodium vivax* was diagnosed in Spain. The case occurred in Aragon, north-eastern Spain, where the vector *Anopheles atroparvus* is present. Although the source of infection could not be identified, this event highlights that sporadic autochthonous transmission of vector-borne diseases in continental Europe is possible and calls for enhanced surveillance and vector control measures.

**Background**

Malaria is a mosquito-borne parasitaemic disease caused by parasites of the *Plasmodium* genus and endemic in Africa, Asia, Central and South America. According to the World Health Organization (WHO), there were 247 million cases of malaria and nearly one million deaths worldwide in 2008, mostly among children living in Africa [1]. Four species of *Plasmodium* have long been recognised to infect humans in nature: *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Recently, the simian parasite *P. knowlesi* has been found as a cause of human malaria in some areas of south-east Asia [2]. Worldwide, *P. falciparum* and *P. vivax* are the most common causes of malaria. The malaria parasites are transmitted by female *Anopheles* mosquito vectors. Of the approximately 430 *Anopheles* species, only 20 species are important for transmission.

Infection with malaria parasites may result in a wide variety of symptoms, ranging from absent or very mild symptoms to severe disease and even death in the case of *P. falciparum* malaria. The main symptoms of malaria include episodes of cyclical or irregular fever, chills, headache, weakness, vomiting and diarrhoea. The incubation period in most cases varies from seven to thirty days after the infective mosquito bite.

In *P. vivax* malaria, the incubation period usually ranges from 10 to 21 days and sometimes up to a year. Unlike *P. falciparum* malaria, *P. vivax* malaria is rarely fatal. However, for *P. vivax*, clinical relapses may occur weeks to months after the first infection. These new episodes arise from dormant forms in the liver, and special treatment with primaquine – targeted at these liver stages – is mandatory for a complete cure.

**Situation in Europe and Spain**

Within the WHO European Region, six countries reported autochthonous malaria infections in 2008 caused by *P. vivax*: Azerbaijan, Georgia, Kyrgyzstan, Tajikistan (the only country in the Region reporting *P. falciparum* malaria), Turkey and Uzbekistan [3]. In the European Union (EU) and European Economic Area (EEA) countries, malaria has been eradicated since 1975 and nearly all reported malaria cases are imported. In 2008, 5,848 malaria cases were reported; the vast majority of cases for which the species was known were caused by *P. falciparum* (78%) while less than 10% were caused by *P. vivax* [4]. During the last 10 years, less than 20 cases of autochthonous transmission of malaria have been reported in the EU/EEA [3,5]. Despite the presence of potential anopheline vectors in some countries, sustained local transmission has not been identified in continental EU countries [5].

In Spain, the last autochthonous case of malaria was reported in 1961 [6] and malaria was officially declared eradicated in 1964. According to the Spanish National Surveillance Network, an average of 400 imported malaria cases are reported each year (with less than 5% due to *P. vivax*). The Spanish population is susceptible to malaria infection given the absence or disappearance of the immunity acquired in the past by contact with the parasite.
The principal potential anopheline vector of malaria in Spain is *Anopheles atroparvus* which is widely distributed throughout Spain (Figure) and can transmit Asiatic strains of *P. vivax* but is refractory to African strains of *P. falciparum* [7].

**Case report**

On 5 October 2010, the Regional Health Authorities of Aragon reported to the Coordinating Centre for Health Alerts and Emergencies at the Spanish Ministry of Health one laboratory-confirmed case of *P. vivax* malaria in a patient in their 40s living in the province of Huesca (Region of Aragon). The patient had developed fever on 20 September 2010 and was diagnosed on 25 September with acute tonsillitis and started treatment with amoxicillin and ibuprofen. Four days later, the patient was hospitalised because of clinical deterioration with fever and jaundice. On the same day, *Plasmodium* spp. parasites were detected in the blood smear, and antimalarial treatment with chloroquine and primaquine was initiated. On 1 October the patient was dismissed in good clinical condition.

**Laboratory results**

Detection of macrocytosis on the first blood sample taken upon hospital admission led to a Giemsa staining where *Plasmodium* spp. parasites were unexpectedly identified. Further tests (Rapid Test Binax, chromatography) diagnosed *Plasmodium* spp. (non-*falciparum*). On 4 October, the National Centre for Microbiology in Madrid (National Reference Laboratory) confirmed the presence of *P. vivax* by microscopy and multiplex PCR. Genomic analysis of the parasite is still ongoing.

**Epidemiological investigation**

According to the epidemiological investigation, the patient did not have any travel history to an endemic/epidemic area ever, or contact to persons visiting or residing in such areas. There was no history of surgeries, invasive examinations or diagnostics, or blood transfusions. The patient was never an injecting drug user or had any treatments involving injections. The patient reported two visits to airports, Barcelona airport in summer 2008 and Zaragoza airport in summer 2009.

In the vicinity of the patient’s residence there were swine exploitations and was frequently exposed to mosquito bites. Furthermore, the patient lives in an area of the province of Huesca where *An. atroparvus* is present in several nearby localities. No malaria cases have been reported amongst the case’s contacts or residents in the locality. There have been no reports of imported malaria cases from this area in recent years, including 2010.

**Control measures**

The implemented control measures included testing household members for malaria, active case finding in the neighbourhood of the case and through alerting healthcare centres (including hospitals) in the area, as well as entomological survey and vector control. The entomological survey carried out so far has not proven the presence of *Plasmodium* parasites in local mosquitoes.

**Risk assessment for Spain**

Although the investigation was very detailed, we have not been able to identify the source of infection. Ongoing genetic analysis of the parasite may help to specify its possible origin. Transmission may have occurred through local *Anopheles* species after infection from people coming from endemic areas carrying gametocytes in their blood. Airport malaria caused by infected mosquitoes imported from endemic areas seems improbable due to the distance to the next international airport (approx. 100 km) and the limited flight range of local anophelines (4.5 km).

The possibility of a secondary case originating from the reported case is unlikely as the patient has been treated, comprehensive control measures have been implemented, and the person had never donated blood.

In Spain, the situation following the eradication of malaria in 1964 is defined as ‘anophelism without malaria’ with the presence of potential vectors for the parasite (mainly *An. atroparvus*, which is a species refractory to *P. falciparum*) and environmental conditions favourable for the breeding, development and permanence of the vector [7]. The risk for local transmission of malaria will depend on the presence of parasitaemic individuals and competent vectors at a given time and place. This risk is reduced by early and appropriate detection and treatment of cases and vector control activities in place. However, it is still possible that other sporadic autochthonous cases could still be identified.
Conclusions

Given the described conditions in Spain, an autochthonous case of malaria is not unexpected. Previous events, including the occurrence of several emerging vector-borne disease outbreaks in different countries in Europe, indicate that sporadic autochthonous transmission of vector-borne diseases in continental Europe is possible [9-11].

The available epidemiological information does not suggest that there is a risk for human health in the area. The epidemiological investigation suggested that this was a sporadic case with no evidence of further local transmission. With the current information, this event does not pose a significant risk to EU/EEA citizens. Despite the fact that autochthonous cases have been reported sporadically in the EU in the past, such cases never resulted in established local transmission involving more than a few cases.

Given the presence of competent vectors for malaria in the EU, we cannot exclude similar events in the future. Continued monitoring of the situation in areas where *Anopheles* mosquito populations are present is needed, including increased awareness among clinicians, to rapidly identify and report suspected malaria cases to respective authorities, and ensure an appropriate public health response.

References

Infection with Mayaro virus in a French traveller returning from the Amazon region, Brazil, January, 2010

Mayaro virus (MAYV) disease is a mosquito-borne zoonosis endemic in humid forests of tropical South America. MAYV is closely related to other alphaviruses that produce a dengue-like illness accompanied by long-lasting arthralgia. A French tourist developed high-grade fever and severe joint manifestations following a 15-day trip in the Amazon basin, Brazil, and was diagnosed with MAYV infection in January 2010. This case is the first reported in a traveller returning from an endemic South American country to Europe.

Introduction
Mayaro virus (MAYV) (family Togaviridae, genus Alphavirus) is an arthropod-borne zoonotic pathogen circulating only in tropical South America [1]. The transmission cycle of MAYV in the wild is nearly similar to the continuous sylvatic cycle of yellow fever and is believed to involve wild primates (monkeys) as the reservoir and the tree-canopy-dwelling Haemagogus mosquito as the vector. Thus, human infections are strongly associated with recent exposure to humid tropical forest environments [1,2]. MAYV disease is an acute, self-limited dengue-like illness of three to five days’ duration. Moreover, MAYV is closely related to chikungunya virus and produces a nearly indistinguishable, highly debilitating arthralgic disease [1-3].

Here we report the case of MAYV disease that recently occurred in a French citizen who presented with severe rheumatologic disorders after visiting the Brazilian Amazon. This report illustrates that with increasing travel to remote areas, travellers are at risk of acquiring and importing rare diseases that are not indigenous to Europe.

Case report
The patient, a man in his late 20s, came to the travel clinic of the Department of Internal Medicine and Tropical Diseases of the University Hospital Centre, Bordeaux, France on 4 January 2010 with persistent incapacitating arthralgia for a two-month period and predominating in his knees and joints of the hands.

FIGURE
Timeline for travel history and symptoms in a French traveller with Mayaro virus disease, October 2009 - January 2010

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The patient had travelled in the Amazon forest region for two weeks in October and November 2009 for the purpose of fishing and butterfly hunting. He stayed for two days in Manaus, Amazonas, Brazil, and for a further two days at Barcelos, Amazonas, north-western Brazil, before travelling in a dugout canoe along the Rio Negro River for ten days to the confluence area of the Demini River with the Araca River, a forest place situated 70 miles north of Barcelos. After another two days in Barcelos, he returned to France via Manaus and Sao Paulo (Figure).

During his second stay in Barcelos, in early November, he developed symptoms assumed to be related to dengue virus infection, with high-grade fever, headache, generalised myalgia and diffuse arthralgia. Macular and partially confluent transient exanthema mainly on his arms appeared around the fifth day of illness. After his return to France, the patient had increasingly difficulty walking and was severely impaired in daily activities because of severe recurrent joint pains.

The patient had received yellow fever vaccine 10 years before. During the trip to the Amazon, he had taken doxycyclin as prophylaxis for malaria.

When he presented to our centre on 5 January 2010 (two months after onset of symptoms), the patient complained of persistent headache, myalgia and severe symmetrical joint pains (wrists and ankles). At the time of presentation, laboratory tests showed a leukocyte cell count of 6,600 cells/µL and a thrombocyte count of 177,000 platelets/µL. No markers of autoimmunity were found, notably anti-citrullin peptide antibodies or anti-nuclear antibodies. He was negative for the major histocompatibility complex HLA B27 gene. Concurrently, serologic status for dengue, chikungunya and yellow fever viruses as well as MAYV was evaluated using IgM capture and IgG sandwich ELISA at the National Reference Centre for Arboviruses, Institut Pasteur, Paris. Serology for MAYV revealed positive results for specific IgM (optical density [OD]=0.34; serum control OD=0.122). OD values for specific IgG were negative (OD=0.082; serum control OD=0.092). The other serological results were negative, as well as tests for leptospirosis, rickettsiosis, Q fever, cytomegalovirus and Plasmodium falciparum malaria. Five weeks later, on 8 February 2010, MAYV antibody serology showed persistence of specific IgM (OD=0.494; serum control OD=0.116) and a lack of immunoglobulin switching from IgM to IgG (OD for IgG=0.076; serum control OD=0.084).

The patient recovered completely, although severe joint pain persisted for eight further weeks until 10 April despite symptomatic treatment. The diagnosis of a presumptive case of MAYV infection diagnosed by serology was established.

Discussion

To the best of our knowledge, this case is the first published report of MAYV disease in a traveller returning to Europe. The presenting symptoms and signs were almost identical to those reported in previous clinical descriptions of the disease [2,4,5]. In this case, the decision to test for a rather exotic virus such as MAYV was based on several factors: the patient’s detailed travel history in tropical South America, which allowed risk factors to be identified such as potential exposure to vectors carrying diseases endemic in that area; the clinical presentation with incapacitating arthralgia following acute febrile illness; and finally, the expertise and technical tools available in the specialist clinic for tropical medicine where the patient was treated. Other viral infections with similar clinical presentation and geographical distribution were ruled out by laboratory tests.

The case illustrates the challenge of clinically differentiating MAYV disease from classical dengue fever and other febrile exanthematous diseases that also circulate in South America, as well as the role of laboratory confirmation in establishing a correct diagnosis. Indeed, dengue fever was initially suspected considering its occurrence in most cities and places on tropical America, including the Amazon basin. The pathogenesis of debilitating symptoms in MAYV disease is still a poorly understood phenomenon [5], although persistent infection of synovial macrophages has been documented for other closely related and also arthritogenic alphaviruses [6]. The results of serological studies of the two consecutive convalescent-phase serum samples showed that the patient did not seroconvert with a switch from IgM to IgG. In most acute arboviral infections, IgM class-specific antibodies are generally no longer detectable after a period of 6-12 months post infection [7,8]. Considering the period for seroconversion in MAYV infection, we can therefore assume that the time between disease onset and the last late-phase blood sampling in this patient was not long enough for to allow Ig class switching.

Interestingly, this report highlights the need for increased awareness MAYV disease as a differential diagnosis in travellers or migrants returning from endemic areas of tropical South America with febrile illnesses involving peripheral rheumatism and persistent arthralgia. Finally, it illustrates how travellers can act as signals for alert that can provide insights into the risk of transmission of infections in certain geographical areas.

References


**Rapid Communications**

**Epidemiological analysis of mosquito-borne Pogosta disease in Finland, 2009**

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Citation style for this article:

Pogosta disease is a viral disease caused by a mosquito-borne alphavirus, Sindbis virus (SINV), and large human outbreaks of SINV infection have emerged in Finland every seven years. After a major outbreak in 2002 an epidemic was expected to take place in 2009. Data from the National Infectious Disease Registry showed a small outbreak in humans in 2009 with a total of 105 reported cases but the seven-year cycle did not recur as anticipated.

**Introduction**

Sindbis virus (SINV) is a mosquito-borne alphavirus (of the family Togaviridae), present in Eurasia, Africa and Oceania [1,2]. Antibodies to SINV are detected in humans in various geographical areas but clinical infections caused by SINV are reported mostly from Finland where SINV is associated with fever, rash and arthritis, known as Pogosta disease [3]. The treatment is symptomatic. Clinically similar diseases are found in Sweden (Ockelbo disease) and in Russia (Karelian fever) [4,5]. The majority of clinical cases occur in Finland during August and September when the primary vectors, the ornithophilic late summer mosquito species Culex and Culiseta, are abundant. The incidence of Pogosta disease has been highest in the eastern parts of Finland in recent decades.

Remarkably, outbreaks of Pogosta disease have thus far emerged every seven years since the first outbreak was noted in 1974, and the cause for this phenomenon is yet to be discovered. Tetraonid birds such as grouse, might contribute to this pattern [3]. Grouse have previously shown population cycles with population “crashes” coinciding with SINV outbreaks [7]. Antibodies to SINV have been detected in grouse and migratory birds [3,6].

The last major epidemic in Finland took place in 2002 with almost 600 reported cases and it was anticipated that an outbreak would occur again in 2009. This paper describes the characteristics of the Pogosta disease in Finland from June through October 2009 and discusses the findings in relation with the previous epidemic.

**Methods**

Since 1995, all confirmed diagnoses of SINV infection have been reported to the National Infectious Disease Registry (NIDR) at the National Institute for Health and Welfare (THL). Notifications include information on date of sample collection, date of birth, sex, and on place of treatment. Multiple notifications of persons with the same date of birth, sex and place of treatment received within a 12-month period were combined as one case. The place of treatment refers to the health care center or hospital (in particular hospital district) where the diagnosis has been made. Data were analysed by sex, age, week and month of disease onset.

**Figure 1**

Number and incidence rates of laboratory confirmed Sindbis virus cases, Finland, 1995-2009 (n=3,041)
and by hospital district of treatment. Finland has a population of 5.3 million and is divided into 20 hospital districts. Laboratory diagnosis is based on enzyme immunoassays (EIA) and/or in some cases a haemagglutination inhibition test (HI) (5).

**Results**
From June through October 2009, a total of 105 laboratory confirmed cases were reported to the NIDR and the incidence of SINV infection was two cases per 100,000 inhabitants per year (Figure 1). Most of the cases occurred in September (n=60) followed by August (n=33). Sixty percent (n=63) of the cases were females. The highest incidence (4.6/100,000/year) was among persons aged 50-59 years. Only two of the cases were aged under 18 years.

**Figure 2**
Number and incidence rates of laboratory confirmed Sindbis virus cases, by health care districts, Finland 2009 (n=105) and 2002 (n=597)

**Figure 3**
Sindbis virus cases in north Karelia and central Finland, 2009 (n=29) and 2002 (n=212)
The incidence was highest in north Karelia, followed by east Savo, central Ostrobothnia and central Finland together with southern Ostrobothnia (Figure 2). The incidence rates in 2009 were considerably lower than those in 2002.

The number of cases was highest in central Finland (n=15). The majority of the cases (n=10) in central Finland occurred in July-August whereas only one case was reported from north Karelia during this time period (Figure 3). On the contrary, 13 cases were reported from north Karelia and five from central Finland during the months of September-October. In 2002, cases peaked in September in both of the hospital districts.

Discussion

A major Pogosta disease outbreak has occurred every seven years in Finland since 1974, with hundreds or even thousands of patients. Following this pattern, another outbreak was expected for 2009. However, the number of cases was substantially lower than in previous epidemics in 1995 and 2002 when 1301 and 597 cases were reported respectively [6]. The 105 cases reported in 2009 exceed the average number of cases (n=77) in the non-epidemic years during 1995-2009. However, in some intermediate years, 1997-1998, 2000 and 2003, the number of cases exceeded the number reported in 2009. In comparison, five SINV infections were reported in Sweden in 2009 (Sirkka Vene, personal communication, 1 December 2009).

The factors behind the puzzling cycles in the epidemiology of Pogosta disease are unclear but recently attention has focused on tetraonid birds. In the epidemic years of 1974 and 1981, grouse population crashed in north Karelia [4]. Further, detection of SINV antibodies in one quarter of grouse examined in the year following the 2002 epidemic, indicated vast exposure of grouse to SINV, and suggested that the virus may have an endemic cycle in tetraonid birds [6]. The density of grouse was above average in 2007 but the population crashed in 2008. The recovery of grouse population can be rapid and was anticipated to occur in 2009. However, the density of their population continued to decline to an all time low (since the measurements from 1980s) [8]. Hence, it is plausible that grouse play a significant role in the human epidemiology of SINV. It is possible that the continuing decline in the grouse population in 2009 diminished the role of grouse as amplifying hosts and that therefore, a milder outbreak than expected was observed.

Similar to previous findings, the incidence of SINV was highest in the hospital district of north Karelia. The difference in incidence of this hyperendemic region with other hospital districts was not, however, as prominent as previously. In 2009 many of the hospital districts with high incidence were located in central and northwestern parts of Finland. These observations may point towards a geographical shift in the incidence of SINV virus infection. The relatively low incidence in north Karelia compared to the epidemic in 2002 may also reflect the increased human seroprevalence towards SINV which indicates immunity. The district of Kainuu had no cases, which was surprising since previous studies showed high seroprevalence in that area [6].

This could be attributable to significant underdiagnosis or acquired immunity, which may result from high number of cases during the 1970s and 1980s.

Most cases in central Finland occurred in August whereas in north Karelia the number of cases peaked in September. This could reflect the variations in mosquito activity and population size due to differences in weather conditions in these areas. The month of May was drier than normally in Joensuu (the largest city in north Karelia) but the rainfall in June-July was considerably higher than on average [9]. Perhaps the dry May in Joensuu contributed to fewer cases in July-August but the high rainfalls in June and July created better environmental conditions for mosquito development and thus, more human cases of SINV occurred in September-October. Weather conditions are also likely to influence human outdoor activities, and thereby exposure to SINV.

In summary, a limited outbreak of SINV in humans took place in Finland in late summer and autumn of 2009 but the expected seven-year cycle did not recur. The data suggest a geographical shift in disease incidence. It is likely that fluctuations in grouse populations play a major role in the occurrence of SINV epidemics. To further elucidate the role of tetraonid birds and other factors, such as weather and climate variations, more epidemiological studies and proper mathematical modeling of SINV epidemics is needed.

References

Between early July and 22 August 2010, 81 cases of West Nile neuroinvasive disease were reported in the region of Central Macedonia, northern Greece. The median age of cases was 70 years. Encephalitis, meningoencephalitis or aseptic meningitis occurred mainly in patients aged 50 years or older. This is the first time that West Nile virus (WNV) infection has been documented in humans in Greece. Enhanced surveillance and mosquito control measures have been implemented.

Introduction
On 4 August 2010, physicians from the Infectious Disease Hospital in Thessaloniki, northern Greece, informed the Hellenic Centre for Disease Control and Prevention (KEELPNO) about an increase in the number of hospitalised cases with encephalitis in the previous month (13 patients with encephalitis were hospitalised in July 2010, compared with a mean of five hospitalised cases in the same month of the previous three years). Despite several laboratory tests, no aetiological factor had been identified. Most patients were elderly (over 65 years of age) and resided in the region of Central Macedonia, northern Greece. On the same day, 11 serum and three cerebrospinal fluid (CSF) specimens from 11 patients with encephalitis and/or aseptic meningitis were sent for further testing to the Reference Laboratory for Arboviruses at the Aristotle University of Thessaloniki. The following day, the results showed that IgM antibodies against West Nile virus (WNV) had been detected in 10 of the 11 serum specimens and in all three CSF specimens. WNV infection in humans had not been previously documented in Greece.

WNV is a positive-sense RNA virus of the Flaviviridae family, belonging to the Japanese encephalitis antigen group of viruses [1]. WNV is maintained in an enzootic cycle between birds and mosquitoes, mainly Culex species, while humans, horses and other mammals are incidental or dead-end hosts. Most human WNV infections are subclinical, and approximately 20% of infected individuals develop a febrile illness, while in less than 1%, the disease progresses to neuroinvasive disease, with the most severe form seen among elderly and immunocompromised individuals [2].

Although the virus was first isolated in 1937, interest in its impact on humans increased in 1996, when a large outbreak of West Nile neuroinvasive disease (WNND) was observed in Romania and in 1999, when WNV was introduced into the United States [3,4]. Several cases of WNV infection have been reported in horses and humans in Mediterranean countries [5-8], while WNND has been recently reported in humans in Hungary and Italy [7-9].

Methods
Surveillance
Following an alert issued by the Ministry of Health and KEELPNO on 6 August 2010 about 11 reported WNV infection cases, physicians in Greece were asked to notify KEELPNO of all confirmed or probable cases of WNV infection using a standardised reporting form, which included information on the demographic characteristics, clinical manifestations, underlying chronic medical conditions, potential risk factors and laboratory results of cases. The exact address of cases’ place of residence was obtained from hospital registries. In addition, active surveillance to identify cases included daily telephone inquiries to the hospitals of the region of Central Macedonia, from where the cases had been reported.

Case definition
The 2008 European Union case definition of WNV infection [10] was used, with slight modifications. A confirmed case was defined as a person meeting any of the following clinical criteria: encephalitis, meningitis, fever without specific diagnosis and at least one of the four laboratory criteria: (i) isolation of WNV from blood or CSF, (ii) detection of WNV nucleic acid in blood or CSF, (iii) WNV-specific antibody response (IgM) in CSF,
and (iv) WNV IgM high titre, and detection of WNV IgG, and confirmation by neutralisation.

A case was considered probable if the patient met the above clinical criteria and a WNV-specific antibody response was demonstrated in his or her serum sample. Epidemiological criteria were not used in the case definition due to the absence of recent surveillance data in animals.

**Laboratory methods**

Serum and CSF specimens were tested for the presence of WNV-specific IgM and IgG antibodies using commercial ELISA kits (Focus Technologies, Cypress, CA, USA). Reverse transcription-polymerase chain reaction (RT-PCR) was performed on RNA from 15 of 99 specimens, because the remaining samples had been taken between three and 15 days after the onset of illness, when viraemia is usually over. Primer sets specific for WNV and degenerate primers (able to detect flavivirus RNA) were used [11,12].

**Data analysis**

Data were entered in a database designed using Epidata software (Epidata association, Denmark, version 3.1) and were analysed using the GNU R software environment. Incidence was calculated using the 2007 mid-year estimated population from the Hellenic Statistical Authority as denominator [13].

**Results**

By 22 August 2010, 99 cases of WNV infection had been notified to KEELPNO. Of these, 81 had central nervous system manifestations (West Nile neuroinvasive disease, WNND) and 18 (eight probable and 10 confirmed cases) had only mild symptoms of fever and headache. We analyse here the 81 cases of WNND. Of these, 39 were confirmed and 42 were probable cases. The overall incidence of WNND was 0.72 cases per 100,000 population.

In total, 77 serum and 47 CSF specimens were available; for 45 of the 81 WNND patients both CSF and serum specimens were provided, while for four patients only CSF was available. WNV-specific IgM antibodies were

**Figure 1**

Reported cases of West Nile neuroinvasive disease by date of symptom onset, Greece, 1 July – 22 August 2010 (n=81)

**Table 1**

Characteristics of reported cases of West Nile neuroinvasive disease, Greece, 1 July – 22 August 2010 (n=81)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of cases</th>
<th>Incidence (per 100,000 population)</th>
<th>Risk ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>3</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>3</td>
<td>0.20</td>
<td>1.44 (0.96–10.95)</td>
</tr>
<tr>
<td>30–39</td>
<td>2</td>
<td>0.11</td>
<td>0.83 (0.18–7.37)</td>
</tr>
<tr>
<td>40–49</td>
<td>2</td>
<td>0.12</td>
<td>0.90 (0.29–3.85)</td>
</tr>
<tr>
<td>50–59</td>
<td>12</td>
<td>0.85</td>
<td>6.16 (1.93–29.40)</td>
</tr>
<tr>
<td>60–69</td>
<td>14</td>
<td>1.18</td>
<td>8.56 (1.29–33.72)</td>
</tr>
<tr>
<td>70–79</td>
<td>37</td>
<td>3.49</td>
<td>25.39 (6.92–101.72)</td>
</tr>
<tr>
<td>≥80</td>
<td>8</td>
<td>1.79</td>
<td>13.05 (2.81–43.94)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>36</td>
<td>0.64</td>
<td>Reference</td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td>0.81</td>
<td>1.27 (0.92–1.92)</td>
</tr>
<tr>
<td><strong>District (prefecture) of residence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thessaloniki</td>
<td>27</td>
<td>2.37</td>
<td>Reference</td>
</tr>
<tr>
<td>Imathia</td>
<td>21</td>
<td>14.57</td>
<td>6.15 (1.32–11.64)</td>
</tr>
<tr>
<td>Kilkis</td>
<td>9</td>
<td>10.44</td>
<td>4.44 (1.29–12.34)</td>
</tr>
<tr>
<td>Pella</td>
<td>17</td>
<td>11.72</td>
<td>4.95 (2.12–8.14)</td>
</tr>
<tr>
<td>Pieria</td>
<td>2</td>
<td>1.56</td>
<td>0.66 (0.32–2.54)</td>
</tr>
<tr>
<td>Serres</td>
<td>3</td>
<td>1.59</td>
<td>0.67 (0.17–3.25)</td>
</tr>
<tr>
<td>Larisa</td>
<td>2</td>
<td>0.70</td>
<td>0.30 (0.12–1.85)</td>
</tr>
<tr>
<td><strong>Total (in country)</strong></td>
<td>81</td>
<td>0.72</td>
<td>–</td>
</tr>
</tbody>
</table>

CI: confidence interval.
detected in all 77 serum and in 39 of the 47 CSF specimens, while WNV-specific IgG antibodies were detected in 42 of the 77 serum and 17 of the 47 CSF specimens. In 39 of the 45 patients for whom both types of specimen were available, the presence of IgM in both CSF and serum was seen, proving autochthonous antibody production; for IgG this was not tested. As cross-reactions are common among flaviviruses, specimens were also tested for tick-borne encephalitis (TBE) virus (although TBE is not prevalent in the area and none of the patients reported tick bites): all were negative. Low cross-reactivity was seen with dengue virus; however, when a positive result was obtained for dengue virus, the titres were very low compared with the high titres seen for WNV. None of the patients had been vaccinated for yellow fever. RT-nested PCR was negative in all specimens tested.

The first cases of WNND had onset of symptoms in early July (Figure 1).

**Figure 2**
Place of residence of reported cases of West Nile neuroinvasive disease, Greece, 1 July – 22 August 2010 (n=80)

*For one of the 81 cases of West Nile neuroinvasive disease, the place of residence has not been confirmed and is not included on the map. Each dot represents one case; the grey areas represent towns or cities; the blue lines represent rivers. The district of Larissa belongs to the region of Thessalia. All other districts from where cases have been reported belong to the region of Central Macedonia.*
The median age of the WNND cases was 70 years (range: 12–86 years), with most (n=71) aged 50 years or older (Table 1). Of all WNND cases, 45 (56%) were males.

The incidence, 1.7 per 100,000 population, of WNND among those aged 50 years or older was almost 12 times higher (risk ratio: 12.2; 95% confidence interval: 4.9 to 28.5) than that of individuals aged under 50 years. The risk of WNND was 27% higher among males compared with females (Table 1).

The place of residence of the WNND cases is presented in Figure 2: 30 resided in an urban area and 51 in a rural setting. The vast majority (n=79) lived in districts (prefectures) of the region of Central Macedonia and only two were reported from the region of Thessalia (district of Larissa). It is of note that a large number of cases (n=58) lived near rivers and/or on the irrigated plains between and surrounding the Aliakmonas and Axios rivers (Figure 2).

None of the notified cases reported travel to a known WNV-endemic area during the two weeks before onset of symptoms. Information on outdoor activities was gathered from 55 of the cases: 27 cases reported spending many hours outdoors in the countryside every day. None of the cases had a history of blood transfusion or tissue/organ transplant during the two weeks before the onset of symptoms.

All notified cases with central nervous system manifestations were hospitalised. Of those, 65 had encephalitis or meningoencephalitis, and 16 had aseptic meningitis (Table 2).

Of the 65 WNND cases with encephalitis and/or meningoencephalitis, 60 were aged 50 years or older. Information on underlying chronic medical conditions was available for 60 of the 81 patients. These included hypertension (n=26), a history of immunosuppression (n=17), coronary artery disease (n=11), and diabetes mellitus (n=9). Ten cases were admitted to an intensive care unit. As of 22 August 2010, eight cases had died, giving a case fatality rate of 9.9% among the reported WNND cases. All deceased patients were aged over 70 years, and suffered from hypertension and diabetes.

**Discussion and conclusions**

We describe here 81 cases of WNND reported between early July and 22 August in the region of Central Macedonia, northern Greece. The fact that a small proportion of infected individuals develop WNND [2] suggests that this ongoing outbreak may in fact be larger. As of 26 August 2010, more cases of WNND have been reported bringing the total number of WNND cases to 108. In addition, information was gathered through enhanced surveillance, and a degree of under-reporting, particularly at the beginning of the outbreak, is expected.

Serological surveys conducted in humans in the 1980s and in 2007 in Greece identified WNV antibodies in approximately 1% of selected populations (i.e. farmers, wood-cutters, shepherds) in the region of Central Macedonia. Of 392 serum samples collected from residents in a selected urban area in the district of Imathia (central Greece) in 2007, six were positive for WNV, of which four were confirmed by microneutralisation assay [14,15]. The authors concluded that WNV or related viruses circulate in endemic cycles in rural areas in Greece. In contrast, a survey of 9,590 blood donations and 115 CSF samples from patients with aseptic meningitis in Greece between 2005 and 2007 revealed no positive results for WNV by nucleic acid test. However, the sources of the clinical samples were major laboratories and/or blood banks in the cities of Athens and Ioannina [16].

The presence of WNV in animals is not monitored routinely in Greece. However, a few *ad-hoc* studies have been conducted. In a seroprevalence survey in animals in 1980, antibodies to WNV were found in 8.8% of sheep, 8.7% of goats, 3.9% of cattle, 20.4% of horses, 1.4% of pigs, and 24.5% of birds [17]. In an unpublished survey conducted from May 2001 to December 2004, 302 of 7,549 (4%) equine serum samples were found positive for WNV using neutralisation tests; positive samples in equines were found in 36 of 49 districts (prefectures) studied from all parts of Greece (O. Mangana, Ministry of Agriculture, personal communication, 10 August 2010).

In conclusion, previous studies in humans and animals suggest that WNV has probably been circulating in the region of Central Macedonia and possibly in other parts of Greece for many years. Increased rainfall, high temperatures and humidity during recent months, as well as the geographical features (i.e. river deltas, rice fields, irrigated plains) of some parts of the region of Central Macedonia, have probably favoured the multiplication of *Culex* species, leading to the occurrence of numerous cases of WNV infection in humans.

**Table 2**

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Number of cases with encephalitis or meningoencephalitis</th>
<th>Number of cases with aseptic meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>20–29</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>30–39</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>40–49</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>50–59</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>60–69</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>70–79</td>
<td>31</td>
<td>6</td>
</tr>
<tr>
<td>≥80</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>16</td>
</tr>
</tbody>
</table>

Clinical manifestations of reported cases of West Nile neuroinvasive disease by age group, Greece, 1 July – 22 August 2010 (n=81)
Public health measures

After the initial notification of the cluster of WNV infection cases, the Hellenic Centre for Disease Control and Prevention alerted physicians throughout the country and prepared a set of guidelines for health professionals, with the necessary instructions for laboratory diagnosis. Surveillance of human WNV infection cases has been established and the public has been given guidance about personal protective measures.

The Hellenic Centre for Blood Transfusion prepared specific guidelines and measures for blood and blood products safety, including a 28-day deferral policy for all donors residing in the specific districts (prefectures) where WNV infection cases were detected, as well as any blood donor who had visited the same districts (prefectures) for one or more days. Blood units that had been collected in the same region since the beginning of July were quarantined until tested for WNV by PCR. They were all found negative for WNV and were released for use. Finally, specific advice was provided to all blood donors asking them to inform the blood donation department they attended, if they develop a fever within 15 days after their donation. The National Organisation for Transplantations has also been informed and is requesting WNV testing of donors depending on residence area or travel history.

The Ministry of Health and Social Solidarity coordinates the intensification of mosquito control programmes at district level, which are already being implemented. The Ministry also undertook the coordination of the health sector preparedness and the collaboration with the veterinary services of the Ministry of Agriculture. Surveillance for WNV in mosquitoes (using bait traps) has been put in place.

Updates on reported WNV infection cases and deaths in Greece are published in the daily epidemiological surveillance reports (available in English from http://www.keelpno.gr/eng/wnv).

Acknowledgements

We would like to thank all hospital physicians and local public health authorities who contributed to the surveillance of WNV infections in Greece. The technical support of the personnel in the Reference Laboratory for Arboviruses in Thessaloniki is highly appreciated.

References


Since the occurrence of West Nile virus (WNV) infection in humans in 2008 in Italy, concerns have been raised about the potential risks associated with solid organ transplantation (SOT). A nationwide retrospective survey showed that 1.2% of SOT donors in 2009 were WNV-seropositive and demonstrated that human WNV infection is distributed throughout several Italian regions. Transmission of WNV or other arboviruses through SOT is a possibility and risk assessment should be carried out before SOT to avoid infection through transplantation.

**Background**

In 1998, when the first cases of equine West Nile virus (WNV) infection in Italy were detected in Tuscany, no human cases were reported [1]. WNV re-emerged in Italy in 2008, and viral circulation was identified among different vectors and different animal species, including horses and wild birds [2]. The first cases of human WNV neuroinvasive infections in Italy were identified in September 2008 in the Emilia-Romagna region [3].

In the summer of 2009, additional human cases were reported in the same and in other neighbouring Italian regions. In one of the most affected areas in the province of Ferrara, a WNV seroprevalence of 0.68% was observed in healthy blood donors, raising concerns about the potential risks of WNV transmission by blood transfusion and organ transplantation [4,5].

In 2009, as a precautionary measure, the National Transplant Centre issued guidance that potential donations from all donors of solid organs, tissues and cells from the Bologna, Ferrara, Modena and Reggio-Emilia provinces who were screened by nucleic acid amplification tests (NAAT) for the presence of WNV viraemia, and tested positive, had to be rejected. Donors from other regions who had spent at least one overnight stay in the above-listed provinces during the 28 days before donation should not be considered eligible for donation [6].

Two days before screening of donors was implemented, in Bologna, Emilia-Romagna province, transmission of WNV infection through liver transplantation was detected by NAAT before clinical symptoms appeared in the recipient. The transmission of WNV was managed, post-transplant, by administration of hyperimmune serum and viraemia-guided adjustment of the immunosuppressive drug regimen, accompanied by supportive care [7]. The patient recovered from the transplantation and did not develop symptoms of WNV infection or sequelae. Before this case, all reported SOT donor-derived WNV infections were identified retrospectively in symptomatic transplanted patients with severe outcomes in the United States (US) [8]. This post-transplant detection of WNV infection in SOT recipients demonstrates that the absence of a universal screening policy in the immediate pre-transplant period makes it almost impossible to accurately quantify the transmission rate and the subsequent clinical impact of WNV transmission to SOT recipients. In the US, a recent serosurvey carried out among SOT recipients suggested that asymptomatic WNV infection may be common in areas of WNV activity and that the severe clinical presentations of WNV infections are equally frequent in both immunocompromised and immunocompetent subjects [9].

On the basis of this evidence, re-defining the risks for WNV transmission by organ, tissue and cell transplantation was identified by the National Transplant Centre.
as a necessary safety measure before the start of the next WNV season, from late spring to early autumn. The Italian Transplant Network considered this to be a priority in order to establish the factual basis for implementing future strategies for preventing WNV transmission.

A nationwide retrospective survey of WNV seroprevalence was therefore undertaken in all SOT donors recruited by the National Transplant Centre during 2009.

**Methods**

Serum samples from the Italian SOT donors in 2009 stored in the biorepository facilities of the Italian Transplant Network, were analysed by the two reference laboratories identified by the National Transplant Centre for assessment of WNV infection: the Laboratory of Virology at the National Institute for Infectious Diseases ‘L. Spallanzani’ in Rome and the Regional Reference Centre for Microbiological Emergencies of the Microbiology Unit, St Orsola–Malpighi University Hospital, in Bologna.

The presence of WNV-specific IgG and IgM was investigated using a two-step approach: first, a screening test was performed using a commercial enzyme-linked immunosorbent assay (ELISA) method (Euroimmun, AG, Lübeck, Germany); all samples that were positive for either IgG or IgM were then confirmed by an immunofluorescent antibody assay (IFA, Euroimmun). Second, the IgG-positive sera were further evaluated by ELISA to measure the IgG avidity, using the method of Fox et al. [10]. All the IgG- and IgM-positive samples identified in the previous steps were further characterised by microneutralisation assay (MNTA) against WNV as previously described [11], and to rule out possible cross-reactions of the test, serum samples were also tested by MNTA against Usutu virus. All IgM-positive sera samples were retrospectively tested by NAAT using Procleix-WNV assay performed on the TIGRIS system, Novartis (for donors from Emilia Romagna) or Artus Real Art WNV LC RT RCRt kit, QIAGEN (for donors from Piedmont and Tuscany) in order to evaluate the presence of WNV viraemia. During the screening activity for WNV in 2009 no WNV-positive donor was identified before SOT.

**Results**

A total of 1,248 serum samples from SOT donors in 2009 were analysed, accounting for 98.1% of SOT donors recruited during that year. Table 1 lists the number of SOT donors evaluated in this study, by region of residence. WNV-specific antibodies were detected in 15 samples from individual donors at the time of organ donation, thus giving an overall positivity rate of 1.2%. Data from MNTA indicate that seven of 15 samples had

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of donors tested</th>
<th>Number of donors positive WNV</th>
<th>WNV: West Nile virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abruzzo-Molise</td>
<td>12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Basilicata</td>
<td>11</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Calabria</td>
<td>12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Campania</td>
<td>74</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Emilia Romagna</td>
<td>120</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Friuli-Venezia Giulia</td>
<td>45</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lazio</td>
<td>103</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Liguria</td>
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</tr>
<tr>
<td>Lombardy</td>
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<td></td>
</tr>
<tr>
<td>Marche</td>
<td>51</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Piedmont-Aosta Valley</td>
<td>120</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bolzano-Bozen independent provincial administration</td>
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<td></td>
</tr>
<tr>
<td>Trento independent provincial administration</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>Apulia</td>
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<td></td>
</tr>
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<td>Sardinia</td>
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</tr>
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<td>Tuscany</td>
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<td></td>
</tr>
<tr>
<td>Umbria</td>
<td>11</td>
<td>0</td>
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<tr>
<td>Veneto</td>
<td>109</td>
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<td><strong>Total</strong></td>
<td><strong>1,248</strong></td>
<td><strong>15</strong></td>
<td></td>
</tr>
</tbody>
</table>

*IgG - and/or IgM-positive, by serological screening (see Table 2).*
neutralisation activity against WNV. However, eight samples did not show appreciable neutralisation titre against WNV. Among these, two samples were reverse transcription-polymerase chain reaction (RT-PCR) positive and two other samples probably showed neutralisation activity against the related Usutu virus (Table 2).

The inability to confirm by MNTA all the samples testing positive by ELISA and IFA might be due to the cross-reactivity to closely related flaviviruses or might be consistent with the notion that the neutralising response to WNV in humans is variable and that only a subset of infected individuals generate antibodies against high-neutralising epitopes [12]. Furthermore, from a technical point of view, maturation state of WNV particles used in the MNTA might have been important for determining whether antibodies in a given serum samples were judged WNV-specific by MNTA, as already reported by Nelson et al. [13]. The highest number of WNV-seropositive donors were from the regions of Emilia-Romagna and Tuscany (Table 1 and Figure).

Table 2 shows the place of residence, demographic information and laboratory results obtained for these 15 patients.

Two donors (from Piedmont and Emilia-Romagna) were identified as IgG- and IgM-positive. One donor, from Tuscany, was positive only for IgM. All these three IgM-positive samples were also positive for viral RNA, showing quite a low viral load (equivalent to a genome copy number of $3 \log$ copies/ml). We speculate that such a low level of viral concentration in blood is likely to pose a limited risk of viral transmission through SOT. Four IgG-positive specimens showed an antibody avidity of 80% to 90% suggesting that exposure to WNV probably occurred more than six months before the organ donation; another four IgG-positive samples (all from donors who donated organs after June 2009) showed an antibody avidity lower than 40%, suggesting that the infection was probably acquired within six months before the organ donation, a time frame consistent with WNV exposure during the 2009 mosquito season (June to October).

In addition, recipients of a solid organ from donors who were shown to have been positive for WNV genome or to have had IgM-specific antibodies at the time of organ donation were also included in this study. Two recipients from the Piedmont IgM-positive donor (one kidney recipient and one liver recipient) did not show any seroconversion to WNV. The remaining recipient (who received a kidney) was not available for testing. All three recipients are well and have never shown signs consistent with WNV infection. The IgM-positive donor from Tuscany did not in the end donate organs for reasons not related to WNV infection.

Discussion and conclusions

The occurrence of an antibody response in some donors from the Piedmont, Friuli-Venezia Giulia, Marche and Basilicata regions is rather unexpected and shows evidence of WNV infection in humans in several Italian regions. However, it is possible that WNV activity has hitherto been underestimated in some regions, due to an insufficient veterinarian and entomological surveillance system, which may have generated inconsistent data. Evidence from the present study – i.e. the low IgG avidity index, the presence of an IgM-specific response and/or WNV RNA in blood samples – is consistent with the notion that recrudescence of WNV activity in Italy occurred in the last two years, following the report of the first human cases of neuroinvasive disease [3-5]. These findings highlights the need for an accurate nationwide approach to risk assessment related to transplantation, in order to implement appropriate prevention strategies and limit the potential burden of severe neurological complications in the immunocompromised recipients. During the 2009 season, only one case of WNV transmission by SOT was observed in the Emilia-Romagna region [7]. No additional cases of WNV transmission from infected donors were documented retrospectively in our study, suggesting that the transmission of WNV to recipients of SOT from viraemic donors does not always occur. Furthermore,
the positivity in MNTA against Usutu virus in two samples additionally confirms that this virus has to be added to the list of those that can be transmitted to humans [14,15] and its possible transmission through SOT deserves particular attention.

These results indicate that, due to the well-known circulation of WNV in many different areas in Italy, transmission of WNV or other arboviruses through SOT is possible, and that the risk assessment process related to transplantation is a challenging issue that requires a systematic approach [7].

Regional Coordinators of the Italian Transplant Network:
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Table 2
Detailed information of solid organ donors who tested positive by serological screening for West Nile virus

<table>
<thead>
<tr>
<th>Region</th>
<th>Sampling date, 2009</th>
<th>Cause of death</th>
<th>Age at death (years)</th>
<th>Place of residence (province)</th>
<th>Possible place of exposurea (if different from place of residence)</th>
<th>WNV-specific antibody titres</th>
<th>IgG avidity (%)b</th>
<th>MNTA titre against WNVd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basilicata</td>
<td>22 Sep</td>
<td>Stroke</td>
<td>64</td>
<td>Matera</td>
<td>Lake Garda</td>
<td>≥1:160</td>
<td>&lt;1:10</td>
<td>89</td>
</tr>
<tr>
<td>Lazio</td>
<td>7 Jul</td>
<td>Stroke</td>
<td>67</td>
<td>Rome</td>
<td>–</td>
<td>1:80</td>
<td>&lt;1:10</td>
<td>31</td>
</tr>
<tr>
<td>Tuscany</td>
<td>3 Jan</td>
<td>Cranial trauma</td>
<td>57</td>
<td>Lucca</td>
<td>–</td>
<td>1:80</td>
<td>&lt;1:10</td>
<td>NA</td>
</tr>
<tr>
<td>Tuscany</td>
<td>26 Mar</td>
<td>Brain haemorrhage</td>
<td>67</td>
<td>Versilia (Lucca)</td>
<td>–</td>
<td>1:40</td>
<td>&lt;1:10</td>
<td>NA</td>
</tr>
<tr>
<td>Tuscany</td>
<td>15 Jul</td>
<td>Stroke</td>
<td>87</td>
<td>Florence</td>
<td>–</td>
<td>&lt;1:10</td>
<td>1:10</td>
<td>NA</td>
</tr>
<tr>
<td>Tuscany</td>
<td>25 Aug</td>
<td>Brain haemorrhage</td>
<td>74</td>
<td>Siena</td>
<td>–</td>
<td>1:80</td>
<td>&lt;1:10</td>
<td>11</td>
</tr>
<tr>
<td>Tuscany</td>
<td>20 Oct</td>
<td>Brain haemorrhage</td>
<td>40</td>
<td>Prato</td>
<td>–</td>
<td>≥1:160</td>
<td>&lt;1:10</td>
<td>43</td>
</tr>
<tr>
<td>Tuscany</td>
<td>13 Nov</td>
<td>Cranial trauma</td>
<td>85</td>
<td>Livorno</td>
<td>–</td>
<td>≥1:160</td>
<td>&lt;1:10</td>
<td>68</td>
</tr>
<tr>
<td>Piedmont</td>
<td>28 Jul</td>
<td>Asphyxia/hypoxia</td>
<td>57</td>
<td>Mottalciata (Biella)</td>
<td>–</td>
<td>1:80</td>
<td>&lt;1:10</td>
<td>73</td>
</tr>
<tr>
<td>Piedmont</td>
<td>21 Oct</td>
<td>Post-anoxic coma</td>
<td>63</td>
<td>Chieri (Turin)</td>
<td>–</td>
<td>1:160</td>
<td>1:40</td>
<td>13</td>
</tr>
<tr>
<td>Emilia Romagna</td>
<td>23 Feb</td>
<td>Heart failure</td>
<td>71</td>
<td>Modena</td>
<td>–</td>
<td>1:400</td>
<td>&lt;1:10</td>
<td>88</td>
</tr>
<tr>
<td>Emilia Romagna</td>
<td>14 May</td>
<td>Brain haemorrhage</td>
<td>56</td>
<td>Traversetolo (Parma)</td>
<td>–</td>
<td>1:200</td>
<td>&lt;1:10</td>
<td>52</td>
</tr>
<tr>
<td>Emilia Romagna</td>
<td>1 Sep</td>
<td>Brain haemorrhage</td>
<td>78</td>
<td>Naples</td>
<td>Reggio Emilia</td>
<td>≥1:160</td>
<td>1:80</td>
<td>25</td>
</tr>
<tr>
<td>Marche</td>
<td>1 Aug</td>
<td>Brain haemorrhage</td>
<td>48</td>
<td>Rimini</td>
<td>–</td>
<td>1:400</td>
<td>&lt;1:10</td>
<td>84</td>
</tr>
<tr>
<td>Friuli-Venezia</td>
<td>24 Jul</td>
<td>Cranial gunshot wound</td>
<td>63</td>
<td>Gorizia</td>
<td>–</td>
<td>1:160</td>
<td>&lt;1:10</td>
<td>88</td>
</tr>
</tbody>
</table>

MNTA: microneutralisation assay; NA: not applicable, due to low or negative IgG enzyme-linked immunosorbent assay (ELISA) optical density values; WNV: West Nile virus.

a For the donors whose place of residence was not in the WNV activity area identified in 2008 and 2009, the possible place of WNV exposure was provisionally assigned by a retrospective investigation based on consultation of medical records or on direct information received by the donors’ relatives. The dashes (–) in this column indicate that no history of travel in WNV-endemic areas other than those identified in Italy was recorded in the 12 months before donation.

b <40% : less than 6 months after infection; >60% : 6 months or more after infection, according to [10].
c Positive for WNV by reverse transcription-polymerase chain reaction (RT-PCR).
d Samples yielding a neutralisation titre ≥ 1:20 were scored as positive.

Tableau 2
Informations détaillées sur les donneurs d’organes solides qui ont testé positif par examen sérologique pour le virus de la fièvre de West

<table>
<thead>
<tr>
<th>Région</th>
<th>Date de prélèvement, 2009</th>
<th>Cause de la mort</th>
<th>Âge à la mort (ans)</th>
<th>Lieu de résidence (province)</th>
<th>Localisation possible d’exposition (si différent du lieu de résidence)</th>
<th>Titres WNV-ségmentés</th>
<th>IgG avidité (%)</th>
<th>Titre MNTA contre WNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basilicata</td>
<td>22 Sep</td>
<td>Stroke</td>
<td>64</td>
<td>Matera</td>
<td>Lake Garda</td>
<td>≥1:160</td>
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<td>89</td>
</tr>
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<td>Lazio</td>
<td>7 Juil</td>
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</tr>
<tr>
<td>Tuscany</td>
<td>3 Jan</td>
<td>Craniocraque</td>
<td>57</td>
<td>Lucca</td>
<td>–</td>
<td>1:80</td>
<td>&lt;1:10</td>
<td>NA</td>
</tr>
<tr>
<td>Tuscany</td>
<td>26 Mar</td>
<td>Cryo-sanglote</td>
<td>67</td>
<td>Versilia (Lucca)</td>
<td>–</td>
<td>1:40</td>
<td>&lt;1:10</td>
<td>NA</td>
</tr>
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<td>Tuscany</td>
<td>15 Juil</td>
<td>Stroke</td>
<td>87</td>
<td>Florence</td>
<td>–</td>
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<td>Cryo-sanglote</td>
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<td>Asphyxie/hipoxie</td>
<td>57</td>
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<td>–</td>
<td>1:80</td>
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<tr>
<td>Emilia Romagna</td>
<td>23 Febr</td>
<td>Néfartim</td>
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<td>Modena</td>
<td>–</td>
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<td>Friuli-Venezia</td>
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<td>–</td>
<td>1:160</td>
<td>&lt;1:10</td>
<td>88</td>
</tr>
</tbody>
</table>

MNTA: examen de neutralisation à microparticules; NA: non applicable, due to low or negative titre d’avidité de l’IgG dans l’assay ELISA-optique; WNV: virus de la fièvre de West.

a Pour les donneurs dont le lieu de résidence n’était pas dans l’aire d’activité du WNV identifiée en 2008 et 2009, la localisation possible d’exposition au WNV était provisoirement assignée par une investigation rétrospective basée sur la consultation des dossiers médicaux ou sur des informations directes reçues des proches des donneurs. Les dashes (–) dans cette colonne indiquent que le lieu de voyage en zones endémiques d’autres virus que celles identifiées en Italie n’a pas été enregistré dans les 12 mois précédant le don.

b <40% : moins de 6 mois après infection; >60% : 6 mois ou plus après infection, d’après [10].
c Positif pour WNV par RT-PCR.
d Échantillons produisant un titre de neutralisation ≥ 1:20 sont considérés comme positifs.
References


We report about West Nile virus (WNV) infections in a symptomatic traveller returning from Israel and in her asymptomatic travel companion. Knowledge of the current epidemiological situation in Israel from where WNV cases were reported recently enabled a rapid diagnosis. The described cases serve as a reminder for physicians to consider WNV in the diagnosis of patients returning from areas with potential circulation of the virus.

At the end of July 2010, a Dutch woman in her early thirties presented to our first aid department with fever, retro-orbital headache and a macular rash. The day before she had returned from a 10-day holiday to Israel where she noticed several mosquito bites during a camping trip at the Sea of Galilee. There was no history of tick bites. Five days before presentation, she had suddenly fallen ill with extreme fatigue, myalgia, fever and an increasingly severe headache. Movement of the eyes had been painful. The next day she had developed a burning sensation of the skin that was followed by a skin eruption. The rash started on the trunk and spread to arms and legs.

Upon presentation in the first aid department she did not appear ill. Body temperature was 36.4°C. There was a generalised macular rash, sparing hands and feet, and no petechiae or eschars were present. Neurological examination revealed no abnormalities. Potential infection with West-Nile virus was suspected because of the clinical picture and recent reports of West Nile virus cases in Israel [1].

Laboratory examination showed a leukopenia of 1.8x10^9/L with a predominance of large granular lymphocytes; thrombocyte count was 92x10^9/L. Infection with Epstein Barr virus, cytomegalovirus, dengue virus, enterovirus and parechovirus was excluded by serology and antigen test or PCR. A lumbar punction was not performed due to missing neurological symptoms. West Nile virus RNA could not be detected in the EDTA plasma sample taken on day five after onset of disease by a TaqMan reverse transcriptase-PCR assay, using a probe for the WN3'NC [2]. However, in a second blood sample obtained fifteen days later, seroconversion for both serum IgG and IgM antibodies against West Nile virus was detected with an indirect qualitative enzyme-linked immunosorbent assay (ELISA) (Focus Diagnostics, Cypress, California). This assay uses antibody capture technique for the detection of IgM antibodies.

Once the diagnosis was confirmed, serology was performed in the patient’s travel companion who reported having had similar complaints, but who had already recovered by the time he returned to the Netherlands. The laboratory results showed that he was seropositive for WNV IgG and IgM. Recovery was uneventful in both patients.

West-Nile virus is endemic in Israel. The largest recent outbreak in humans in Israel occurred in the year 2000 with more than 400 reported cases [3]. Recently, 12 cases of West Nile fever have been reported, centered around the Tel Aviv area [1]. Culex perexiguus, Cx. pipiens, and Aedes caspius are the vectors of West Nile virus in Israel where most cases of West Nile fever occur between August and October. The seasonal occurrence of human cases reaches a peak one month after the mosquito peak [4].

Measures to avoid mosquito bites such as wearing protective clothes and using repellents are recommended during the whole transmission season and this case report serves as a reminder to physicians to consider West Nile fever in patients with fever returning from Israel.

References


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Introduction and control of three invasive mosquito species in the Netherlands, July-October 2010

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In July 2010, during routine mosquito surveillance inspections at companies that import used tires, three invasive species were found at five locations in the Netherlands: the yellow fever mosquito (Aedes aegypti), the Asian tiger mosquito (Ae. albopictus) and the American rock-pool mosquito (Ae. atropalpus). This is the first time that Ae. aegypti is reported from the Netherlands. Mosquito control was initiated one week after the first invasive mosquito was found, using adulticides and larvicides. The available data suggest that the implemented control measures have been effective for this season.

Introduction
Following the discovery of Aedes albopictus in the Netherlands in 2005 related to companies that import Lucky bamboo [1], continuous surveillance at these companies was started in 2006. Gradually, other national surveillance activities for this mosquito species were initiated, including passive surveillance (since 2007), active surveillance at parking lots along main highways entering the country from the south and east (since 2008), and at companies that import used tires (since 2009). In 2009, during routine surveillance activities, the exotic mosquito species Ae. atropalpus, a North American species that had been encountered several times in Europe [2], but had never established here, was found for the first time in the Netherlands [3].

These surveillance activities are meant to identify as early as possible the presence of exotic mosquito species with the aim to prevent the establishment of invasive exotic mosquito species, especially those that are known to be vectors of pathogens of public health importance such as dengue- and chikungunya virus. Here we report the finding and the successive control of three invasive mosquito species, Ae. aegypti, Ae. albopictus and Ae. atropalpus in the Netherlands.

Methods
A total of 34 companies that import used tires into the Netherlands were included in the invasive mosquito survey. Routine inspections were carried out from April to the last week of October [2]. A qualitative risk assessment on the introduction of invasive mosquito species was performed to determine the frequency of inspection of a company. Parameters in the risk assessment were (i) the type of tires that are imported, (ii) the countries from which tires are imported, and (iii) whether the tire storage is in- or outdoors. Collected larvae and adult mosquitoes were diagnosed either morphologically by using the diagnostic keys from Schaffner et al. [4], or molecularly by PCR sequencing the mitochondrial cytochrome oxidase subunit 1 (CO1) gene [5]. A week after the first finding, infested locations were treated by spraying Bacillus thuringiensis israelensis (B.t.i.) serotype H14 or Bacillus sphaericus (B.s.) against larvae and/or deltamethrin (aqua K-Othrine, Bayer Environmental Sciences) against adult mosquitoes. Larval control of the surrounding area (predefined perimeter of 500 m) consisted of removal of potential larval habitats for container-breeding Aedes spp. when possible, or treatment with either B.t.i. space spray (VectoBac WG, Valent BioSciences), or with B.t.i./Bacillus sphaericus (B.s.) granules (Vectomax, Valent BioSciences). It was decided to perform larvicidal treatment once every two to three weeks, until the first week of November.

Following the discovery of an exotic species at a location, surveillance was intensified to assess the potential spread of the invasive species and the effectiveness of the control activities by placing traps for adult mosquitoes (BG-sentinel, Biogens) and oviposi-
tion traps [6] in the 500 m perimeter surrounding the company site.

**Results**

Three exotic mosquito species (*Ae. aegypti*, *Ae. albopictus*, and *Ae. atropalpus*) were found in five locations in the Netherlands. The first two mosquito larvae, *Ae. atropalpus*, were found on 21 July 2010, during a routine inspection at Location 1 (Heijningen) (Figure, Table 1).

On the next day, during an intensified inspection, one adult *Ae. albopictus* and one adult *Ae. aegypti* were collected, in addition to the two initial *Ae. atropalpus* larvae. The infestation level for *Ae. atropalpus* (in terms of percentage of infested tires and total number of larvae) at this company was relatively high, but less so for *Ae. albopictus* and *Ae. aegypti*, of which no larvae and/or pupae were found. Results of intensified inspection suggest that *Ae. atropalpus* and *Ae. albopictus* (but not *Ae. aegypti*) had spread to the surrounding areas of Location 1. On 3 September 2010, the last exotic species was collected from Location 1 and its surroundings (Table 2).

At Location 2 (Oosterhout), several male *Ae. aegypti* specimens were collected starting with 26 July. The last invasive species were found at this location on 6 August, when two adult *Ae. atropalpus* were collected. Despite intensive surveillance, no immature forms of invasive species were found at the company’s premises or in the surrounding areas.

**Figure**

Locations of tire companies that were positive (n=5) and negative (n=29) for at least one of the invasive mosquito species, the Netherlands, 2010

On 26 July, three adult specimens (but no larvae) of *Ae. atropalpus* were collected from Location 3 (Oss). In addition, another three adult *Ae. atropalpus* were found on 5 August at this location. In the surrounding area, one *Ae. albopictus* was collected in a BG-sentinel trap placed approximately 50 m from the tire platform on 9 August, but no larvae of exotic species were found in the surrounding area. The last specimen (larva) was found at this location on 23 August.

On 24 August, the first larvae (six specimens) of *Ae. atropalpus* were collected from Location 4 (Weert). On 13 September, high numbers of this species (larvae and adults) were found at this location and several larvae were found in the surrounding area, including at the premises of a neighbouring tire-importing company. The two companies are considered as one location (Location 4). In addition, one *Ae. albopictus* specimen was collected from the tire platform on 13 September and one *Ae. albopictus* specimen was found in a BG sentinel trap at approximately 25 m from the infested companies, one week later. The last specimen was found on 27 September.

On 28 September, two *Ae. albopictus* larvae were collected from Location 5 (Montfoort). The third (and last) specimen was collected in an adult trap on the tire platform on 5 October. No specimens were found in the surrounding area.

All infested companies described here belong to the ‘high risk’-category for importing exotic mosquito species, based on the type, origin and storage of the tires that are imported, and are therefore inspected every two weeks. No invasive mosquito species were found at any of the other companies that were included in the survey.

**Discussion and conclusion**

The discovery of *Ae. aegypti* in the Netherlands was unexpected, mostly because, unlike *Ae. albopictus* [3], *Ae. aegypti* is not directly associated with the international trade in used tires [7]. Even without control measures, the tropical *Ae. aegypti* will probably not survive the winter in temperate areas such as the Netherlands and consequently does not pose a direct health risk for the country. This is in contrast with the public health risks related to re-introduction of *Ae. aegypti* into southern Europe [8,9].

In addition, this report describes the discovery of an *Ae. albopictus* for the first time in the outdoor environment in the Netherlands. Although the species is still regularly found in glasshouses as hitchhikers in importation of Lucky bamboo [10], preventive and curative indoor control measures in these glasshouses appear to be effective to prevent indoor or outdoor establishment, since a location never stays positive for *Ae. albopictus* longer than 1.5 month (Scholte, unpublished data).
Back-tracing data of the company at Location 1 suggests introduction of *Ae. albopictus* and *Ae. aegypti* by a shipment of used airplane tires at the end of May 2010, originating from southern Florida, an area inhabited by both species. On 24 July, part of the same shipment was transported to Location 2 (belonging to the same company), and on 4 August to Location 3. Back-tracing information of the companies at Location 4 showed recent tire import from Italy. *Ae. albopictus* from Italy [11] and the United States [12] are considered to display diapause and potentially to survive temperate climates [13,14]. *Ae. atropalpus* had already been found at two sites in the Netherlands in 2009 [2] which indicates that the first introduction of *Ae. atropalpus* was in or before 2009, although more recent introductions are not excluded either. This species had a relatively large population at Locations 1 and 4, and colonised larval habitats in the surrounding areas, other than tires.

The fact that relatively few adults and no other life-stages of *Ae. aegypti* and *Ae. atropalpus* were found at Location 2, indicates a low level of infestation.

The available data for this season (Table 2) suggest that the implemented control measures have been effective, although it is too early at this moment in time to assess if eradication has been achieved. Per location, it took between one and three treatments and a maximum time span of seven weeks between the first treatment and the day when the last exotic species was found. It will be crucial in the years to come to monitor the locations (including the surrounding areas) that had been infested with one or more of the exotic species in 2009 and 2010, in order to restart mosquito control as early as possible.

Having witnessed these introductions of exotic invasive mosquito species that pose a potential threat to public health in Europe, international collaboration and action of medical entomologists, public health experts, policy makers, and the tire-business industry is critical to address this.

### Acknowledgements

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### Table 1

Summary of the results of the invasive mosquito survey at used tire companies by location, the Netherlands, July-October 2010

<table>
<thead>
<tr>
<th>Location</th>
<th>Adults collected</th>
<th>Larvae collected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Ae. aegypti</em></td>
<td><em>Ae. albopictus</em></td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>15</td>
</tr>
</tbody>
</table>

### Table 2

Inspections, mosquito control, and findings of at least one of the three exotic mosquito species for each location per week, the Netherlands, July-October 2010

<table>
<thead>
<tr>
<th>Location</th>
<th>Week (2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43</td>
</tr>
<tr>
<td>1</td>
<td>x x x x x</td>
</tr>
<tr>
<td>2</td>
<td>x x x x x</td>
</tr>
<tr>
<td>3</td>
<td>x x x x x</td>
</tr>
<tr>
<td>4</td>
<td>x x x x x</td>
</tr>
<tr>
<td>5</td>
<td>x x x x x</td>
</tr>
</tbody>
</table>

- **No inspection**
- **No exotic species found (negative)**
- **Larvae and/or adults found of one of the three exotic mosquito species**
- **X Control measures**


<table>
<thead>
<tr>
<th>Country</th>
<th>Bulletin Name</th>
<th>Description</th>
<th>Frequency</th>
<th>Language(s)</th>
<th>URL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Austria</strong></td>
<td>Mitteilungen der Sanitätsverwaltung</td>
<td>Bundesministerium für Gesundheit Familie und Jugend, Vienna.</td>
<td>Monthly</td>
<td>German</td>
<td><a href="http://www.bmgfj.gv.at/cms/site/thema.html?channel=CH0951">http://www.bmgfj.gv.at/cms/site/thema.html?channel=CH0951</a></td>
</tr>
<tr>
<td><strong>Belgium</strong></td>
<td>Vlaams Infectieziektenbulletin</td>
<td>Department of Infectious Diseases Control, Flanders</td>
<td>Quarterly</td>
<td>Dutch, summaries in English</td>
<td><a href="http://www.infectieziektenbulletin.be">http://www.infectieziektenbulletin.be</a></td>
</tr>
<tr>
<td><strong>Czech Republic</strong></td>
<td>Zpravy CEM (Bulletin of the Centre of Epidemiology and Microbiology)</td>
<td>Centrum Epidemiologie a Mikrobiologie Státního</td>
<td>Monthly</td>
<td>Czech, titles in English</td>
<td><a href="http://www.szu.cz/ce/adefaultt.htm">http://www.szu.cz/ce/adefaultt.htm</a></td>
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<td></td>
<td>EPIDAT (Notifications of infectious diseases in the Czech Republic)</td>
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<td></td>
<td><a href="http://www.szu.cz/ce/epidat/epidat.htm">http://www.szu.cz/ce/epidat/epidat.htm</a></td>
</tr>
<tr>
<td><strong>Cyprus</strong></td>
<td>Newsletter of the Network for Surveillance and Control of Communicable Diseases in Cyprus</td>
<td>Medical and Public Health Services, Ministry of Health, Nicosia</td>
<td>Biannual</td>
<td>Greek</td>
<td><a href="http://www.moh.gov.cy">http://www.moh.gov.cy</a></td>
</tr>
<tr>
<td><strong>Denmark</strong></td>
<td>EPI-NEWS</td>
<td>Department of Epidemiology, Statens Serum Institut, Copenhagen.</td>
<td>Weekly</td>
<td>Danish and English</td>
<td><a href="http://www.ssi.dk">http://www.ssi.dk</a></td>
</tr>
<tr>
<td><strong>Finland</strong></td>
<td>Kansanterveys</td>
<td>Department of Infectious Disease Epidemiology, National Public Health Institute, Helsinki.</td>
<td>Monthly</td>
<td>Finnish</td>
<td><a href="http://www.ktl.fi/portal/suomi/julkaisut/kansanterveyslehti">http://www.ktl.fi/portal/suomi/julkaisut/kansanterveyslehti</a></td>
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<tr>
<td><strong>France</strong></td>
<td>Bulletin épidémiologique hebdomadaire</td>
<td>Institut de veille sanitaire, Saint-Maurice Cedex.</td>
<td>Weekly</td>
<td>French</td>
<td><a href="http://www.invs.sante.fr/beh/default.htm">http://www.invs.sante.fr/beh/default.htm</a></td>
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<tr>
<td><strong>Hungary</strong></td>
<td>Epinfo (az Országos Epidemiológiai Központ epidemiológiai információs hezilapja)</td>
<td>National Center For Epidemiology, Budapest.</td>
<td>Weekly</td>
<td>Hungarian</td>
<td><a href="http://www.oek.hu/oek.web?t=839&amp;nid=41&amp;pid=7&amp;lang=hun">http://www.oek.hu/oek.web?t=839&amp;nid=41&amp;pid=7&amp;lang=hun</a></td>
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<tr>
<td><strong>Iceland</strong></td>
<td>EPI-ICE</td>
<td>Landlaeknisambættið Directorate Of Health, Seltjarnarnes</td>
<td>Monthly</td>
<td>Icelandic and English</td>
<td><a href="http://www.landlaeknir.is">http://www.landlaeknir.is</a></td>
</tr>
<tr>
<td><strong>Ireland</strong></td>
<td>EPI-INSIGHT</td>
<td>Health Protection Surveillance Centre, Dublin.</td>
<td>Monthly</td>
<td>English</td>
<td><a href="http://www.ndsc.ie/hpsc/EPI-Insight">http://www.ndsc.ie/hpsc/EPI-Insight</a></td>
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<td></td>
<td>Bolletino Epidemiologico Nazionale (BEN)</td>
<td>Istituto Superiore di Sanità, Reparto di Malattie Infettive, Rome.</td>
<td>Monthly</td>
<td>Italian</td>
<td><a href="http://www.epicentro.iss.it/ben">http://www.epicentro.iss.it/ben</a></td>
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<td><strong>Latvia</strong></td>
<td>Epidemiologiās Biletenti</td>
<td>Sabiedrības veselības agentura</td>
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<td><a href="http://www.sva.lv/epidemiologija/bileten">http://www.sva.lv/epidemiologija/bileten</a></td>
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<td><strong>Lithuania</strong></td>
<td>Epidemiologijos Žinios</td>
<td>Užkreciamų ligų profilaktikos ir kontroles centras</td>
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<td>Lithuanian</td>
<td><a href="http://www.ulac.lt/index.php?pl=26">http://www.ulac.lt/index.php?pl=26</a></td>
</tr>
<tr>
<td><strong>Netherlands</strong></td>
<td>Infectiezieken Bulletin</td>
<td>Rijksinstituut voor Volksgezondheid en Milieu</td>
<td>Monthly</td>
<td>Dutch</td>
<td><a href="http://www.rivm.nl/infectieziekenbulletin">http://www.rivm.nl/infectieziekenbulletin</a></td>
</tr>
<tr>
<td><strong>Norway</strong></td>
<td>MSIS-rapport</td>
<td>Folkehelseinstituttet, Oslo.</td>
<td>Weekly</td>
<td>Norwegian</td>
<td><a href="http://www.folkehelse.no/nyhetsbrev/msis">http://www.folkehelse.no/nyhetsbrev/msis</a></td>
</tr>
<tr>
<td><strong>Poland</strong></td>
<td>Meldunki o zachorowaniach na choroby zakazne i zatrucia w Polsce</td>
<td>Panstwowy Zaklad Higieny, National Institute of Hygiene, Warsaw.</td>
<td>Fortnightly</td>
<td>Polish and English</td>
<td><a href="http://www.pzh.gov.pl/epimeld/index_p.html#01">http://www.pzh.gov.pl/epimeld/index_p.html#01</a></td>
</tr>
</tbody>
</table>
PORTUGAL
Saúde em Números
Ministério da Saúde,
Direção-Geral da Saúde, Lisbon.
Sporadic, print only. In Portuguese.
http://www.dgs.pt

ROMANIA
Info Epidemiologia
Centrul pentru Prevenirea si Controlul Bolilor Transmisibile,
National Centre of Communicable Diseases Prevention and Control, Institute of Public Health, Bucharest.
Sporadic, print only. In Romanian.
http://www.cpcbt.ispb.ro

SLOVENIA
CNB Novice
Inštitut za varovanje zdravja, Center za nalezljive bolezni, Institute of Public Health, Center for Infectious Diseases, Ljubljana.
Monthly, online. In Slovene.

SPAIN
Boletín Epidemiológico Semanal
Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Madrid.
Fortnightly, print and online. In Spanish.
http://www.isciii.es/jsp/sps/centros/epidemiologia/boletinesSemanal.jsp

SWEDEN
EPI-aktuellt
Smittskyddsinstitutet, Stockholm.
Weekly, online. In Swedish.
http://www.smittskyddsinstitutet.se/publikationer/smis-nyhetsbrev/epi-aktuellt

UNITED KINGDOM
England and Wales
Health Protection Report
Health Protection Agency, London.
Weekly, online only. In English.
http://www.hpa.org.uk/hpr

Northern Ireland
Communicable Diseases Monthly Report
Communicable Disease Surveillance Centre, Northern Ireland, Belfast.
Monthly, print and online. In English.
http://www.cdscni.org.uk/publications

Scotland
Health Protection Scotland Weekly Report
Health Protection Scotland, Glasgow.
Weekly, print and online. In English.
http://www.hps.scot.nhs.uk/ewr/index.aspx

OTHER JOURNALS
EpiNorth journal
Norwegian Institute of Public Health, Folkehelseinstituttet, Oslo, Norway
Published four times a year in English and Russian.
http://www.epinorth.org

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“Europa” is the official portal of the European Union. It provides up-to-date coverage of main events and information on activities and institutions of the European Union.
http://europa.eu

European Commission - Public Health
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