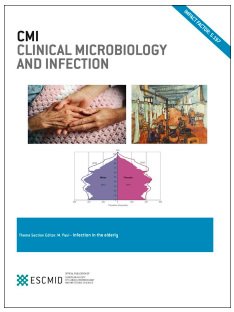
Preparing clinicians for (re-) emerging arbovirus infectious diseases in Europe

Accepted Manuscript



Preparing clinicians for (re-) emerging arbovirus infectious diseases in Europe

Louise Sigfrid, Chantal Reusken, Isabella Eckerle, Veronique Nussenblatt, Sam Lipworth, Janey Messina, Moritz Kraemer, Onder Ergonul, Anna Papa, Marion Koopmans, Peter Horby

PII: S1198-743X(17)30336-1

DOI: 10.1016/j.cmi.2017.05.029

Reference: CMI 984

To appear in: *Clinical Microbiology and Infection*

Received Date: 28 February 2017

Revised Date: 17 May 2017

Accepted Date: 28 May 2017

Please cite this article as: Sigfrid L, Reusken C, Eckerle I, Nussenblatt V, Lipworth S, Messina J, Kraemer M, Ergonul O, Papa A, Koopmans M, Horby P, Preparing clinicians for (re-) emerging arbovirus infectious diseases in Europe, *Clinical Microbiology and Infection* (2017), doi: 10.1016/j.cmi.2017.05.029.

- Louise Sigfrid
  - University of Oxford, Tropical Medicine and Global Health
  - Brighton & Sussex Medical School, Public Health
- Chantal Reusken
  - Erasmus MC, Department of Viroscience, Rotterdam
- Isabella Eckerle
  - University of Bonn Medical Centre, Institute of Virology
- Veronique Nussenblatt
  - Labaratory of Medical Microbiology, University of Antwerp,
- Sam Lipworth
  - University of Oxford, Tropical Medicine and Global Health
- Janey Messina
  - University of Oxford, School of Geography and the Environment, School of Interdisciplinary Area Studies
- Moritz Kraemer
  - University of Oxford , Department of Zoology
- Onder Ergonul
  - Koc Universitesi, School of Medicine, Infectious Diseases
- Anna Papa
  - Aristotle University of Thessaloniki, A Dept. of Microbiology
- Marion Koopmans
  - Erasmus MC, Department of Viroscience, Rotterdam
- Peter Horby
  - University of Oxford, Tropical Medicine and Global Health

Abstract

Background: Arthropod-borne virus (Arbovirus) infections are considered an emerging threat for Europe, with an increase in cases in recent decades. The increase in global travel and trade has contributed to the introduction of vectors and viruses into new geographical areas. Tropical arboviruses such as dengue and chikungunya have re-emerged causing local, sporadic outbreaks ignited by travel-imported cases. The recent Zika virus outbreak in the Americas highlighted a need to strengthen preparedness to (re-)emerging arbovirus infections globally.

Aims: To strengthen preparedness for the early identification of (re-)emerging arbovirus outbreaks in Europe and highlight areas for research.

Sources: An evidence review of published and grey literature together with consultations with European arbovirus experts.

Content: This paper presents an overview of endemic and travel-imported arboviruses of clinical significance in Europe. The overview includes syndromic presentation, risk factors for infection and risk of transmission. Moreover, an update on treatments and vaccinations and surveillance notifications and reporting. The paper also presents predictive modelled risks of further geographical expansion of vectors and viruses.

Implications: There are a range of arboviruses of clinical significance to Europe. There has been an increase in notifications of endemic and travel-imported arbovirus cases in recent years and an increased geographical range of vectors and viruses. The heterogeneity in surveillance reporting indicates a risk for the early identification of (re-)emerging outbreaks. The data presented shows a need to strengthen preparedness to (re-)emerging arbovirus infections and a need for research into neglected arboviruses, risks of non-vector transmission and effective therapeutics and vaccinations.

Introduction

Arboviruses use arthropod vectors as their main transmission route, predominantly mosquitoes, ticks, midges and sandflies.(1) They are sustained in a transmission cycle between arthropods as vectors and vertebrate animal reservoirs as the main amplifying hosts.(1) For some arboviruses, for instance West Nile virus (WNV), humans are a dead-end host, not generating enough viremia to infect vectors and contribute to onward transmission. Humans are the main reservoir for others, including dengue (DENV), chikungunya (CHIKV), and Zika virus (ZIKV), with a subsequent risk of local outbreaks without the need for an animal reservoir.

A number of arboviruses are endemic in Europe. Surveillance data shows an increase in geographical spread of both their vectors and viruses, but is likely to be biased by a lack of detection and vector surveillance capacity. Arboviruses have attracted renewed attention in Europe since the realisation that invasive mosquitoes were being re-introduced into Europe through travel and trade.(2, 3) *Aedes albopictus,* one of the most invasive mosquitoes is now endemic across southern Europe and was the main vector for the first outbreak of CHIKV in Italy (2007). (4, 5) *Aedes albopictus* is continuously expanding its range and eggs have now been found as far north as England (2016). (6) *Aedes aegypti*, introduced to Madeira in 2005 caused a large outbreak with >2000 cases of dengue there (2012), (7) the largest outbreak in Europe since an outbreak in Athens (1927-28) with >1000 mortalities.(8)

Surveillance data shows increased geographical spread of sandflies, the vectors for Toscana virus (TOSV), which is emerging as a leading cause of aseptic meningitis in southern Europe. (9-11) At the same time, ticks transmitting tick-borne encephalitis virus (TBEV) have extended their range into higher latitudes and altitudes. (12-14) Additionally, the main vector for Crimean- Congo haemorrhagic fever virus (CCHFV), *Hyalomma marginatum* ticks*,* traditionally endemic to south-east Europe, has extended further south-west. (15) The extended geographic range of vectors poses a risk of virus introduction into new areas, illustrated by the first two cases of CCHF in Spain in 2016.(16) Predictive modelling of vectors and viruses indicates risk of further geographical expansion. (17-19) The potential for non-vector transmission from body fluids, including blood transfusion transmission and most recently sexual transmission of ZIKV is another cause of concern (20).

Due to their complex transmission cycles, arboviruses require multidisciplinary surveillance and control schemes. Despite the known presence of significant arbovirus related disease, there are still gaps in surveillance data.(21) Reporting is heterogeneous; due to differing national surveillance systems and not all countries are covered by the European surveillance system.(22-26) Given the evidence that arboviruses are an increasing problem in Europe and the announcement in 2015 that ZIKV in Latin America was a public health emergency of international concern, here we review arboviruses of clinical significance in Europe. We also review current surveillance and reporting, with the aim to strengthen awareness and early identification of (re-) emerging arboviruses with epidemic potential and highlight areas for policy and research.

Arboviruses of clinical importance to Europe (Tables 1 and 2)

Europe is host to several endemic arboviruses of significant clinical importance. WNV, TBEV and TOSV can cause syndromes of neuroinvasive disease. CCHFV can lead to severe haemorrhagic fever and Sindbis virus (SINV) to syndromes of fever and arthralgia. DENV, CHIKV and ZIKV virus are not endemic to Europe, but there has been an increase of travel-imported cases in recent years. This has resulted in sporadic, local outbreaks of DENV and CHIKV in southern Europe, and reports of sexual and mother-child transmission of ZIKV across Europe.

Flaviviridae

Flaviviruses are the most important arboviruses globally. WNV re-emerged with an outbreak in Romania 1996 and has since caused regular outbreaks in southeast Europe (Fig.1). (27) There was a > 60% increase in case notifications at the EU level in 2016 (n=206, as of 18 Nov.16) compared to 2014 (n=74) (28). Austria reported its first case in 2014 (29) and Portugal its first laboratory-confirmed case in 2015.(30) WNV is maintained in an enzootic cycle with birds as amplifying hosts and *Culex* spp. as transmitting vectors. *Culex* spp. are the most widespread mosquito in Europe and are prevalent worldwide, except for the extreme northern parts of the temperate zone.(31) Approximately 20% of infected people develop mild West Nile fever; < 1% neuro-invasive disease. (32)

The numbers of recognized human cases of TBE in all endemic regions of Europe have increased by almost 400% in the last 30 years. (33) Notification rates are highest in the Baltic States, with the highest number of cases reported from the Czech Republic.(24) Greece reported its first autochthonous case in 2014, (24) followed by the Netherlands in 2016. (34) Eighteen EU/EEA member states reported cases of TBE (n=2,057) in 2014. (24) There are three subtypes of TBEV; the European subtype mainly transmitted by *Ixodes ricinus* ticks is widespread across Europe (Fig. 2), *Ixodes persulcatus* is the main vector of the Siberian and Far Eastern subtypes. In the Baltic countries and Finland there is an overlap of vectors and subtypes. (35) Infections with the European sub-type range from asymptomatic, mild flu-like illness to a bi-phasic course with severe neurological disease. (36)

There has been a large increase in dengue fever cases globally in the past decades. Travel-imported cases are frequently reported in Europe, which has caused sporadic, local outbreaks in regions with competent mosquito vectors (*Ae.aegypti*, *Ae.albopictus*), (37) including in Croatia (2010), Madeira (2012) and France (2010, 2014, 2015).(4, 23, 38-41) Twenty EU/EEA countries reported cases (n=1,796) in 2014, including four locally acquired in France. This was fewer compared to 2013, but higher compared to earlier years. (23) The frequency of DENV diagnoses in travellers is associated with travel behaviour. (42) Under-diagnosis is likely given the lack of standardisation of diagnostics for unexplained febrile illness syndromes and restrictive diagnostic testing.(42) Human DENV infection can range from asymptomatic (40-80%), to dengue fever, to severe hemorrhagic dengue. There are four serologically distinct DENV; sequential DENV infection can increase the risk of severe disease.(23)

There have not been any reports of vector-borne transmission of ZIKV in Europe,(43) but there has been a massive increase in travel-imported cases (n=2,078, from 21 countries) with 102 cases in pregnant women. Seven EU

countries have reported sexual transmission of ZIKV (as of 19 Jan. 2017). (43, 44) Worldwide 71 countries have reported mosquito-borne transmission of ZIKV since 2015 (as of 17 Jan. 2017), 29 countries reported potentially associated microcephaly and other CNS malformations in newborns and 21 countries an increased incidence of Guillain-Barre syndrome. Most infections are asymptomatic (80%) or cause a mild rash-illness. (45) ZIKV is mainly transmitted by *Aedes* spp. mosquitos: *Ae. aegypti* is the only species for which transmission outside Africa has been confirmed. *Ae. albopictus* has shown competence for ZIKV dissemination in laboratory studies but has not been implicated in ZIKV epidemiology in the field outside of Africa.(46)

Bunyaviridae

There has been an increase in notifications of CCHF cases in southeast Europe. More than 9,500 cases have been reported from Turkey (2002-2016).(47) Outbreaks have also been reported from Albania, Kosovo and Bulgaria.(48-50) The first two autochthonous cases detected in Spain (2016), highlights the risk of geographical spread and nosocomial transmission.(16) Probability modelling of risk of human CCHF occurrence indicates potential further areas at risk (Fig.3).(51) In 2014, nine cases likely from Bulgaria were reported with one detected in the UK (26). Human infections range from a febrile illness to severe haemorrhagic syndromes. (52) CCHFV is mainly transmitted by *Hyalomma* spp. ticks (26) and through animal blood or tissues e.g. during slaughter. Wild and domesticated animals act as reservoirs without developing disease. (51)

TOSV, transmitted by sandflies, mainly*Phlebotomus perniciosus*, *Phlebotomus perfiliewi,* is emerging as one of the leading causes of aseptic meningitis during the summer in regions in southern Europe, with increasing TOSV circulation reported around the Mediterranean basin (Fig.4) (53-55). Despite its clinical significance, it is a neglected disease with limited data available.(1) The role of vertebrates in the transmission cycle remains unclear. Seroprevalence studies indicate that a significant proportion of infections are asymptomatic or mild. A regional study from Italy found that 40% of meningitis/encephalitis cases in children and 52% of aseptic meningitis cases in adults were associated with TOSV infection (2010-12) (53, 56). A severe encephalitis case caused by TOSV lineage C has been reported in Greece. (57) A study from Granada, Spain found a 24.9% seroprevalence; several studies from Greece reported seroprevalence of between zero and 60%, with higher levels in coastal regions. (58-60)

Togaviridae

SINV is transmitted by mosquitoes (genus:*Culex* and *Culiseta*). Clinical infection is mainly reported in Northern Europe where SINV causes regular outbreaks of sindbis fever, known as Pogosta (Finland), Ockelbo (Sweden) and Karelia fever (Russia). Cases are seen yearly with larger outbreaks in Finland approximately every seven years (61). There is limited epidemiological data available. SINV or antibodies to SINV have been identified in wildlife across Europe[1]. The virus is maintained by transmission between bird hosts and mosquito vectors.(61) The viremic window is narrow, with low titers and there is no evidence of human-to-human transmission.

[1] Austria, Belarus, Bulgaria, Czech Republic, Estonia, Finland, Germany, Hungary, Italy, Moldova, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Spain, Sweden, Ukraine, and the UK.

(62) Infections can be asymptomatic or result in fever and arthralgia which can be chronic.(61)

The related CHIKV is transmitted by *Ae. aegypti* and *Ae. albopictus* mosquitos. CHIKV is not endemic to Europe, but there has been a steep increase in travel-imported cases (31, 63) which resulted in the first autochthonous outbreak in continental Europe in Italy (2007) and a smaller outbreak in France (2014) . (25, 64, 65) Thirteen EU/EEA countries reported cases in 2014 (n=1,461), a 20-fold increase compared with 2013 (n=72). Eleven cases were locally acquired in France. (25) Infections can present with syndromes of febrile illness and arthralgia. (25)

Arbovirus disease surveillance (Table 3)

The surveillance data reported to the European surveillance system (TESSy, 2014) shows that the number of EU/EEA countries providing reports has increased since the previous annual epidemiological report (2012). (66) However, there are still gaps in reporting: not all countries provide reports and reporting for some arboviruses was heterogeneous due to use of different case-definitions. (23, 25) Specific EU-level case-definitions exists for TBEV, WNV and an interim case-definition for ZIKV (24, 67, 68). A majority used the generic case-definition for viral haemorrhagic fever for CCHFV, DENV and CHIKV infections. (23, 25, 26) (Appendix 1-4). TOSV and SINV are not notifiable at EU-level.

Risk factors

Risk factors for infection include spending time outdoors and working with animals in endemic areas. A majority of autochthonous and travel-imported cases in Europe are reported in May-October, with peaks in July–September. Chikungunya cases were reported until December, (25) and a smaller peak of imported DENV in January. The highest peak in Sweden and Finland was in January-April, reflecting travelling patterns. (23) EU notifications of endemic arboviruses were highest in men and older age-groups, travel-imported viruses in younger age-groups likely relecting population travel-patterns. (22, 24) There is a lack of demographic data for TOSV, CCHFV and SINV infections. Studies have shown highest number of TOSV cases in adults >25 years old, but higher seroprevalence in older age-groups, (69) SINV infections in 30-69 year olds. (61) There is limited data about the risk of long-term sequelae and risk-factors for severe disease.

Non-vector transmission

Non –vector transmission has been documented for several (Table 1). TBEV has been linked to consuming unpasteurized milk products, CCHFV to slaughter, human-to-human and nosocomial transmission from close contact with bodily fluids or improper sterilization of medical equipment.(70) Sexual transmission (male-to-female), up to >six weeks post-onset of symptoms and possibly by other human body fluids has been reported for ZIKV.(45, 71-75) Blood transfusion, (76, 77) organ transplant (37, 77, 78) and vertical transmission (77, 78) have been reported for DENV and WNV. However, there are limited studies on the risk of transmission from different body fluids.

Treatment

No specific antiviral treatment exists for any of the arboviruses, and supportive care, fluid and electrolyte management and haematological support for haemorrhage, are

the mainstays of clinical management. Observational data suggest that ribavirin may be beneficial against CCHFV, but there have been no randomised-controlled trials.(79-81) Ribavirin is recommended as CCHFV post-exposure prophylaxis for health care workers, (82-84) but with limited studies on effectiveness. Corticosteroids have been indicated to be beneficial among a few severely ill CCHF patients (80) and tested as treatment for dengue-related shock and patients at an early stage to prevent complications, but with insufficient data available to make recommendations on their use.(85) There is an ongoing clinical trial evaluating the use of an α-glucosidase I inhibitor and a platelet-activating factor antagonist against dengue fever. (86)

Vaccinations

There are effective vaccines available for TBEV; (87) vaccination campaigns have effectively reduced the incidence of TBE in targeted areas.(88) Human ZIKV, DENV, WNV and CHIKV vaccines are undergoing clinical trials.(89-91) The first human dengue vaccine is recommended by WHO for use only in regions with high burden of disease.(92) Phase 3 trials showed varying vaccine-efficacy against different serotypes, by age and previous DENV exposure. (92)

Conclusion

European health systems are increasingly confronted with the challenges of arbovirus infections, with an increase in notifications and geographical distribution of vectors and viruses in recent decades. A rise in global travel and trade, poses a risk of introduction of arboviruses into new geographical areas. Models of the predicted distribution of *Ae. albopictus* and *Ae. aegypti* mosquitoes based on surveillance data and environmental modelling, indicates risk of further geographic expansion across south and south-eastern Europe (Fig. 5). (17-19) The expansion of DENV and CHIKV globally has been preceded by the spread of their vectors. (18) The first two cases of autochthonous CCHFV infections detected in southwestern Europe in 2016, together with modelling of suitability of CCHF occurrences, also highlights the risk of geographical expansion of endemic arboviruses.(19)

The identification of emerging outbreaks of arboviruses in Europe, and globally, is challenging. A large proportion of infections are asymptomatic, subclinical or presents with non-specific symptoms. Together with variations in access to diagnostic testing this indicates that many cases are undiagnosed and the true burden of arbovirus infections is largely unknown. The gaps and heterogeneity in surveillance reporting is another cause of concern indicating risk of delayed detection of (re-) emerging outbreaks.

The risk of localized outbreaks of travel-imported arboviruses and of non-vector transmission highlights the importance of early identification of cases. Access to up-to-date information together with detailed travel and vaccination history and exposure to ticks or insects, can aid identification of cases and diagnostics.(1) A combination of vector surveillance and case-based reporting is used at the EU level to alert blood banks and provide recommendations on blood donor deferral and systematic screening of visitors from endemic areas.(93-95)

This data highlights a need to strengthen preparedness to (re-) emerging arbovirus infections across Europe for the early identification of outbreaks. There is a need to

strengthen integrated surveillance through awareness raising and access to diagnostics and harmonized case-definitions. Furthermore, a need for research into neglected arboviruses, non-vector transmission routes, as well as effective therapeutics and vaccinations.

Transparency declaration

The authors declare no conflicts of interest.

Funding

This work forms part of the PREPARE (Platform for European Preparedness against (re-) emerging epidemics funded by the European Commission under grant number 602525.

MUGK is supported by a Training Grant from the National Institute of Child Health and Human Development, T32HD040128.

Family Genus	Virus	Transmission	Syndromes	European regions and risk(1)	Occurrence
Flavivirus **Flavivirus**	West Nile virus (WNV)	**Mosquito** Blood transfusion Organ transplant Vertical (rare) Breast-feeding (rare)	Febrile illness Rash Neurological syndrome	Southern, South-east and Central Europe (high risk)	Endemic
	Tick-borne encephalitis virus (TBEV)	**Ticks** Animal tissue(96) Blood transfusion(96) Breastfeeding(96)	Febrile illness Rash Neurological syndrome	Northern, Central and Eastern Europe (high risk)	Endemic
	Dengue virus (DENV)	**Mosquito** Anthroponotic* Blood transfusion Transplant Vertical(97) Breast milk(97)	Febrile illness Rash and/or arthralgia Haemorrhagic syndrome Neurological syndrome	Madeira and Southern Europe (low risk)	Sporadic, localised outbreaks**
Bunyaviridae **Nairovirus**	Crimean-Congo Haemorrhagic fever (CCHFV)	**Tick** Animal- & human-fluids Nosocomial	Febrile illness Rash and/or arthralgia Haemorrhagic syndrome	South-east and Eastern Europe (low risk)	Endemic
Bunyaviridae **Phlebovirus**	Toscana virus (TOSV)	**Sandfly**	Febrile illness Rash Neurological syndrome	Southern and South-east Europe (high risk)	Endemic
Togaviridae **Alphavirus**	Chikungunya virus (CHIKV)	**Mosquito** Anthroponotic(98) Vertical(98)	Febrile illness Arthralgia	Southern Europe (low risk)	Sporadic, localised outbreaks**
	Sindbis virus (SINV)	**Mosquito**	Rash and arthralgia	Northern Europe	Endemic

*****Anthroponotic**:** Human-vector-human transmission ** Risk of local outbreaks through travel-imported cases into regions with *Aedes* spp. mosquitoes

Table 1. Arboviruses of clinical importance to Europe with risk of vector-borne transmission in Europe
The table shows an overview of arboviruses including reported transmission routes, main syndromes and risks by region. (1)

Virus	Incubation (days,range)	Mild infection	Severe infection	Mortality rate*
WNV	3-14	Fever with headache, body aches, joint pains, vomiting, diarrhea, or rash (erythematous maculopapular or morbilliform	< 1% (mainly elderly): Encephalitis, meningitis. Long term sequela.	Approx. 5%^
TBEV	7 (4–28)	1st phase: Febrile illness with headache, myalgia, and fatigue. Lasts for several days and may be followed by an afebrile and relatively asymptomatic period (1-33 days) after which 1/3 develop a second phase of more severe disease.	Second phase: aseptic meningitis, encephalitis, or myelitis. Meningeal signs, altered mental status, cognitive dysfunction, ataxia, rigidity, seizures, tremors, cranial nerve palsies, and limb paresis. Long-term sequela in 30%.	0.5–2%^^
CCHFV	3–7 (1 -13)**	Febrile illness with headache, myalgia, backache, joint, abdominal pain and vomiting.	Haemorrhagic syndrome, from petechiae to ecchymoses on the mucous membranes & the skin; most common bleeding sites: nose, gastrointestinal system, uterus, urinary and respiratory tracts. Necrotic hepatitis may occur.	2 – 10% (Europe) (52)
TOSV	3 - 14	Febrile illness	Aseptic meningitis, facial paralysis, tremors, rash	None reported
SINV	<7days (not established)	Maculopapular, often pruritic rash (trunk and limbs), mild fever, joint symptoms, (mainly wrists, hips, knees, ankles), nausea, headache, myalgia.	Chronic arthritis	None reported
DENV	4–7 (3–14)	High fever, severe headache, retro-orbital pain, myalgia, arthralgia, a maculopapular rash and minor haemorrhage, which can follow a 'saddleback' sequence with brief remission on day3.	Haemorrhagic syndrome with severe plasma leakage; shock or fluid accumulation, respiratory distress; severe bleeding; or severe organ impairment, impaired consciousness or heart impairment.	Approx. 2.5 % (highest risk children and adolescents)
CHIKV	3-7 (1-12)	Fever, headache, myalgia, nausea, photophobia, incapacitating joint pain and petechial or maculopapular rash. Recurrent symmetric joint pain (30-40%) can last for years.	Symmetric arthralgia, neurological, haemorrhagic and ocular manifestations, myocarditis, hepatitis. Meningoencephalitis (neonates).	Approx. 0.02%
ZIKV	3-12	Maculopapular rash (+/-itchy), with or without mild fever, arthralgia, fatigue, non-purulent conjunctivitis/conjunctival hyperaemia, myalgia, headache.	Guillain-Barre syndrome, Microcephaly and other CNS malformations (fetuses)	None reported.

*with supportive treatments ** generally shorter following nosocomial infection. ^ 4% to 14% if neuro-invasive disease, increased risk with age ^^ up to 35% in Far Eastern subtype
WNV: West Nile virus; TBEV: tick-borne encephalitis virus; CCHFV: Crimean-Congo haemorrhagic fever virus; TOSV: Toscana virus; SINV: Sindbis virus; DENV: Dengue fever; CHIKV: Chikungunya virus

Table 2: Clinical manifestation (33, 38, 45, 56, 61, 99-101)

Clinical infection	Countries reporting data (n (%))	Cases reported (n)	Locally acquired (n)	Confirmed cases (n)	Notification rate (per 100,000 population)	Countries using an EU case definition* (n (%))	Highest rate: Age group (years)	Highest rate: (Male: Female ratio)
WNF	25 (81%)	77	74	63	0.01	21 (84%)	>65	2.3:1
TBE	24 (77%)	2057	NR	986	0.42	16 (67%)	>45	1.4:1
CCHF	25 (81%)	9	8	4	_	20 (80%)	45-64	Men (89%)
Dengue fever	25 (81%)	1796	4	1510	0.42	16 (67%)	25-44	1.1:1**
Chikungunya	23 (74%)	1461	11	875	0.31	16 (70%)	15-44	0.7:1

NR: not reported * Proportion of EU/EEA countries reporting. The generic EU case definition for VHFs where used for CCHF, Dengue fever and Chikungunya. **in the age group 15-24years old the proportion of cases was higher in females

Table 3. Surveillance data on infections reported by EU/EEA countries (2014)
The table shows surveillance data reported to the European surveillance system TESSy in 2014 by the 31 EU/EEA countries. (22-26)

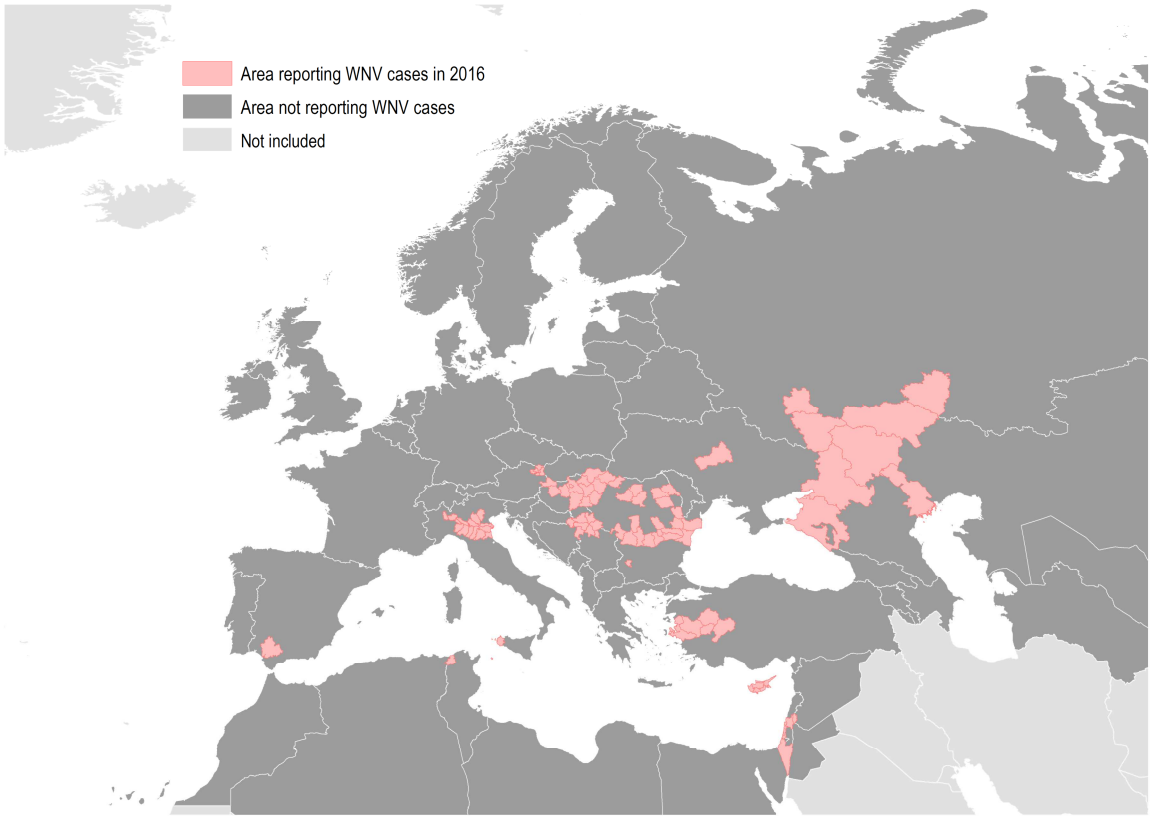


Figure 1. West Nile fever surveillance data

The map shows countries in Europe reporting human cases of West Nile virus infections in 2016.(102)

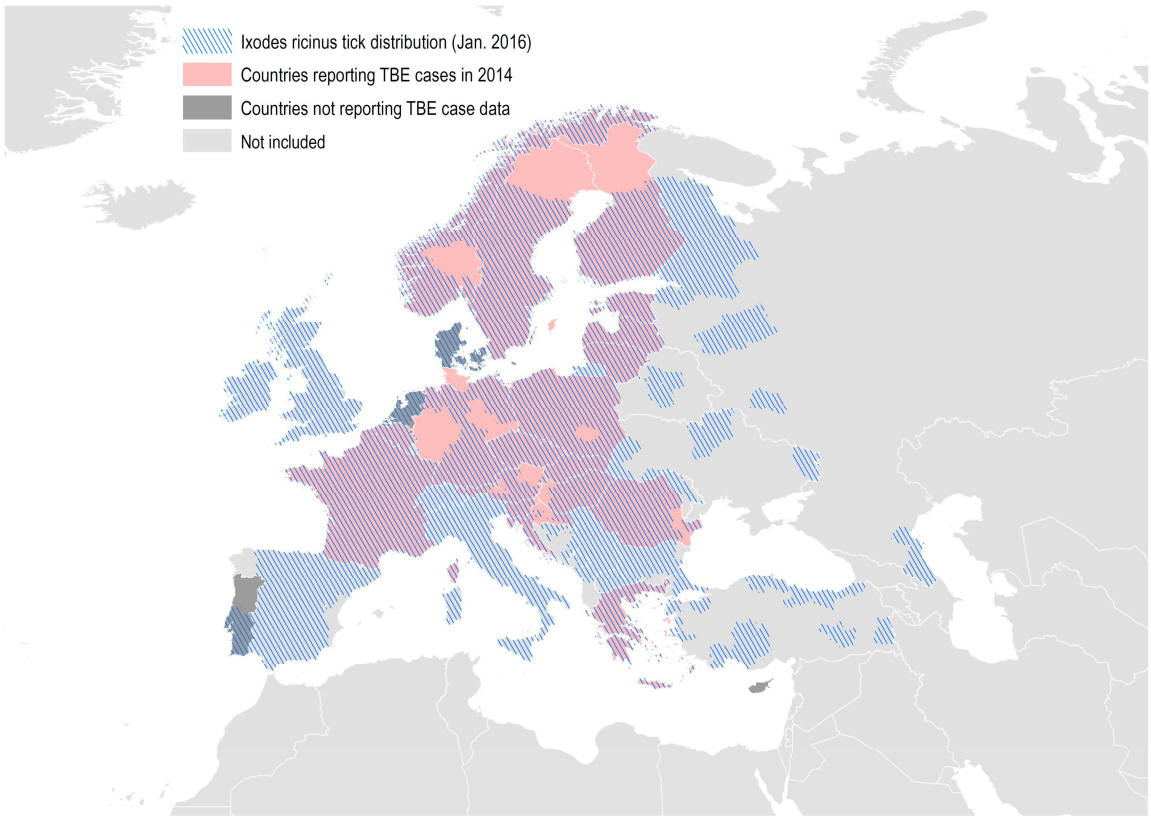


Figure 2. Tick-borne encephalitis and *Ixodes ricinus* surveillance data
The map shows EU/EEA countries reporting cases of TBEV infections at the EU level in 2014 and the current known distribution of *Ixodes ricinus* ticks in Europe (as of Jan.2016)(13, 24)

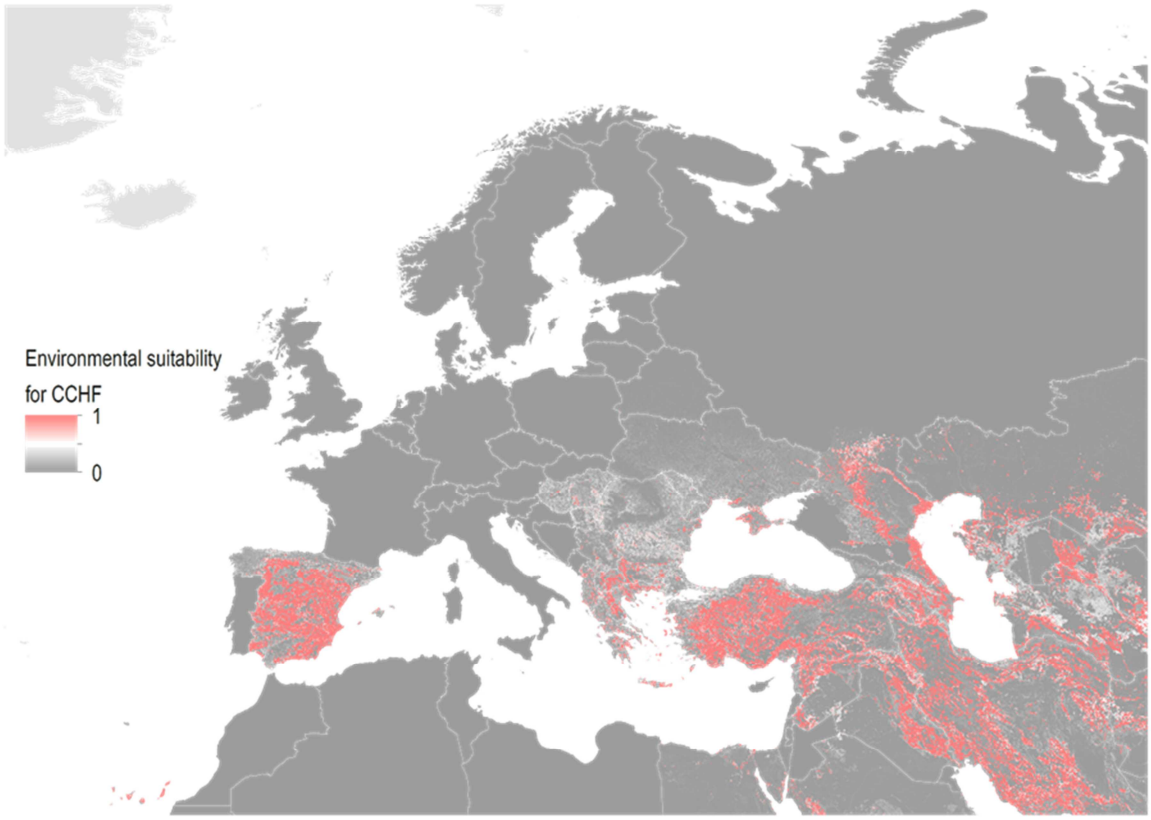


Fig 3. Probability of human CCHF occurence
The maps show the probability of human CCHF occurence based on an exhaustive data base of CCHF cases and an ecological niche modelling framework (methodology described in Messina et al, 2015). Areas in red are those most suitable for transmission. (19)

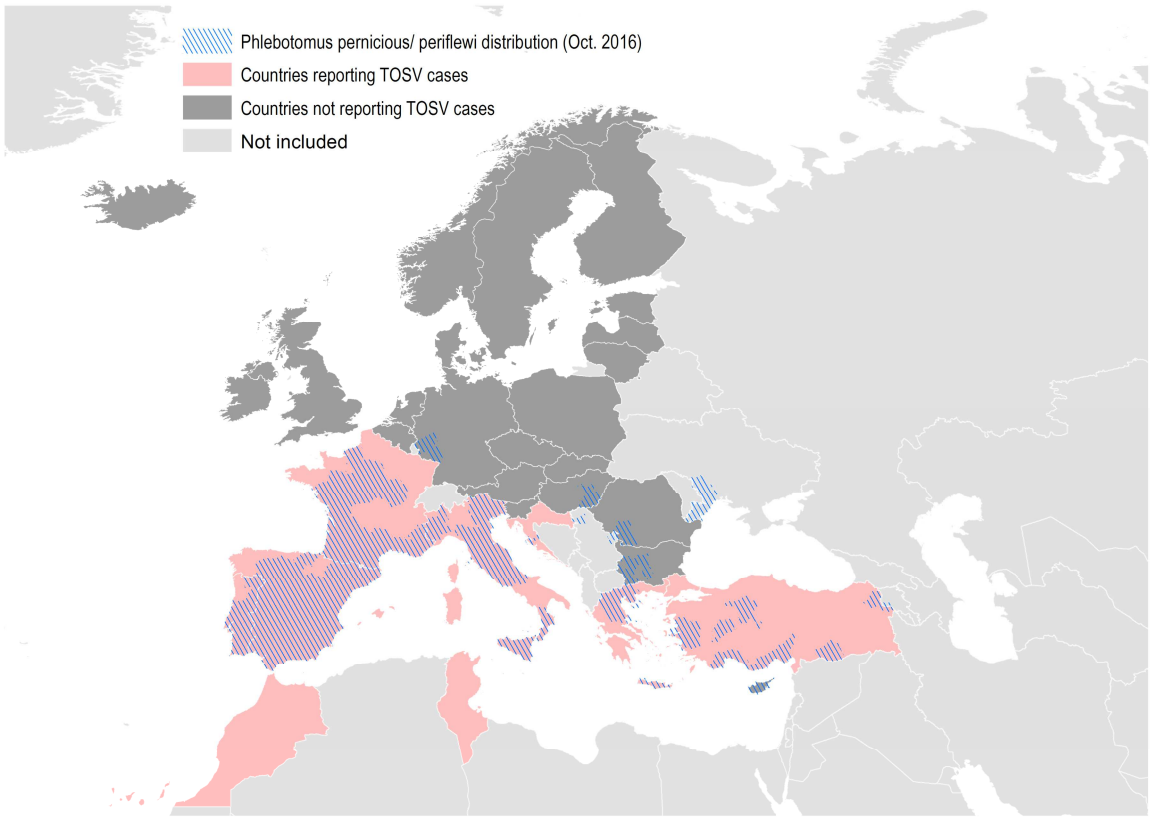


Figure 4: Toscana virus case reports and vector distribution
The map shows countries with reports of human cases of TOSV infections and the current known distribution of the *Phlebotomus pernicious* and *Phlebotomus perfiliewi* sandflies (VectorNet data as of Oct. 2016).(53, 56, 103, 104) *Phlebotomus neglectus* has recently been identified as another vector in Croatia (Charrel & Ayhan 2017, unpublished results). Note: the VectorNet data covers Europe only and not neighbouring regions around the Mediterranean basis, such as North Africa where TOSV and sandfly vectors, including *Phlebotomus sergenti* and *Phlebotomus longicuspis* are known to be present (105).

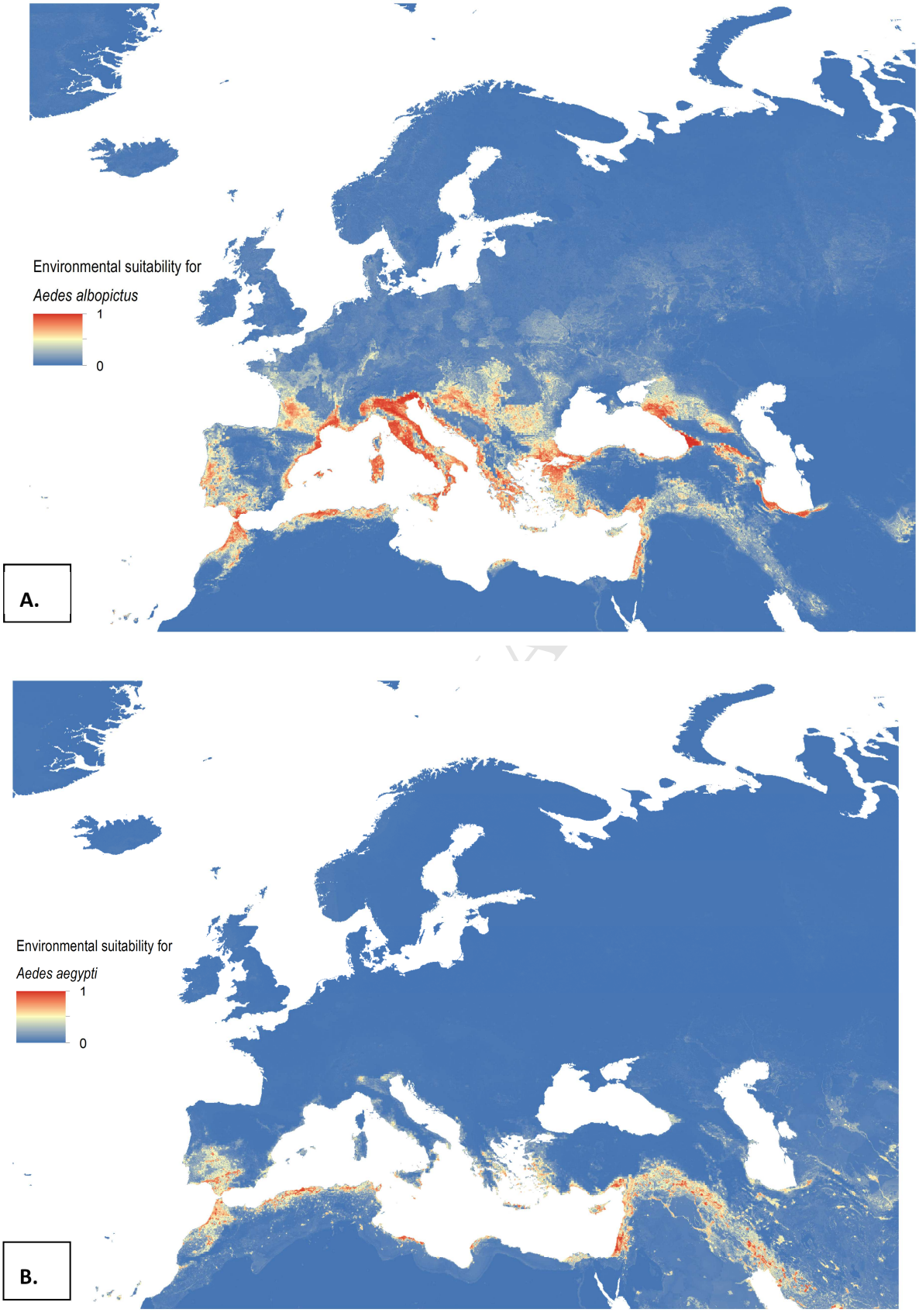


Figure 5. Predicted distribution of *Aedes albopictus and Aedes aegypti*
The maps show the predicted distribution of *Ae. albopictus* (A) and of *Ae. aegypti* (B) in Europe, based on data on the known locations of the species combined with information on environmental conditions (methodology described in Kraemer et al, 2015). The map depicts the probability of occurrence (from 0 blue to 1 red) at a spatial resolution of 5 km × 5 km. (18)

References

1. Cleton N, Koopmans M, Reimering J, Godeke G, Reusken C. Come fly with me Review of clinically important arboviruses for global travelers. Journal of Clinical Virology. 2012.

2. Scholte EJ, Dijkstra E, Blok H, De Vries A, Takken W, Hofhuis A, et al. Accidental importation of the mosquito Aedes albopictus into the Netherlands: a survey of mosquito distribution and the presence of dengue virus. Medical and veterinary entomology. 2008;22(4):352-8.

3. Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: global risk of invasion by the mosquito Aedes albopictus. Vector Borne Zoonotic Dis. 2007;7(1):76-85.

4. Rezza G. Dengue and other Aedes-borne viruses: a threat to Europe? Eurosurveillance. 2016;21(21).

5. Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, et al. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet. 2007;370(9602):1840-6.

6. PHE. Mosquito: nationwide surveillance: PHE; 2016 [cited 2016 1 Dec]. Available from: https://www.gov.uk/government/publications/mosquito-surveillance/mosquito-nationwide-surveillance.

7. Seixas G, Salgueiro P, Silva AC, Campos M, Spenassatto C, Reyes-Lugo M, et al. Aedes aegypti on Madeira Island (Portugal): genetic variation of a recently introduced dengue vector. Memórias do Instituto Oswaldo Cruz. 2013;108(Suppl 1):3-10.

8. Louis C. Daily Newspaper View of Dengue Fever Epidemic Athen, Greece 1927–1931. Emerg Infect Dis 2012;18(1):78-82.

9. ECDC. Phlebotomine sand flies Stockholm: ECDC; [cited 2016 4 Nov].

10. Ballart C, Barón S, Alcover MM, Portús M, Gállego M. Distribution of phlebotomine sand flies (Diptera: Psychodidae) in Andorra: First finding of P. perniciosus and wide distribution of P. ariasi. Acta Tropica. 2012;122(1):155-9.

11. Maroli M, Rossi L, Baldelli R, Capelli G, Ferroglio E, Genchi C, et al. The northward spread of leishmaniasis in Italy: evidence from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. Tropical medicine & international health : TM & IH. 2008;13(2):256-64.

12. Danielová V, Schwarzová L, Materna J, Daniel M, Metelka L, Holubová J, et al. Tick-borne encephalitis virus expansion to higher altitudes correlated with climate warming. International Journal of Medical Microbiology. 2008;298, Supplement 1:68-72.

13. ECDC. Ixodes ricinus Stockholm: ECDC; [cited 2016 2 Dec].

14. Jaenson TG, Jaenson DG, Eisen L, Petersson E, Lindgren E. Changes in the geographical distribution and abundance of the tick Ixodes ricinus during the past 30 years in Sweden. Parasites & Vectors. 2012;5(1):8.

15. ECDC. Hyalomma marginatum Stockholm: ECDC; [cited 2016 2 Dec].

16. ECDC. Rapid risk assessment Crimean-Congo haemorrhagic fever in Spain Stockholm: ECDC; 2016 [cited 2016 15 Nov].

17. Kraemer MU, Sinka ME, Duda KA, Mylne A, Shearer FM, Brady OJ, et al. The global compendium of Aedes aegypti and Ae. albopictus occurrence. Scientific data. 2015;2:150035.

18. Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. eLife. 2015;4:e08347.

19. Messina JP, Pigott DM, Golding N, Duda KA, Brownstein JS, Weiss DJ, et al. The global distribution of Crimean-Congo hemorrhagic fever. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2015.

20. Yakob L, Kucharski A, Hue S, Edmunds WJ. Low risk of a sexually-transmitted Zika virus outbreak. The Lancet Infectious Diseases.16(10):1100-2.

21. Rizzo C, Napoli C, Venturi G, Pupella S, Lombardini L, Calistri P, et al. West Nile virus transmission results from the integrated surveillance system in Italy 2008 to 2015. Eurosurveillance. 2016;21(37).

22. ECDC. Annual epidemiological report West Nile fever. Stockholm: ECDC; 2016.

23. ECDC. Annual epidemiologica report 2016 Dengue fever Stockholm: ECDC; 2016 [cited 2016 29 Dec.]. Available from: http://ecdc.europa.eu/en/healthtopics/dengue_fever/Pages/Annual-epidemiological-report-2016.aspx.

24. ECDC. Annual epidemiological report Tick-borne encephalitis Stockholm: ECDC; 2016 [cited 2016 4 Nov].

25. ECDC. Annual epidemiological report Chikungunya fever Stockholm: ECDC; 2016 [cited 2016 4 Nov]. Available from: http://ecdc.europa.eu/en/healthtopics/chikungunya_fever/Pages/Annual-epidemiological-report-2016.aspx.

26. ECDC. Annual epidemiological report Crimean-Congo haemorrhagic fever Stockholm: ECDC; 2016 [cited 2016 4 Nov].

27. ECDC. West Nile fever Stockholm: ECDC; [cited 2016 8 Nov].

28. ECDC. West Nile fever maps Stockholm: ECDC; 2016 [cited 2016 22 Nov]. Available from: http://ecdc.europa.eu/en/healthtopics/west_nile_fever/West-Nile-fever-maps/Pages/index.aspx.

29. Jungbauer C, Hourfar MK, Stiasny K, Aberle SW, Cadar D, Schmidt-Chanasit J, et al. West Nile virus lineage 2 infection in a blood donor from Vienna, Austria, August 2014. Journal of Clinical Virology.64:16-9.

30. Ze-Ze L, Proenca P, Osorio HC, Gomes S, Luz T, Parreira P, et al. Human case of West Nile neuroinvasive disease in Portugal, summer 2015. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2015;20(38).

31. ECDC. An emerging threat Mosquito borne diseases in Europe Stockholm: ECDC; [cited 4-Nov-2016.

32. WHO. West Nile Virus Infection (WNV) in Europe 2011 [cited 2015 Jan]. Available from: http://www.who.int/csr/don/2011_08_16/en/.

33. ECDC. Tick borne encephalitis Factsheet for health professionals Stockholm: ECDC; [cited 2016 29 Dec.].

34. Graaf J, Reimering J, Voorn G, Vaate E, Vries A, Rockx B, et al. First human case of tick-borne encephalitis virus infection acquired in the Netherlands July 2016. Euro Surveillance. 2016;21(33).

35. Heinz F, Stiasny K, Holzmann H, Kundi M, Sixl W, Wenk M, et al. Emergence of tick-borne encephalitis in new endemic areas in Austria: 42 years of surveillance. Eurosurveillance. 2015;20(3).

36. Mantke O, Schädler R, Niedrig M. A survey on cases of tick-borne encephalitis in European countries Eurosurveillance. 2008;13(17).

37. ECDC. Dengue fever Stockholm: ECDC; [cited 2016 4 Nov].

38. ECDC. Dengue fever Factsheet for health professionals Stockholm: ECDC; [cited 2016 6 Nov].

39. Wilder-Smith A, Quam M, Sessions O, Rocklov J, Liu-Helmersson J, Franco L, et al. The 2012 dengue outbreak in Madeira: exploring the origins 2014;19(8).

40. La Ruche G, Souares Y, Armengaud A, Peloux-Petiot F, Delaunay P, Despres P, et al. First two autochthonous dengue virus infections in metropolitan France, September 2010. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2010;15(39):19676.

41. Gjenero-Margan I, Aleraj B, Krajcar D, Lesnikar V, Klobucar A, Pem-Novosel I, et al. Autochthonous dengue fever in Croatia, August-September 2010. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2011;16(9).

42. Cleton NB, Reusken CBEM, Wagenaar JFP, van der Vaart EE, Reimerink J, van der Eijk AA, et al. Syndromic Approach to Arboviral Diagnostics for Global Travelers as a Basis for Infectious Disease Surveillance. PLOS Neglected Tropical Diseases. 2015;9(9):e0004073.

43. ECDC. Zika epidemics 2014 onwards Epidemiological situation Stockholm: ECDC; 2016 [cited 2016 16 Nov].

44. ECDC. Epidemiological update Outbreaks of Zika virus and complications potentially linked to the Zika virus infection Stockholm: ECDC; 2016 [cited 2016 4 Nov].

45. ECDC. Zika virus Factsheet for health professionals Stockholm: ECDC; 2016 [cited 2016 29 Dec.].

46. Charrel R, Leparc-Goffart I, Pas S, Lamballerie X, Koopmans M, Reusken C. Background review for diagnostic test development for Zika virus infection. Bulletin of the World Health Organization 2016;94:574-584D. 2016;94:574-84.

47. Leblebicioglu H, Ozaras R, Irmak H, Sencan I. Crimean-Congo hemorrhagic fever in Turkey: Current status and future challenges. Antiviral Research. 2016;126:21-34.

48. ECDC. Consultation on Crimean-Congo haemorrhagic fever prevention and control Stockholm: ECDC; 2009 [cited 2015 Jan].

49. Maltezou HC, Andonova L, Andraghetti R, Bouloy M, Ergonul O, Jongejan F, et al. Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2010;15(10):19504.

50. Papa A, Pappa S, Panayotova E, Papadopoulou E, Christova I. Molecular epidemiology of Crimean-Congo hemorrhagic fever in Bulgaria--An update. Journal of medical virology. 2016;88(5):769-73.

51. Messina JP, Pigott DM, Golding N, Duda KA, Brownstein JS, Weiss DJ, et al. The global distribution of Crimean-Congo hemorrhagic fever. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2015;109(8):503-13.

52. Ergönül Ö. Crimean-Congo haemorrhagic fever. ecLancet Infectious diseases. 2006;6:203-14.

53. Charrel RN, Bichaud L, de Lamballerie X. Emergence of Toscana virus in the mediterranean area. World journal of virology. 2012;1(5):135-41.

54. Calzolari M, Angelini P, Finarelli A, Cagarelli R, Bellini R, Albieri A, et al. Human and entomological surveillance of Toscana virus in the Emilia-Romagna region, Italy, 2010 to 2012. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2014;19(48).

55. Charrel R. Toscana virus infection. Ergonul O, Can F, Akova M, Madoff L, editors2014.

56. Charrel RN, Gallian P, Navarro-Mari JM, Nicoletti L, Papa A, Sanchez-Seco MP, et al. Emergence of Toscana virus in Europe. Emerging Infectious Diseases. 2005;11(11):1657-63.

57. Papa A, Paraforou T, Papakonstantinou I, Pagdatoglou K, Kontana A, Koukoubani T. Severe encephalitis caused by Toscana virus. Emerg Infect Dis 2014.

58. Anagnostou V, Papa A. Seroprevalence of Toscana virus among residents of Aegean Sea islands, Greece. Travel medicine and infectious disease. 2013;11(2):98-102.

59. Anagnostou V, Papa A. Prevalence of antibodies to phleboviruses within the sand fly fever Naples virus species in humans, northern Greece. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2013;19(6):566-70.

60. Papa A, Andriotis V, Tzilianos M. Prevalence of Toscana virus antibodies in residents of two Ionian islands, Greece. Travel medicine and infectious disease. 2010;8(5):302-4.

61. ECDC. Sindbis fever Factsheet for health professionals Stockholm: ECDC; [cited 2016 16 Nov].

62. ECDC. Tick maps Stockholm: ECDC; 2016 [cited 2016 15 Nov].

63. ECDC. Chikungunya risk assessments Stockholm: ECDC; 2015 [cited 2016 11 Nov].

64. Delisle E, Rousseau B, Broche I, Leparc-Goffart I, L'Ambert G, Cochet A, et al. Chikungunya outbreak in Montpellier France September to October 2014. 20. 2015(17).

65. Angelini R, Finarelli A, Angelini P, Po C, Petropulacos K, Macini P, et al. An outbreak of chikungunya fever in the province of Ravenna Italy. Eurosurveillance. 2007;12(36).

66. ECDC. Annual epidemiological report Emerging and vector-borne diseases 2014. Stockholm: ECDC; 2014.

67. ECDC. West Nile fever EU case defintion Stockholm: ECDC; [cited 2016 4 Nov].

68. ECDC. Zika virus case definition Stockholm: ECDC; 2016 [cited 2016 6 Dec]. Available from: http://ecdc.europa.eu/en/healthtopics/zika_virus_infection/patient-case-management/Pages/case-definition.aspx.

69. Cusi MG, Savellini GG, Zanelli G. Toscana virus epidemiology: from Italy to beyond. Open Virol J. 2010;4:22-8.

70. WHO. Crimean-Congo haemorrhagic fever Fact sheet No208: WHO; 2013 [cited 2016 10 Nov].

71. ECDC. Rapid risk assessment Zika virus disease epidemic Ninth update 28 October 2016. Stockholm: ECDC; 2016.

72. Foy B, Kobylinski K, Foy J, Blitvich B, Ravassos da Rosa A, Haddow A. Probable Non–Vector-borne Transmission of Zika Virus Colorado USA. Emerg Infect Dis. 2011;17(5):880-2.

73. D'Ortenzio E, Matheron S, de Lamballerie X, Hubert B, Piorkowski G, Maquart M, et al. Evidence of Sexual Transmission of Zika Virus. New England Journal of Medicine. 2016;374(22):2195-8.

74. Atkinson B, Hearn P, Afrough B, Lumley S, Carter D, Aarons EJ, et al. Detection of Zika Virus in Semen. Emerg Infect Dis. 2016;22(5):940.

75. Shrestha P HP, Carson G. [Submitted]. . . Non-vector transmission of flaviviruses, with implications for the Zika virus. Bull World Health Organ2016.

76. Pozzetto B, Memmi M, Garraud O. Is transfusion-transmitted dengue fever a potential public health threat? World journal of virology. 2015;4(2):113-23.

77. WHO. West Nile virus Fact sheet N°354 World Health Organisation; 2011 [cited 2017 May]. Available from: http://www.who.int/mediacentre/factsheets/fs354/en/.

78. Gupta RK, Gupta G, Chorasiya VK, Bag P, Shandil R, Bhatia V, et al. Dengue Virus Transmission from Living Donor to Recipient in Liver Transplantation: A Case Report. Journal of clinical and experimental hepatology. 2016;6(1):59-61.

79. Ergonul O. Treatment of Crimean-Congo hemorrhagic fever. Antiviral Research. 2008;78(1):125-31.

80. Dokuzoguz B, Celikbas AK, Gok SE, Baykam N, Eroglu MN, Ergonul O. Severity scoring index for Crimean-Congo hemorrhagic fever and the impact of ribavirin and corticosteroids on fatality. Clin Infect Dis. 2013;57(9):1270-4.

81. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infectious Diseases. 2010;10:207-.

82. Canada. Phao. Crimean-Congo Haemorrhagic fever virus pathogen safety data sheet infectious substances: Public health agency of Canada; [cited 2016 13 Nov].

83. Celikbas AK, Dokuzoguz B, Baykam N, Gok SE, Eroglu MN, Midilli K, et al. Crimean-Congo hemorrhagic fever among health care workers, Turkey. Emerg Infect Dis. 2014;20(3):477-9.

84. Guner R, Hasanoglu I, Tasyaran MA, Yapar D, Keske S, Guven T, et al. Is ribavirin prophylaxis effective for nosocomial transmission of Crimean-Congo hemorrhagic fever? Vector Borne Zoonotic Dis. 2014;14(8):601-5.

85. Zhang F, Kramer C. Corticosteroids for dengue infection: DfID; 2014 [cited 2016 1 Dec]. Available from: https://www.gov.uk/dfid-research-outputs/corticosteroids-for-dengue-infection.

86. ClinicalTrialsgov. Celgosivir or Modipafant as Treatment for Adult Participants With Uncomplicated Dengue Fever in Singapore: US National Institutes of Health; 2015 [cited 2016 23 Jan.]. Available from: https://clinicaltrials.gov/ct2/show/NCT02569827?term=dengue+AND+antivirals&rank=1.

87. WHO. Vaccines against tick-borne encephalitis. WHO; 2011.

88. Šmit R. Cost-effectiveness of tick-borne encephalitis vaccination in Slovenian adults. Vaccine. 2012;30(44):6301-6.

89. ClinicalTrialsgov. Dengue vaccination: US National Institutes of Health; 2016 [cited 2017 1 Jan.]. Available from: https://clinicaltrials.gov/ct2/results?term=Dengue+vaccination&Search=Search.

90. ClinicalTrialsgov. Zika vaccination: US National Institutes of Health; 2016 [cited 2016 1 Jan.]. Available from: https://clinicaltrials.gov/ct2/results?term=Zika+vaccination&Search=Search.

91. ClinicalTrialsgov. Chikungunya vaccination: US National Institutes of Health; 2016 [cited 2016 3 Jan].

92. WHO. Immunization Vaccines and Biologicals WHO; 2016 [cited 2017 1 Jan.]. Available from: http://www.who.int/immunization/research/development/dengue_q_and_a/en/.

93. ECDC. Epidemiological situation of West Nile virus infection in the European Union. Stockholm: ECDC; 2012.

94. WNV Twgobsa. West Nile Virus and blood safety introduction to a preparedness plan in Europe. The EU Satellite Meeting of the Working Group on Blood Safety and WNV; 2012.

95. European directorate for the quality of medicines and healthcare. Guide to the preparation use and quality assurance of blood components. France: EDQM; 2015.

96. Fischer M, Rabe I, Rollin P. Infectious diseases related to travel TBE CDC; [cited 2016 13 Nov].

97. Tomashek K, Sharp T, Margolis H. Infectious diseases related to travel Dengue: CDC; [cited 2016 14 Nov].

98. Staples J, Hills S, Powers A. CDC; Infectious diseases related to travel Chikungunya [cited 2016 14 Nov].

99. ECDC. Chikungunya Factsheet for health professionals Stockholm: ECDC; [cited 2016 Nov.].

100. ECDC. Crimean-Congo haemorrhagic fever Factsheet for health professionals Stockholm: ECDC; [cited 2016 Nov.].

101. ECDC. West Nile virus Factsheet for health professionals Stockholm: ECDC; [cited 2016 Nov.].

102. ECDC. Epidemiological update West Nile virus transmission season in Europe 2016 Stockholm: ECDC; 2016 [cited 2016 Jan.].

103. ECDC. Plebotomine maps Stockholm: ECDC; 2016 [cited 2016 15 Nov].

104. Papa A, Mallias J, Tsergouli K, Markou F, Poulou A, Milidis T. Neuroinvasive phlebovirus infection in Greece: a case report. Intervirology. 2014;57(6):393-5.

105. Es-sette N, Ajaoud M, Anga L, Mellouki F, Lemrani M. Toscana virus isolated from sandflies, Morocco. Parasites & Vectors. 2015;8(1):205.

106. European Commission. COMMISSION IMPLEMENTING DECISION amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council 8 August 2012 Brussels: EC; 2012 [cited 2016 10 Nov].

Appendix:

1. EU Case definition WNV infection (67)

Clinical criteria: Any person with fever OR at least one of the following two:

Encephalitis OR Meningitis

Laboratory criteria for case confirmation: At least one of the following four:

1) Isolation of WNV from blood or CSF 2) Detection of WNV nucleic acid in blood or CSF

3) WNV specific antibody response (IgM) in CSF 4) WNV IgM high titre AND detection of WNV IgG, AND confirmation by neutralisation

Laboratory test for a probable case:

WNV specific antibody response in serum. Laboratory results need to be interpreted according to flavivirus vaccination status.

Epidemiological criteria: (At least one of the following two epidemiological links):

1) Animal to human transmission (residing, having visited or having been exposed to mosquito bites in an area where WNV is endemic in horses or birds) 2) Human to human transmission (vertical transmission, blood transfusion, transplants)

Case classification:

Probable case

Any person meeting the clinical criteria AND with at least one of the following two:

1) an epidemiological link 2) a laboratory test for a probable case

2. EU case definition: tick-borne encephalitis (106)

Clinical Criteria
Any person with symptoms of inflammation of the CNS (e.g. meningitis, meningo-encephalitis, encephalomyelitis, encephaloradiculitis)
Laboratory Criteria*
— Laboratory criteria for case confirmation:
At least one of the following five:
— TBE specific IgM AND IgG antibodies in blood
— TBE specific IgM antibodies in CSF
— Sero-conversion or four-fold increase of TBE-specific antibodies in paired serum samples
— Detection of TBE viral nucleic acid in a clinical specimen,
— Isolation of TBE virus from clinical specimen
-Laboratory criteria for a probable case:
Detection of TBE-specific IgM-antibodies in a unique serum sample
Epidemiological Criteria
Exposure to a common source (unpasteurised dairy products)
Case Classification
A. **Possible case** NA
B. **Probable case**
Any person meeting the clinical criteria and the laboratory criteria for a probable case,
OR
Any person meeting the clinical criteria and with an epidemiological link
C. **Confirmed case**
Any person meeting the clinical and laboratory criteria for case confirmation

*Serological results should be interpreted according to the vaccination status and previous exposure to other flaviviral infections. Confirmed cases in such situations should be validated by serum neutralisation assay or other equivalent assays.

3: EU interim case definition: Zika virus (68)

Clinical criteria

A person presenting with a rash, with or without fever and at least one of the following signs and symptoms:

- Arthralgia or
- Myalgia or
- Non-purulent conjunctivitis/hyperaemia

Laboratory criteria

Laboratory criteria for ***a probable case:***

- Detection of Zika specific IgM antibodies in serum

Laboratory criteria for ***a confirmed case:***

At least one of the following:

- Detection of Zika virus nucleic acid in a clinical specimen;
- Detection of Zika virus antigen in a clinical specimen;
- Isolation of Zika virus from a clinical specimen;
- Detection of Zika virus specific IgM antibodies in serum sample(s) and confirmation by neutralization test;
- Seroconversion or four-fold increase in the titer of Zika specific antibodies in paired serum samples

Epidemiological criteria

- History of exposure in an area with transmission of Zika virus within two weeks prior to onset of symptoms or
- Sexual contact with a male having been confirmed with a Zika virus infection in the past four weeks or
- Sexual contact with a male who had been in an area with Zika virus transmission in the past four weeks
- A list of Zika affected areas is kept updated on the ECDC website

Classification

Probable case

A person meeting the clinical criteria and the epidemiological criteria.

A person meeting the laboratory criteria for a probable case.

Confirmed case

A person meeting the laboratory criteria for a confirmed case.

4. EU case definition: Viral haemorrhagic fever (38)

- **Clinical Criteria** : Any person with at least one of the following two:

– Fever
– Haemorrhagic manifestations in various forms that may lead to multi-organ failure

- **Laboratory Criteria** : At least one of the following two:

– Isolation of specific virus from a clinical specimen
– Detection of specific virus nucleic acid in a clinical specimen and genotyping

- **Epidemiological Criteria** : At least one of the following:

– Travel in the last 21 days to a region where VHF cases are known or believed to have occurred
– Exposure within the last 21 days to a probable or confirmed case of VHF whose onset of illness was within the last 6 months

- **Case classification**

A. Possible case: NA
B. Probable case: Any person meeting the clinical criteria and with an epidemiological link
C. Confirmed case: Any person meeting the clinical and the laboratory criteria