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Toward Climate Change Impact: Vectors carrying viral infection

What we should know

Diana Conduto António; Isabella Sanseverino; Luca Pozzoli; Teresa Lettieri

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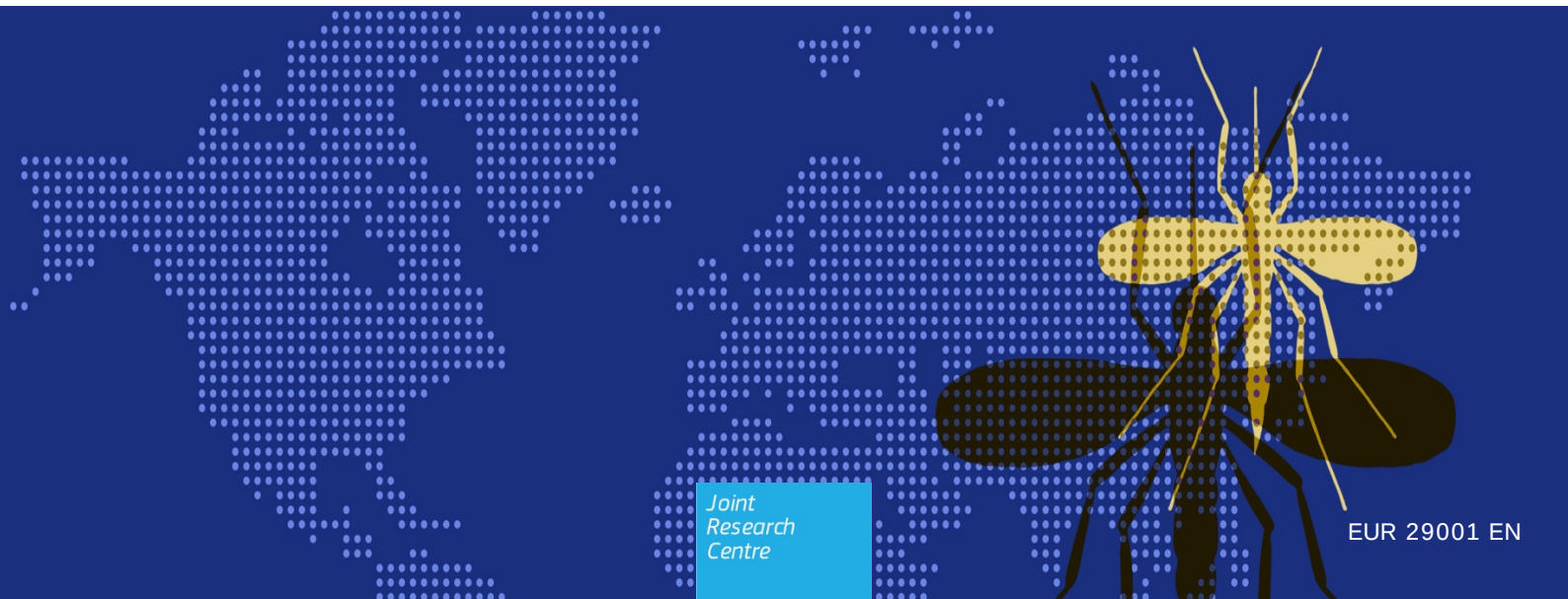

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Acknowledgments

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Abstract

Mosquitoes and ticks can transmit viruses to humans. They can be either direct vectors without any intermediate host (as is the case for mosquitoes) or have an intermediate host (as is the case for ticks).

In recent years, due to global temperature changes, two phenomena have been observed i) the migration of mosquitoes to places that have become warmer, enabling them to adapt to new niches and spread to new areas, and ii) the ability to better survive in different seasons. The increasing incidence of warm winters favours better conditions for mosquitoes and ticks. This increases the exposure of humans to virus infection, especially in urban areas[1,2].

The viruses that cause Zika (ZIK), Dengue (DEN), Chikungunya (CHIK) and tick-borne encephalitis (TBE) belong to the same virus family. ZIK, DEN and CHIK are transmitted by mosquitoes, and TBE is transmitted by ticks. Mosquitoes are responsible for the spread of human diseases such as Dengue fever, Chikungunya and some neurological disorders, while ticks are responsible for the spread of encephalitis. Once infected with the virus, the most common symptoms are usually mild and characterised by fever, skin rash, joint pains and conjunctivitis. In May 2015, an outbreak of Zika virus (ZIKV) infection occurred in Brazil with an estimated total of up to 1.3 million of ZIKV infection cases. This event caught the attention of scientists, the media and the public, and raised awareness of the risk of underestimating mosquito-carried disease and the need to mitigate the spread of the virus by operating at multiple levels (i.e. developing vaccines, mapping the distribution of mosquitoes, and controlling their habitats).

This report provides the public with four pillars of information. The first (chapters 1-3) gives general information about the vectors, viruses and the detection methods, and the second (chapter 4) gives the most recent literature data about their distribution, particularly in Europe. The third (chapter 5) is mainly focused on ZIKV, and compares the recent extensive media attention given to this with the scientific results, in order to avoid the spread of fake news (incorrect scientific news). Finally, the fourth pillar (chapter 6) describes mosquito control strategies, which includes several tactics for limiting the spread of mosquitoes and monitoring their habitats.

1 JRC role in fighting water-borne diseases

The exploratory project "Molecular biosensors & climate data for crisis management to prevent waterborne microorganism impact" (Biocli4crisma), which started in 2015, is coordinated by several Directorates of the Joint Research Centre (JRC)[†].

This project aims to link the detection of waterborne microorganisms with climate data, to ultimately produce a predictive model that can be used for crisis mitigation and management. Biocli4crisma covers climate change impact events, including: i) an increase in episodes of Harmful Algal and Cyanobacterial Blooms (HABs); ii) the impact of storms and floods on public health; iii) the vulnerability of European regions to tropical arboviruses (arthropod-borne viruses).

Collaboration with European and national agencies would allow for the development and regular updating of a predictive model of the spread of arboviruses, which could become a precautionary system similar to the European Flood Awareness System (EFAS)[‡].

The project focuses on detecting the vector-borne Dengue virus (DENV), Chikungunya virus (CHIKV) and Zika virus (ZIKV) in environmental samples to determine their presence and distribution in Europe.

These three viruses belong to the arbovirus family and are carried by different species of Aedes mosquitoes. Since the transmission of the arbovirus to the offspring of mosquitoes has already been reported[3,4], the JRC intends to perform field campaigns to collect mosquitoes in all developmental stages (i.e. the egg, larvae, pupa and adult stages). The field campaigns would allow us to map the distribution of the pathogen-carrying mosquito, and to evaluate the mosquito biomass and the potential risk to the population.

The present report aims to raise public awareness of the threat posed by arboviruses, with a focus on the most recently emerging arbovirus, ZIKV.

[†] Accessible from https://ec.europa.eu/jrc/en.

[‡] https://www.efas.eu/

2 Mosquito: a transmission vector

Several viral diseases are transmitted to humans through arthropods such as mosquitoes and ticks. Viruses that infect both arthropods and vertebrates are generally classified as **ar**thropod-**bo**rne **viruses** (**arboviruses**), and include Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) viruses (Figure 1).

In mosquitoes, arboviruses have the ability to infect the midgut epithelial cells and other tissues, including the salivary glands. To be transmitted to humans and animals, viruses must replicate in the salivary glands and be expelled through the saliva to the blood (Figure 2)[5,6].

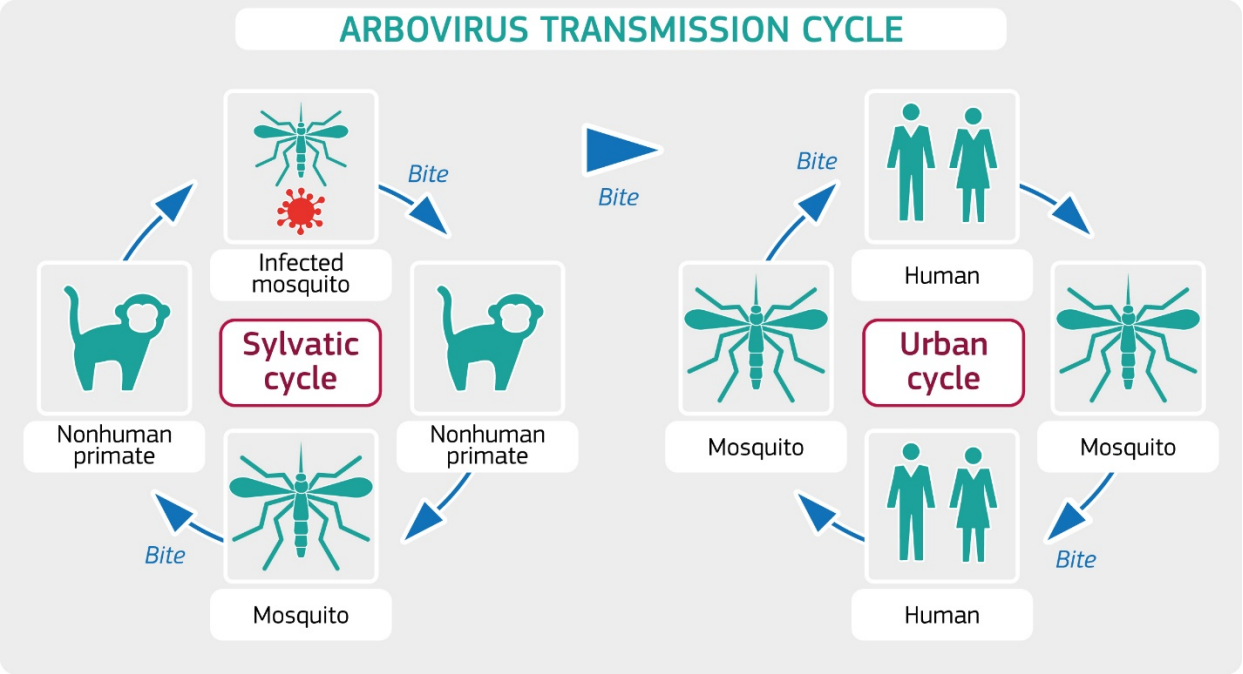

Figure 1. Arbovirus transmission cycle (Source: JRC)

Most arboviruses are transmitted to vertebrates (primarily primates, birds, small mammals) and arthropods in a sylvatic (wild) cycle involving different species of Aedes mosquitoes. In suburban and urban settings, infected Aedes mosquitoes (primary *Aedes aegypti* and *Aedes albopictus*) spread the virus through a human–mosquito–human cycle, which means that the virus can be acquired by mosquitoes from an infected person and then transmitted to other humans.

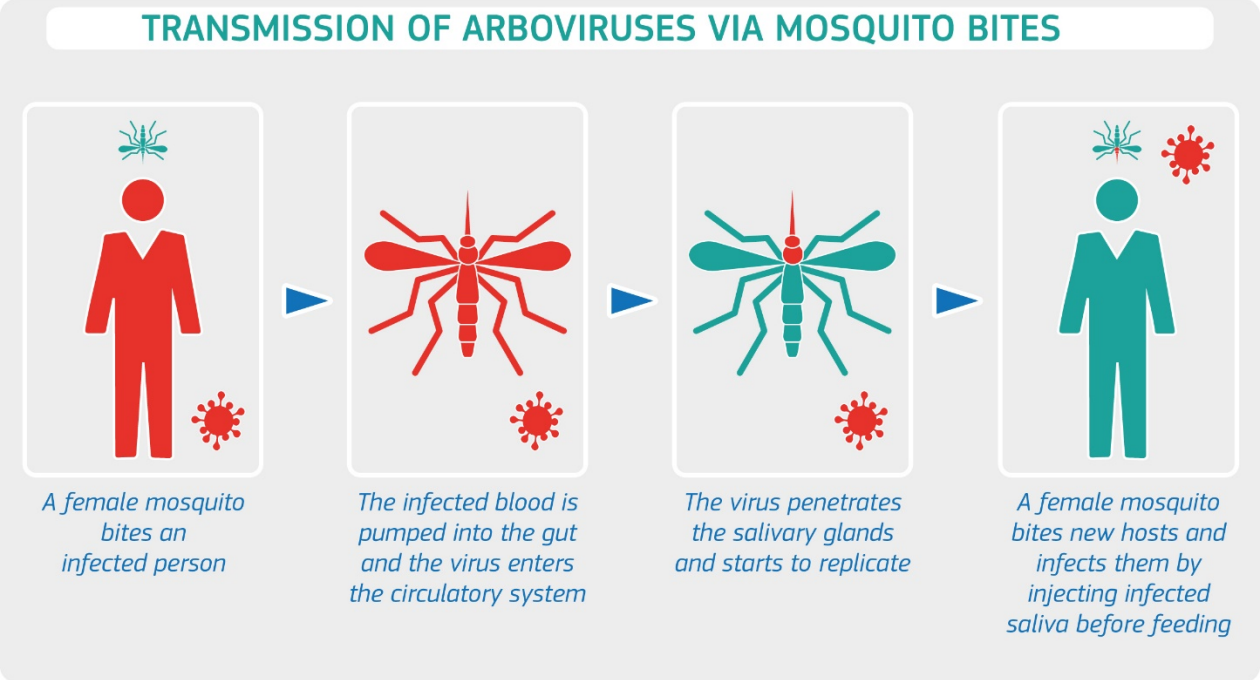

Figure 2. Transmission of arboviruses via mosquito bites (Source: JRC)

Aedes mosquitoes usually proliferate in tropical areas with moderate precipitation, humidity and warm temperatures. Populated urban areas provide optimal conditions for the spread of some species such as *Aedes aegypti* and *Aedes albopictus*[7,8]. In a study conducted in southern Mexico, where Dengue (DEN) is endemic, the authors identified two peaks of activity for mosquitoes in the diurnal activity of both *Aedes aegypti* and *Aedes albopictus* in the sylvan (wild) environment: one in the morning and another in the afternoon. By contrast, no differences were found in domestic areas, where the level of activity remained relatively constant throughout the day[9]. It is well known that the activity of mosquitoes is influenced by different factors, including temperature and light, and these additional observations suggest that some environmental conditions (host availability, time of day) can modify mosquito behaviour[10,11]. Light is a key element which influences the intrinsic factors responsible for the cyclical oviposition of the Aedes species[12].

All mosquitoes develop through four instar stages during their life cycle: egg, larva, pupa and adult (Figure 3). The first three stages develop in water. Eggs laid in water hatch into larvae, which subsequently metamorphose into pupae. After a mosquito is fully developed, it emerges as an adult from the pupa stage[13]. The adult mosquito can survive 30 to 40 days in natural habitats, while in laboratory conditions it can survive up to 117 days[14].

Only female mosquitoes bite humans and other animals. They suck blood in order to obtain the protein and iron needed for egg development. Mosquito eggs are usually laid in water

that contains a moderate amount of organic matter and in vessels such as plant pots, barrels and tyres. Dark water reservoirs with low salinity levels are preferential breeding sites for both *Aedes aegypti* and *Aedes albopictus* mosquitoes[12]. These two species have been widely studied since they are vectors for several arboviruses, including DENV, CHIKV and ZIKV[15,16]. They have been in Africa and Asia for decades, and have more recently become established in some European countries and the Americas. Arboviruses constitute a relevant public health problem, in particular because their presence is becoming more and more established worldwide[17]. While different strategies that target the reduction of population density are currently under investigation, mosquitoes are difficult to eradicate as females can lay sticky eggs in different places, and the larvae can survive for months[18]. Mosquito eggs can also resist suboptimal conditions, such as low humidity and temperature levels (Figure 3)[19-21].

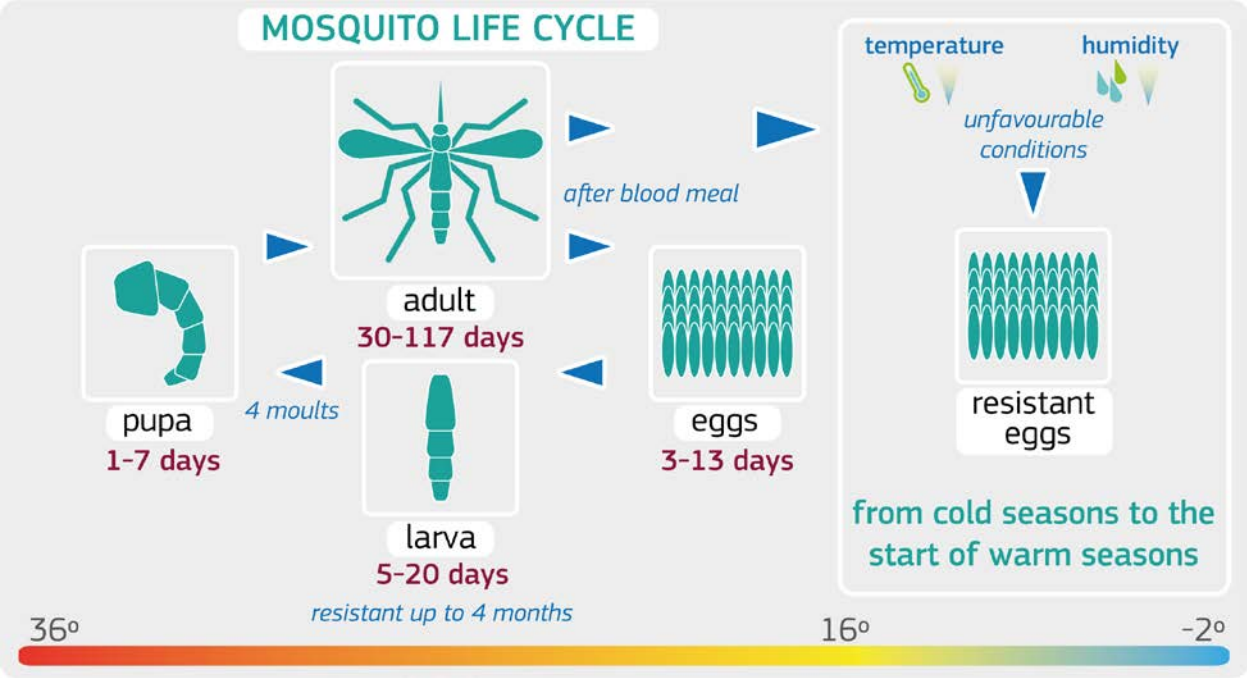

Figure 3. Mosquito life cycle (Source: JRC)

The adult female mosquitoes release their eggs in water where larvae develop. Mature larvae turn into pupae and then become adults. The duration of each developmental stage may vary depending on the environmental conditions. Eggs can take from 3 to 13 days to hatch, larvae mature within 5 to 20 days and pupae may take between 1 and 7 days to metamorphose into adult mosquitoes. Adult Aedes mosquitoes can survive up to 117 days. Oviposition is dependent on protein and iron sources, which are acquired through blood meals. In periods of dehydration or eminent decrease in temperature, the laid eggs are slightly different, and can survive under extreme situations. The resistant eggs will hatch once optimal conditions for development are present. Larvae can also survive for months[20].

3 Pathogenic arboviruses

An arbovirus is a virus transmitted by blood-feeding arthropods such as mosquitoes, sand flies, ticks or gnats. The term arbovirus comprises viruses from different families (see Figure 4 and Table 1).

During the past twenty years, several arboviruses have been considered as emergent disease agents, and are now deemed to be serious public health concerns[17]. Dengue virus (DENV) and Chikungunya virus (CHIKV) are considered to be among the most prevalent arboviruses worldwide.

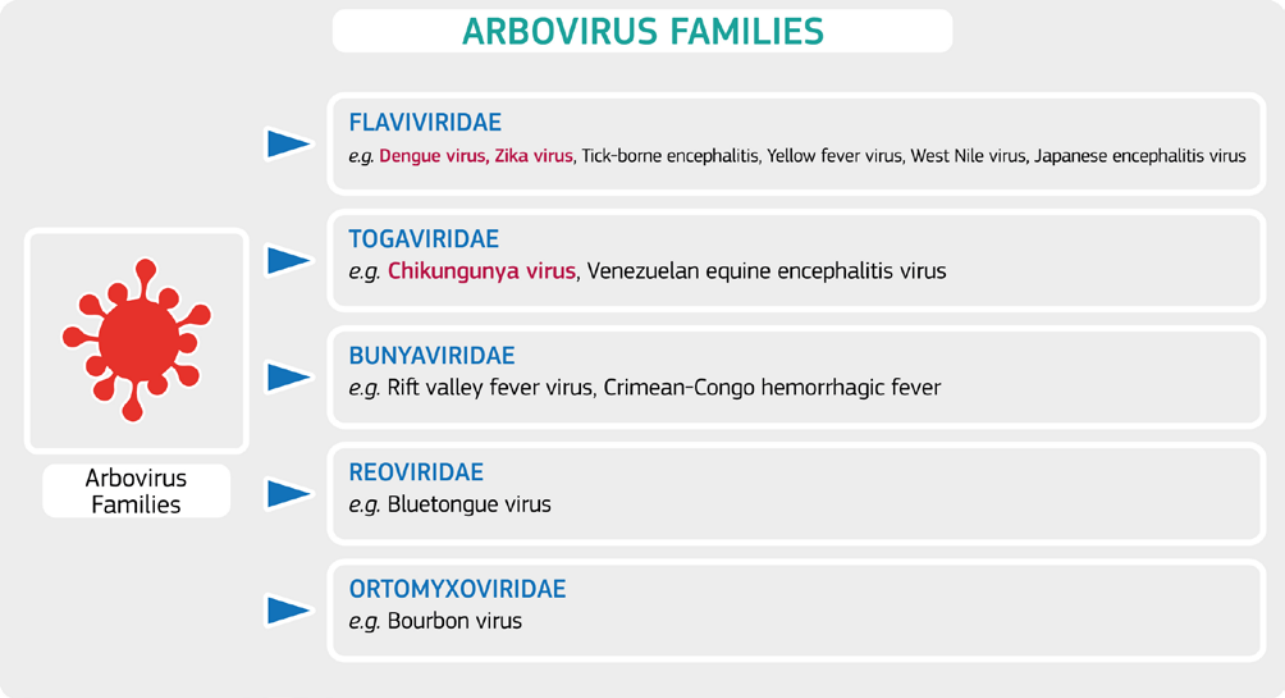

Figure 4. Arbovirus families (Source: JRC)

The figure shows the five different arbovirus families that cause human encephalitis, and some of the most famous viruses belonging to each family.

Table 1. List of pathogenic arbovirus families with examples of the viruses and their transmission cycles (vector and hosts)[22-25].

Virus family	Virus genus	Example	Virus type	Host	Vector	Intermediate host
Flaviviridae	Flavivirus	Dengue virus	RNA	Human	Aedes	n/a
		Zika virus	RNA	Human Monkey	Aedes	n/a
		West Nile virus	RNA	Equine Human	Culex	Birds
		Tick-borne encephalitis	RNA	Human; Wild animals (hares, roe deer, foxes, boar, birds)	Tick	Small wild rodents, but also insectivores and carnivores
		Japanese encephalitis virus	RNA	Equine Human	Culex	Birds Swine
		Yellow fever virus	RNA	Human Monkey	Aedes	n/a
Togaviridae	Alphavirus	Venezuelan equine encephalitis virus	RNA	Equine Human	Culex Aedes Psorophora	Rodents
		Chikungunya virus	RNA	Human	Aedes	n/a
Bunyaviridae	Nairovirus	Crimean-Congo haemorrhagic fever virus	RNA	Human	tick	n/a
	Phlebovirus	Rift Valley fever virus	RNA	Sheep, Cattle	Aedes	Ruminants
Reoviridae	Orbivirus	Bluetongue virus	RNA	Ruminants	Culicoides	Cattle
Orthomyxoviridae	Thogotovirus	Bourbon virus	RNA	Human	Tick	Swine

RNA: ribonucleic acid

n/a: Not applicable

3.1 Dengue virus

Dengue virus (DENV) is responsible for Dengue (DEN) and its severe form, Dengue Haemorrhagic Fever (DHF). It can be caused by five different serotypes: DENV-1, DENV-2, DENV-3, DENV-4 and DENV-5[26]. They are usually transmitted in urban and densely populated areas by the Aedes species found in tropical and subtropical regions, primarily *Aedes aegypti*, but also *Aedes albopictus*. DEN is endemic in more than 100 countries, particularly in Africa, the Americas, South East Asia, Eastern Mediterranean regions and the Western Pacific. Cases of Dengue have been documented in Buenos Aires, south-eastern France, Hong Kong, Madeira, Pakistan and Yemen[27,28]. The transportation of goods, globalisation, travel and migration have helped spread the virus and vectors to different parts of the world (Figure 5). The incidence of the disease is estimated to be 50 million infections per year worldwide, with 90% of the cases of the most severe form of the disease, DHF, reported in children of less than five years of age[29]. The febrile phase lasts for 3 to 7 days, and most patients recover with no major complications. DHF occurs after a secondary infection with a different serotype or multiple infections with other serotypes. Patients with DHF show symptoms of severe thrombocytopenia, plasma leakage and haemorrhagic manifestations[30,31]. Untreated, the disease may lead to severe complications and can even be fatal[32]. There are no specific antiviral agents to treat Dengue infections in humans, and there is no licensed vaccine to prevent the disease[33,34]. Treatment is based on supportive therapy using fluid replacement and platelet transfusion (where there is a rapid decrease in platelet count). Better control of mosquito transmission, the development of a vaccine and increased efforts to produce antiviral and therapeutic drugs are needed in order to reduce Dengue infections.

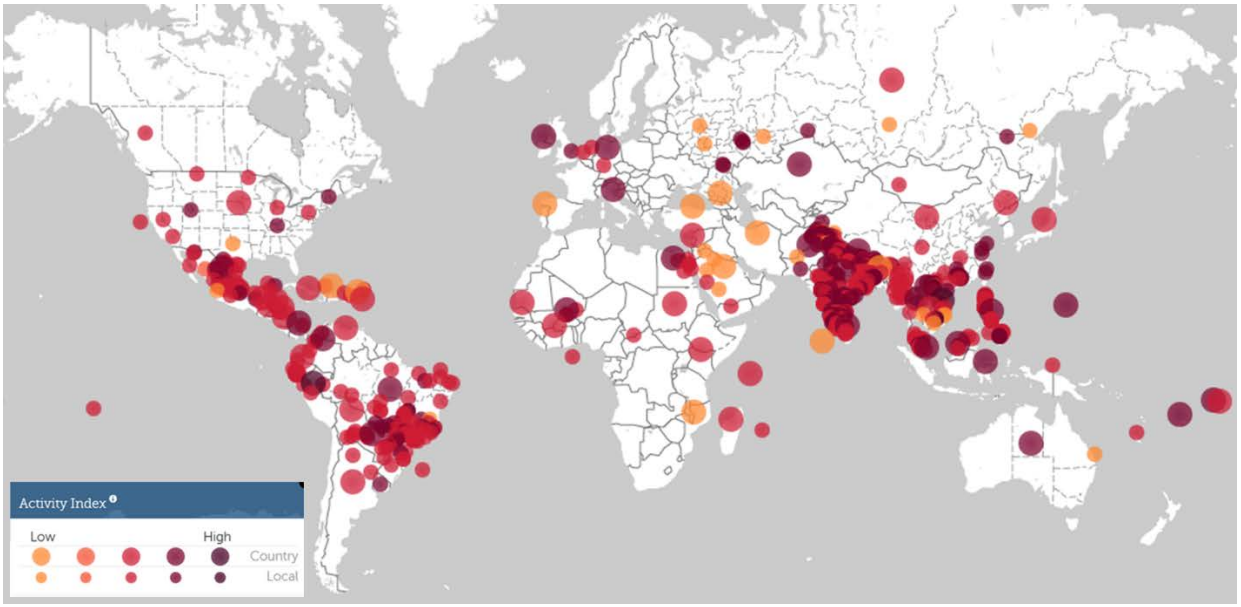

Figure 5. Global distribution of alerts of Dengue virus (DENV) in the past 3 months (August-November 2017)

The large circles indicate a country-level alert, while state, province and local alerts are indicated by the small circles. Marker colour reflects the importance of events at a particular location during a given time window. An event's degree of importance is based on the significance rating of the alert provided by HealthMap users.[§]

[§] Data source: http://www.healthmap.org/, last accessed on 13/11/2017.

3.2 Chikungunya virus

Chikungunya virus (CHIKV) is an alphavirus that causes Chikungunya (CHIK), a severe illness with symptoms similar to those of Dengue virus (DENV), which can lead to its misdiagnosis and underestimations of its frequency. The clinical picture that characterises CHIK is a notable presence of acute arthritis and severe joint pains[35,36]. CHIKV is associated with low mortality rates[37].

The first outbreak of CHIK was reported in Tanzania in July 1952[38]. At present, three genotypes of CHIKV have been identified: West African, East/Central/South African (ECSA) and Asian[39]. Following its first isolation, CHIKV has been detected in India and Southeast Africa. Since 2004, the virus has spread to many other countries, including Kenya, Italy, France and, more recently, Spain (Figure 6)[40,41].

CHIKV is primarily transmitted by the tropical and subtropical mosquito *Aedes aegypti*, but a CHIKV variant, characterised by mutations in the genes encoding envelope glycoproteins, is preferentially transmitted by *Aedes albopictus* mosquitoes[42]. The first European CHIK outbreak reported in Italy was caused by this ECSA variant, whose adaptation to the new vector *Aedes albopictus* likely contributed to the diffusion of CHIK in temperate climates and in rural habitats where *Aedes aegypti* mosquitoes are less common[43].

In October 2013, CHIKV was detected on the island of Saint Martin (Caribbean Sea). The viral strain involved in spreading the infection belonged to the Asian genotype that subsequently expanded its geographic range to circulate in the Caribbean, Central America, northern South America and, more recently, Florida[44,45].

These recent findings about the spread of CHIKV underline the possibility that the viruses may hit not only tropical and subtropical zones but also temperate regions. CHIK infections arise from a human-mosquito-human cycle (Figure 1). Unfortunately, specific therapies or vaccines against CHIKV infection are not yet available, although many studies are addressing these issues[36,44]. Treatment is supportive and includes increased fluid intake and the use of medicines to relieve pain (anti-inflammatory drugs and immunomodulators)[36,44].

The spread of CHIK in recent years highlights the need for more efficient mosquito control programmes and better characterisation of the CHIKV biology for therapy purposes. Looking at Europe, on September 2017, the Italian Ministry of Health confirmed nine cases of CHIKV, three in the city of Anzio (Lazio region) and six in the capital city of Rome, Lazio. As of the first week of October, 146 out of a total of 239 confirmed cases had been reported in the Lazio region, and six cases had been confirmed in the city of Guardavalle Marina, in Calabria region[46]. There have been additional CHIK cases in other Italian regions including Le Marche, Emilia-Romagna and Lombardy, as well as in other European countries including

France and Germany[46,47]. Italy experienced the first outbreak of autochthonous Chikungunya in the Emilia-Romagna region in 2007. France was the only other European country to have reported CHIKV outbreaks, in 2010 and 2014[48,49].

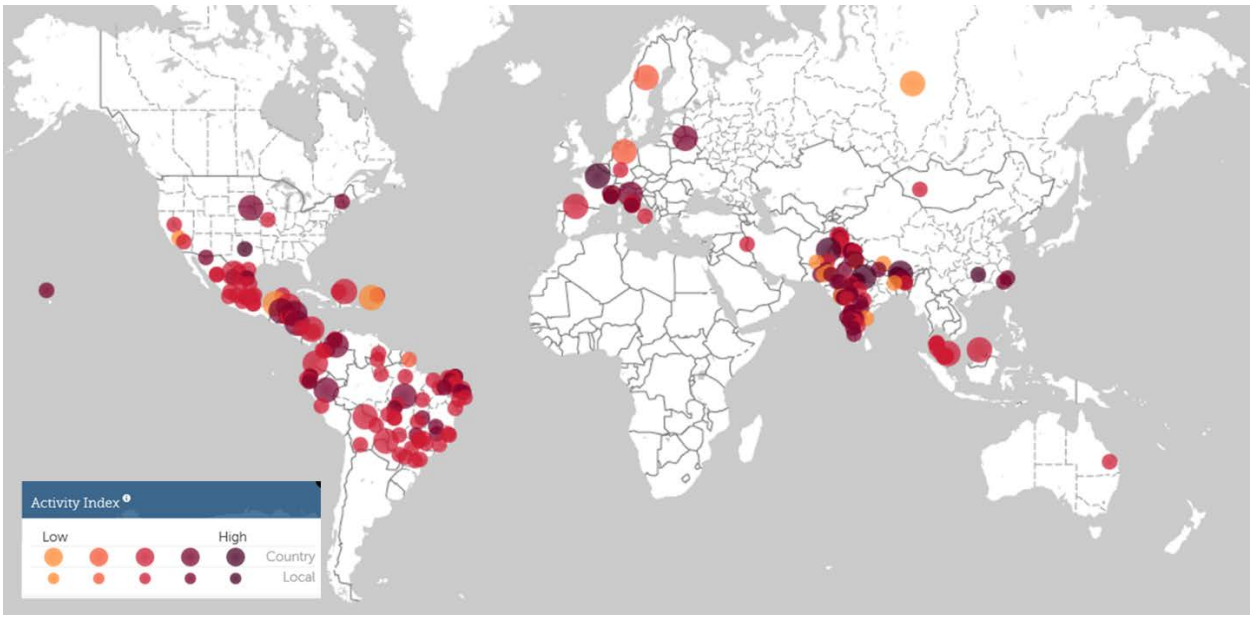

Figure 6. Global distribution of alerts of Chikungunya virus (CHIKV) in the past 6 months (May-November 2017)
The large circles indicate a country-level alert, while state, province and local alerts are indicated by the small circles. Marker colour reflects the importance of events at a particular location during a given time window. An event's degree of importance is based on the significance rating of the alert provided by HealthMap users.[**]

[**] Data source: http://www.healthmap.org/, last accessed on 13/11/2017.

3.3 Zika virus

Zika virus (ZIKV) is an arbovirus belonging to the same family as Dengue virus (DENV). Until recently, the few ZIKV infections that were reported were always associated with mild symptoms, similar to flu symptoms and skin rash, which spontaneously disappeared within a week[50]. Zika (ZIK) infection can be asymptomatic and, unlike Dengue (DEN), is associated with conjunctivitis[51]. This virus has been associated with neurological disorders such as Guillain-Barré Syndrome (GBS)[52]. Sexual and perinatal transmission routes were observed in this virus; indeed the virus has been associated with the development of microcephaly in foetuses[53,54].

There is no cure or vaccine available to prevent ZIKV infection. Treatment is often supportive and mainly based on rest and avoiding dehydration. Nonsteroidal and anti-inflammatory medicines should be discouraged to reduce the risk of the haemorrhagic syndrome reported with other flaviviruses, and antihistamines should be used to relieve pruritic rash[55].

Fourteen vaccine developers in France, USA, Brazil, India and Austria are currently working on the development of a vaccine against ZIKV[56,57]. Vaccines are already in the trial phase, although their commercialisation is not expected for some time yet[b]. Several potential therapeutic agents are also being evaluated[58,59]. More information about the origin, spread and characteristics of the virus is given in Chapter 5.

3.4 Tick-borne encephalitis virus

Tick-borne encephalitis virus (TBEV) and West Nile virus (WNV) are flaviviruses considered to be pathogenic for humans. They have been present in Europe for a long time (Figure 7)[60,61].

Three known subtypes of TBEV have been described so far: European or Western, Siberian and Far Eastern[62]. In Europe, TBEV has been reported in countries including Austria, the Czech Republic, Germany, Lithuania, Poland, Slovakia, and Sweden. More cases were reported in 2016 (1 900 cases) than in 2015 (1 258 cases)[63]. The first case of TBEV endemic transmission was recently reported in the Netherlands, demonstrating the spread of the disease to regions previously denoted as being TBEV-free[64]. TBEV transmission is mainly mediated by the vectors *Ixodes ricinus* and *Ixodes persulcatus*, although the thick *Dermacentor reticulatus* is increasingly reported as being a vector involved in the spread of the disease[65]. *Dermacentor reticulatus* is a vector of over 40 pathogenic agents, and is rapidly spreading in Europe. Two of its biological features are its high reproduction rate and the capacity to survive under unfavourable conditions. It is cold resistant and can live underwater for months[66].

TBEV is transmitted to the vector through 3 possible routes: i) co-feeding on infected hosts; ii) transtadial and transovarial transmission and iii) sexual transmission[67]. Indeed, ticks acquire the virus after feeding on small wild rodents (reservoir hosts) during the nymph and the adult stages, and the infected ticks can transfer the pathogen to other adult ticks through mating[68]. Transovarial transmission also occurs in ticks (also known as vertical transmission through the eggs to the larval stage), but this does not seem to play a central epidemiological role in TBEV transmission. TBEV can multiply on the tick vector, which thereby acts as a long-term reservoir for the virus. Humans and wild animals (including hares, roe deer, foxes, boar and birds) are the indicator hosts of TBEV. They are not able to transmit the virus back to the vectors, but they indirectly sustain the circulation of the virus, allowing the ticks to survive and reproduce. Seroprevalence in these vertebrates is also an indicator of TBEV within a geographical area[69]. Humans can be infected through the bite of an infected tick or by consuming unpasteurised products from infected goats, sheep, or cows. In Slovakia, the incidence of alimentary-acquired infections may account for nearly a quarter of the total reported cases[63]. Humans can develop a viral disease, the most common symptoms of which include fever, headache, nausea and vomiting. Severe headache and encephalitis, meningitis or meningoencephalitis occur in 20-30% of people affected by Tick-borne encephalitis (TBE). Approximately 1-2% of people die from this infection[70]. The risk of

TBEV infection is linked to occupational exposure, outdoor leisure activities and travel to endemic regions. Climate change, including the growth of mean annual air temperature, is also expected to play a role in the geographic distribution and abundance of infected ticks and infection cases[71,72].

Vaccination against TBE is available, but public awareness of the disease and its prevention measures is low[63,73]. Cross-protection against other subtypes has been shown for vaccination based on the European subtype[73,74]. Vaccination against TBEV is recommended for people who live or work in, or travel to TBEV-risk areas.

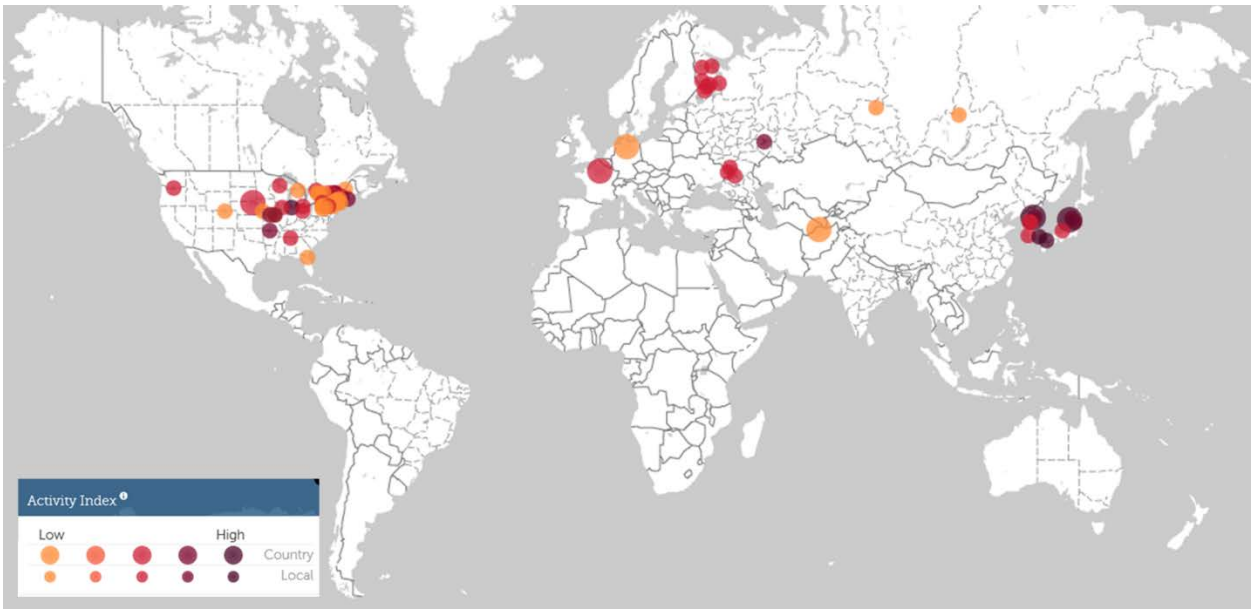

Figure 7. Global distribution of alerts of Tick-borne encephalitis virus (TBEV) in the past 6 months (May-November 2017)

The large circles indicate a country-level alert, while state, province and local alerts are indicated by the small circles. Marker colour reflects the importance of events at a particular location during a given time window. An event's degree of importance is based on the significance rating of the alert provided by HealthMap users.[††]

[††] Data source: http://www.healthmap.org/, last accessed on 13/11/2017.

3.5 West Nile virus

West Nile virus (WNV) is globally widespread (Figure 8) and has been reported in Europe since the 1960s[75]. Nowadays, WNV outbreaks in Europe are irregular and temporally limited phenomena. In 2016, a total of 225 human cases of infections were reported in Europe, mostly in Romania and Italy[76].

WNV belongs to at least two main lineages (although four other WNV lineages have been also identified that show genetic differences to the major known ones)[69]. Lineage 1 is composed of WNV strains from Africa, Australia, Eastern Asia, Europe, North America and India[77]. Lineage 2 has been isolated in sub-Saharan Africa and Madagascar, but it has recently been reported in Hungary, Greece and Italy[77]. Lineage 2 strains were initially considered to be non-pathogenic in humans but, since 2008, these countries have reported human outbreaks caused by WNV lineage 2, resulting in neuroinvasive diseases that are sometimes fatal[78,79]. In Italy, in particular, WNV outbreaks occurred in Sardinia, in the Po river area and in Central Italy[80-82].

WNV has been detected in several genera of mosquitoes, which are the primary vectors for WNV transmission [69]. In Europe, *Culex pipiens*, *Culex perexiguus*, and *Culex modestus* are important vector species, but *Culex pipiens* is the primary global WNV vector due to its propensity to feed on birds[83]. Wild birds are indeed considered the reservoir hosts for the virus, which is maintained in nature through a bird-mosquito cycle. Infected mosquitoes can transmit the virus to humans and animals such as horses, which are considered as dead-end hosts for this infection because they cannot transmit the virus back to the vector. Infection in humans is mainly asymptomatic, and only 20-40% of the infected patients develop West Nile Fever (WNF), which is characterised by mild symptoms, including fever and headache[84]. Severe symptoms such as meningitis, encephalitis, and meningoencephalitis are reported in less than 1% of people affected, with nearly 10% mortality[85].

No human vaccines are currently available and there are no specific treatments for this infection. The best way to prevent WNV infection is to reduce exposure to mosquito bites by wearing clothing that covers the arms and legs, control the spread of the vector by reducing the number of water-filled containers that provide breeding habitats, and use insect repellents.

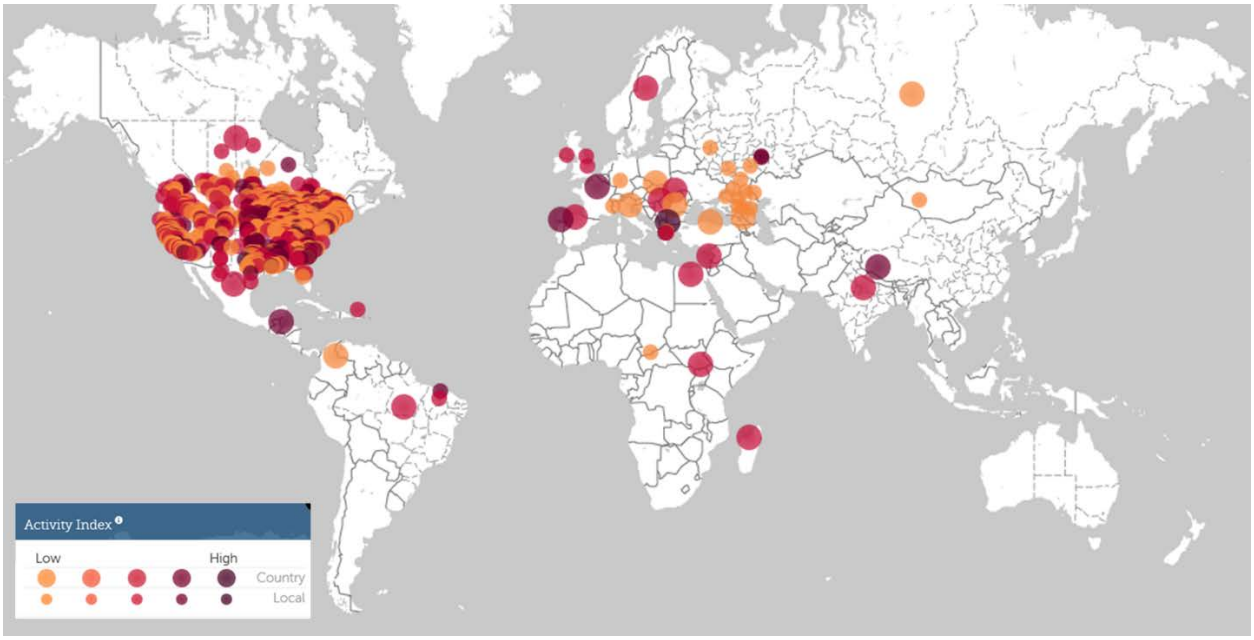

Figure 8. Global distribution of alerts of West Nile virus (WNV) in the past 6 months (May-November 2017)

The large circles indicate a country-level alert, while state, province and local alerts are indicated by the small circles. Marker colour reflects the importance of events at a particular location during a given time window. An event's degree of importance is based on the significance rating of the alert provided by HealthMap users.[‡‡]

[‡‡] Data source: http://www.healthmap.org/, last accessed on 13/11/2017.

4 Clinical signs and virus detection

4.1 Clinical signs

Table 2 summarises the symptomatic profiles of the emerging Dengue (DENV), Chikungunya (CHIKV), Zika (ZIKV), Tick-borne encephalitis (TBEV) and West Nile (WNV) viruses. Figure 9 shows the number of cases for each virus reported in European countries and the total number of cases in the 31 countries in the European Economic Area. Note that the number of reported cases refer to different time periods for the different viruses, based on the data in the Surveillance Atlas of Infectious Diseases of the European Centre for Disease Prevention and Control (ECDC). The reported cases may be associated with travel, local transmission, or unknown causes. The data clearly point out the difference in transmission dynamics between TBEV and the other arboviruses. The reported cases of DENV, CHIKV, ZIKV and WNV are mostly associated with travel, while TBEV is principally reported to have local causes. This means that global interconnections provide the perfect conditions for the spread of DENV, CHIKV, ZIKV and WNV.

Table 2. Emerging viruses and their symptomatic profiles

Virus	Symptoms	Comments
Dengue	High temperature, headache, joint pain, myalgia/arthralgia, skin rash, leukopenia, thrombocytopenia	Symptoms last for 3 to 7 days
Dengue (re-infection, DHF)	Severe thrombocytopenia, plasma leakage, haemorrhage	Occurs by multiple DENV serotypes infection
Chikungunya	Fever, headache, skin rash, thrombocytopenia and leukopenia, acute arthritis, severe joint pains, Guillain-Barré Syndrome	Low mortality rate
Zika	Fever, joint pain, skin rash, conjunctivitis, Guillain-Barré Syndrome, microcephaly	80% of asymptomatic infections. Transmission also by sexual and perinatal routes
West Nile	Fever, headache, joint pains, vomiting, body aches, muscle weakness	Low mortality rate
Tick-borne encephalitis	Fever, headache, nausea, vomiting, fatigue, malaise, myalgia, gastrointestinal symptoms, leukocytopenia	70-95% of asymptomatic infections. Low mortality rate

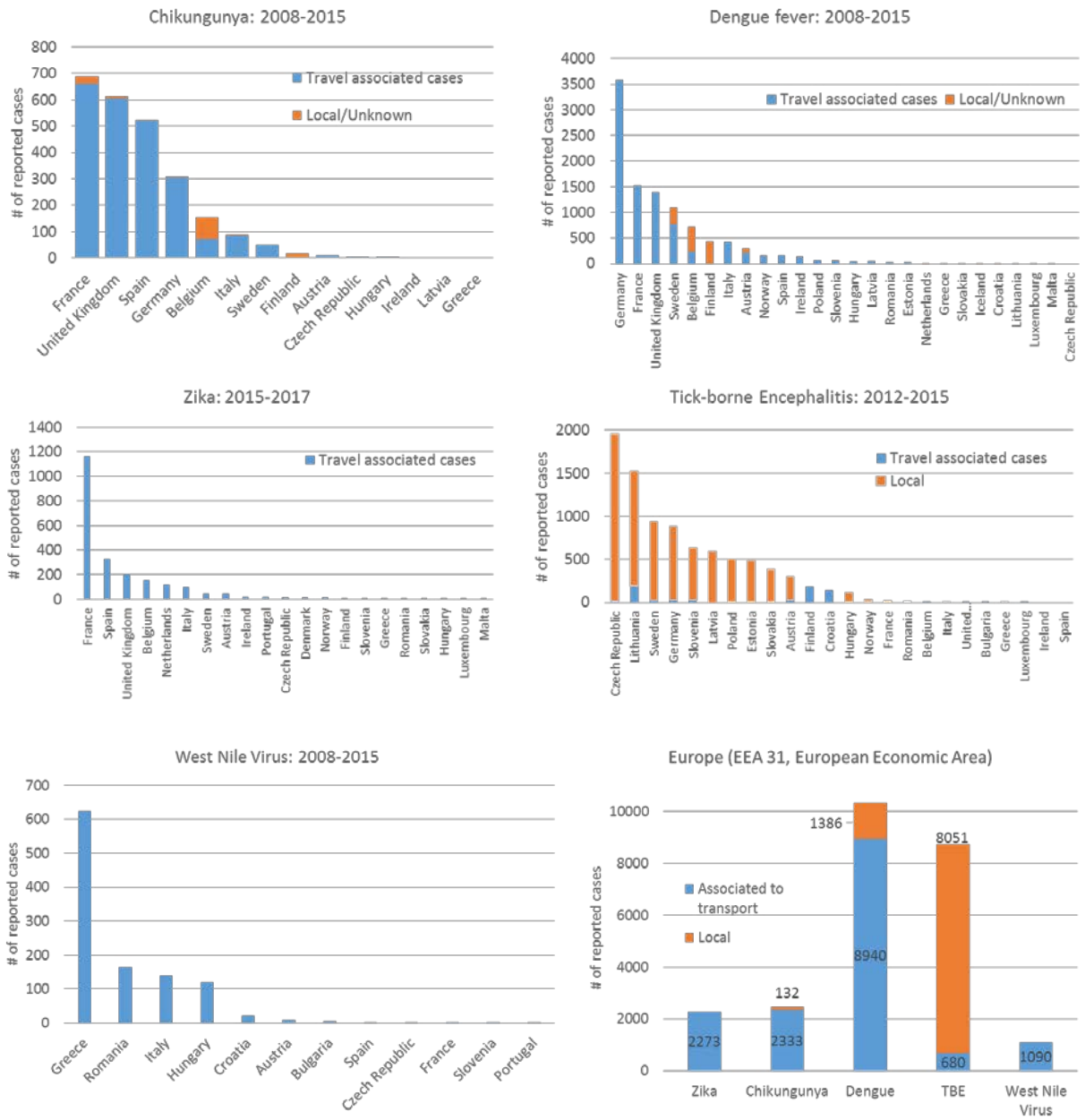

Figure 9. Number of reported cases in Europe of Chikungunya virus (CHIKV), Dengue fever virus (DENV), Zika virus (ZIKV), Tick-borne encephalitis virus (TBEV), and West Nile virus (WNV)[§§]

[§§] Data source: https://ecdc.europa.eu/en/surveillance-and-disease-data, last accessed on 1/12/2017. Dataset provided by ECDC based on data provided by WHO and Ministries of Health of the affected countries. The views and opinions of the authors expressed herein do not necessarily state or reflect those of the ECDC. The accuracy of the authors' statistical analysis and the findings they report are not the responsibility of the ECDC. The ECDC is not responsible for conclusions or opinions drawn from the data provided, or for the correctness of the data, data management, data merging and data collation after provision of the data. The ECDC shall not be held liable for improper or incorrect use of the data.

4.2 Virus detection

The detection arboviruses such as Zika (ZIKV), Dengue (DENV), Chikungunya (CHIKV), West Nile (WNV) and Tick-borne encephalitis (TBEV) viruses is mainly based on Enzyme-Linked Immunosorbent Assays (ELISA) or quantitative Polymerase Chain Reactions (qPCR) (Figure 10)[86-89]. Quantitative PCR is a technique used to quantify the expression of ZIKV-associated genes, while ELISA analyses are performed to identify virus-specific immunoglobulin and neutralising antibodies[90,91]. Quantitative PCR detection is specific to ZIKV, although it can generate false negatives due to virus mutation or the presence of amplification inhibitors[92]. Specific serological tests aimed at identifying immunoglobulins and antibodies may present cross-reactivity between ZIKV and other flaviviruses (i.e. DENV and Yellow Fever virus), which can lead to false positive results and misdiagnoses[90]. However, the detection of virus-specific antibodies does not always indicate an active infection. Positive results may be due to previous exposure to the virus or to a past vaccination. This means that these results could be difficult to interpret.

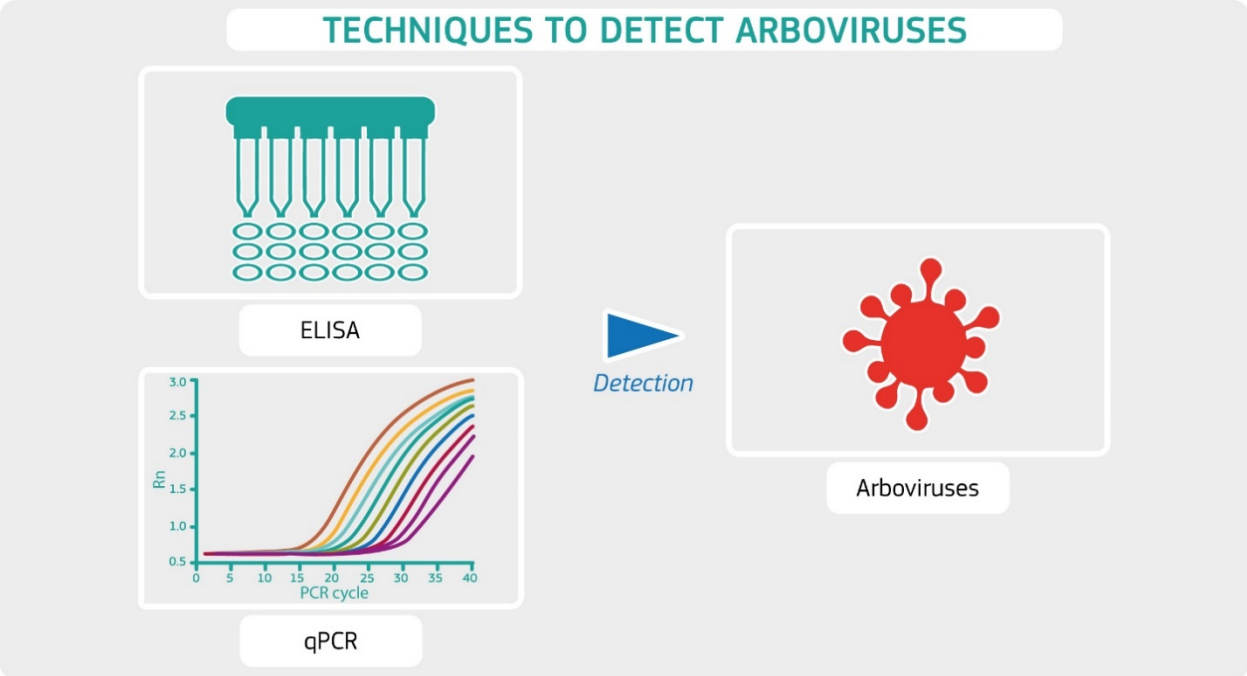

Figure 10. Techniques to detect arboviruses (Source: JRC)
The Enzyme-Linked Immunosorbent Assay (ELISA) and the quantitative Polymerase Chain Reaction (qPCR) are the techniques most frequently used to detect arboviruses in infected samples.

5 Zika: an emerging virus

5.1 Origin and spread of Zika virus

Zika virus (ZIKV) was first described in the 1940s and took the name from the forest where it was found, in Uganda[93]. The virus was isolated for the first time from *Aedes africanus* mosquitoes during a study of monkey resistance to Yellow Fever virus (YFV) and it was later isolated from *Aedes aegypti* in Malaysia[93,94]. At present, thirteen species of mosquito have already been found to carry ZIKV in nature, which corroborates the potential for more mosquito species to become vectors of this disease[95]. Even though ZIKV is especially transmitted to humans by *Aedes aegypti*, *Aedes albopictus* mosquitoes have also been implicated in outbreaks of the virus[55,96]. *Aedes albopictus,* better known as the tiger mosquito, has a wide distribution in temperate zones, beyond the tropical and subtropical regions to which *Aedes aegypti* mosquitoes are confined. At present, the distribution of *Aedes albopictus* in different areas such as Europe and America makes the local spread of ZIKV a serious concern. Major recommendations are addressed to individuals that are considering travel to places where ZIKV is present, because they need to take protective measures to avoid mosquito bites and prevent ZIKV transmission after returning to their countries[a].

After its detection in 1947, the first documented outbreak of a ZIKV infection was reported in Yap Island (Micronesia) many years later, in 2007[97]. Since then, the virus spread to French Polynesia where it caused more than 29 000 infection cases from October 2013 to February 2014, infecting about 11% of the population[98]. From there, the virus was introduced into Brazil. The Brazilian Ministry of Health estimated that between 0.4 and 1.3 million people were infected in 2015 and, as of 29 March 2016, all Brazilian states reported ongoing cases of Zika (ZIK) infections[99]. Since the outbreak, more than seventy territories worldwide have confirmed autochthonous cases of ZIK (Figure 11)[99-101]. To these must be added reports of episodes of non-vector-borne transmission in countries such as France, Italy and Portugal (Madeira)[99]. In these countries, virus transmission occurred through routes that may include sexual or perinatal transmission.

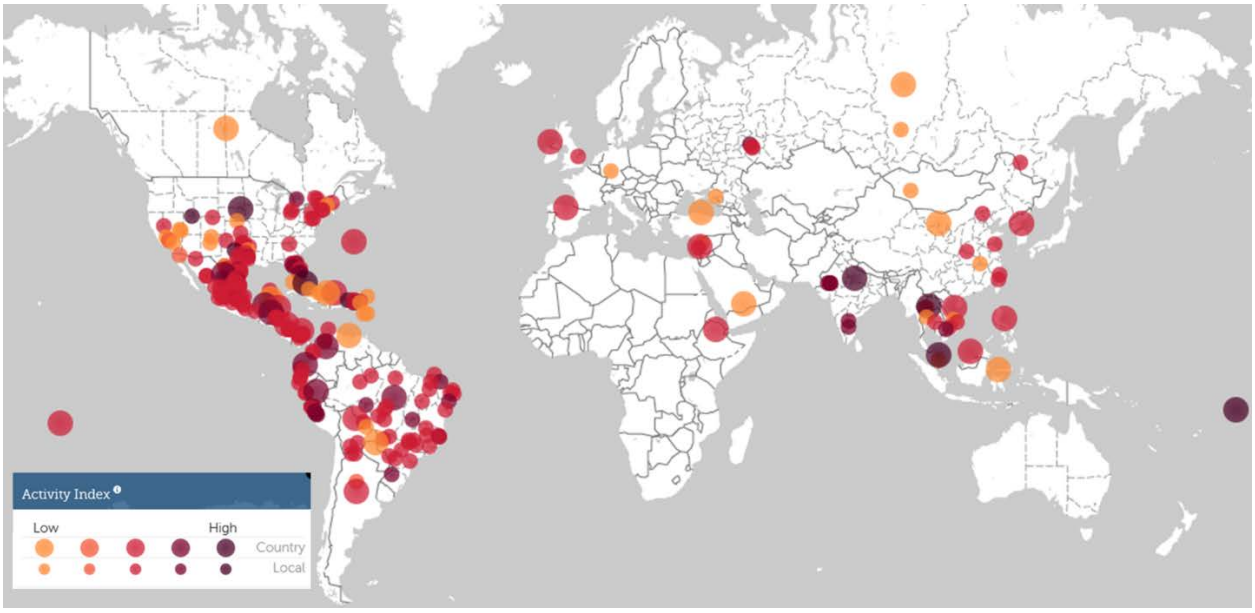

Figure 11. Global distribution of alerts of Zika virus (ZIKV) in the past 6 months (May-November 2017)

The large circles indicate a country-level alert, while state, province and local alerts are indicated by the small circles. Marker colour reflects the importance of events at a particular location during a given time window. An event's degree of importance is based on the significance rating of the alert provided by HealthMap users.[***]

[***] Data source: http://www.healthmap.org/, last accessed on 13/11/2017.

5.2 Clinical signs, transmission and relationship with neurological disorders

When the first Zika virus (ZIKV) epidemic was reported in the Federal State of Micronesia in 2007, it was assumed to be related to Dengue virus (DENV). Although ZIKV is more often associated with conjunctivitis, there are no other features to distinguish between these two infections[51]. In addition, asymptomatic cases of ZIKV are estimated at 80%, making its diagnosis and prevention even more difficult[97].ZIKV has been associated with serious neurological disorders such as Guillain-Barré Syndrome (GBS) and microcephaly[52,54,102,103]. Mutations in ZIKV have recently been described and may be associated with the increased severity of the symptoms reported in the latest outbreaks[104].

In suburban and urban settings, ZIKV is primarily transmitted through the bite of infected mosquitoes from the Aedes genus in a human–mosquito–human transmission cycle (Figure 12). Other routes of transmission have been investigated, and many reports now indicate that ZIKV can be transmitted through perinatal and sexual transmission (Figure 12)[53,105-107]. Recognising the impact of sexual transmission, the World Health Organization (WHO) has released interim guidance regarding its prevention. By March 2017, 2 130 Europeans were reported to have travel-associated ZIKV infections, of which 20 were infected through sexual transmission[108]. ZIKV was isolated in blood, urine, saliva, cerebrospinal fluid, amniotic fluid and breast milk, but further investigation is needed to understand if these incidences could be considered other forms of contagion[51]. Transfusion-transmissible infections have also been suggested[109,110].

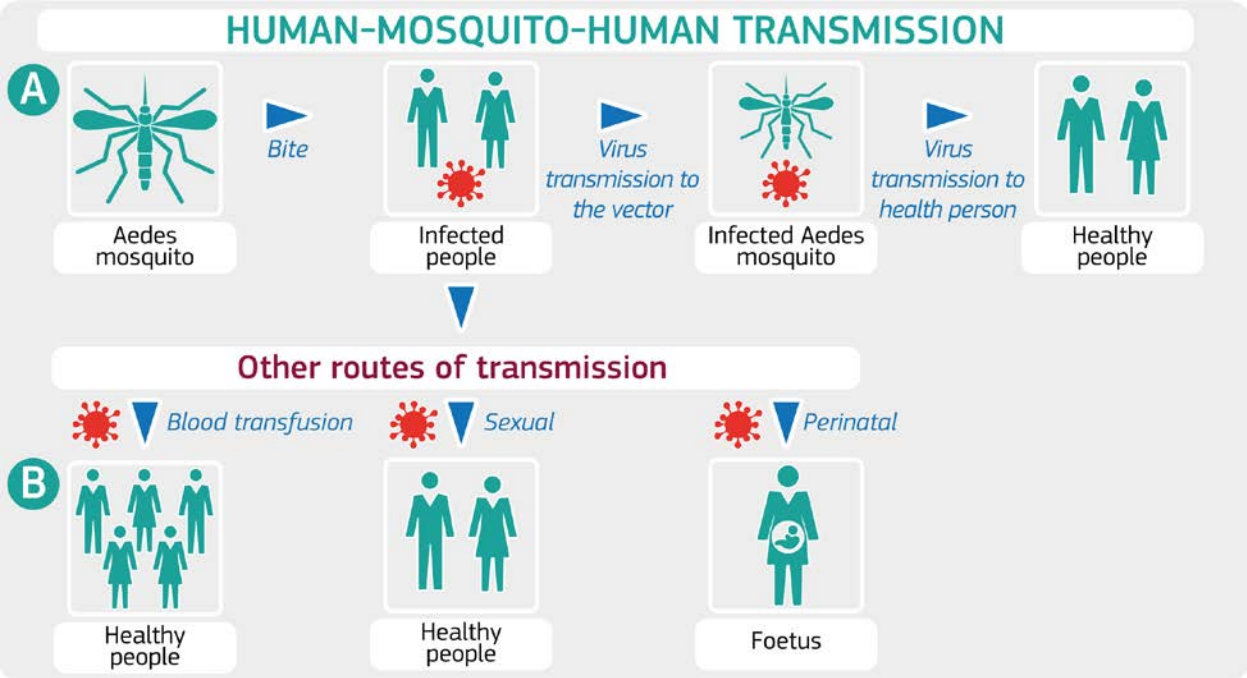

Figure 12. Human-mosquito-human transmission cycle (Source: JRC)

In suburban and urban settings, the transmission of Zika virus (ZIKV) occurs primarily through a human–mosquito–human transmission cycle. The upper panel (Figure 12A) describes the main route of transmission where the virus can pass from humans to humans by the bites of infected Aedes mosquitoes. Figure 12B shows the other routes of ZIKV transmission, including sexual and perinatal transmission. ZIKV may also be spread through blood transfusion.

An association between ZIKV and the neurological disorder GBS was first noted during the large outbreak that occurred in French Polynesia in 2013[111]. GBS is a severe acute paralytic neuropathy that affects the peripheral nerve myelin. It is characterised by muscle weakness and paralysis (Figure 13)[112]. This neurological disorder is more common in adults (particularly in males), and is usually preceded by an infection that prompts an autoimmune response against peripheral nerve tissue. Since October 2015, GBS has been detected at an increasing rate in several countries, including Brazil, Colombia, the Dominican Republic and Venezuela. In each country, at least one GBS case was confirmed as being associated with ZIKV infection. WHO Emergency Committee on Zika has agreed that ZIKV is one of the causes of GBS, although other viruses have been also associated with this disease[52,55,99,113].

In September 2015, researchers reported an increase in the number of cases of neonates born with microcephaly in north-eastern Brazil, and subsequently also in the south-eastern part of the country. Different studies have analysed the association between ZIKV and microcephaly after the first reported cases of perinatal transmission. Microcephaly and/or Central Nervous System (CNS) malformation episodes have since been documented in many other countries where ZIKV was also circulating (Figure 13)[99,114]. Microcephaly is

characterised by incomplete brain development, which results in some degree of mental retardation. At present there is no cure for ZIKV infection[115]. ZIKV has been identified in the amniotic liquid of pregnant women and in body tissues of the foetal brain, the brain tissues of newborns with microcephaly, and placental tissues of miscarriages[115,116-119].

The neurotropism of ZIKV was first discovered in infected mice, where the virus was isolated from brain tissues and seemed to cause a decrease in brain volume during the embryonic stage[120-121-123]. In addition, a laboratory study confirmed that ZIKV has the capacity to target neural cells by efficiently infecting Human Cortical Neural Progenitor Cells (hNPCs) and attenuating their growth[124]. An epidemiological study performed on a group of Brazilian women suggests that ZIKV infection during pregnancy appears to be related not only to microcephaly but also to other grave severe neurological outcomes, including cerebral calcifications, intrauterine growth restriction and abnormal umbilical artery flow[119].

Currently, substantial evidence suggests a causal link between ZIKV and foetal brain anomalies, including microcephaly, especially during the first gestational trimester. However, the mechanism by which mothers can transmit the virus to their babies and the foetus neuronal alteration are still unknown.

It is recommended that people, particularly pregnant women, avoid travelling to areas where ZIKV is circulating. Individuals who live in ZIKV-infected zones should limit mosquito bites by using skin repellents, bed nets and staying in places with air conditioning to keep mosquitoes outside. In the absence of specific treatments, protection against mosquito exposure is the only prevention method available to reduce the impact of the infection.

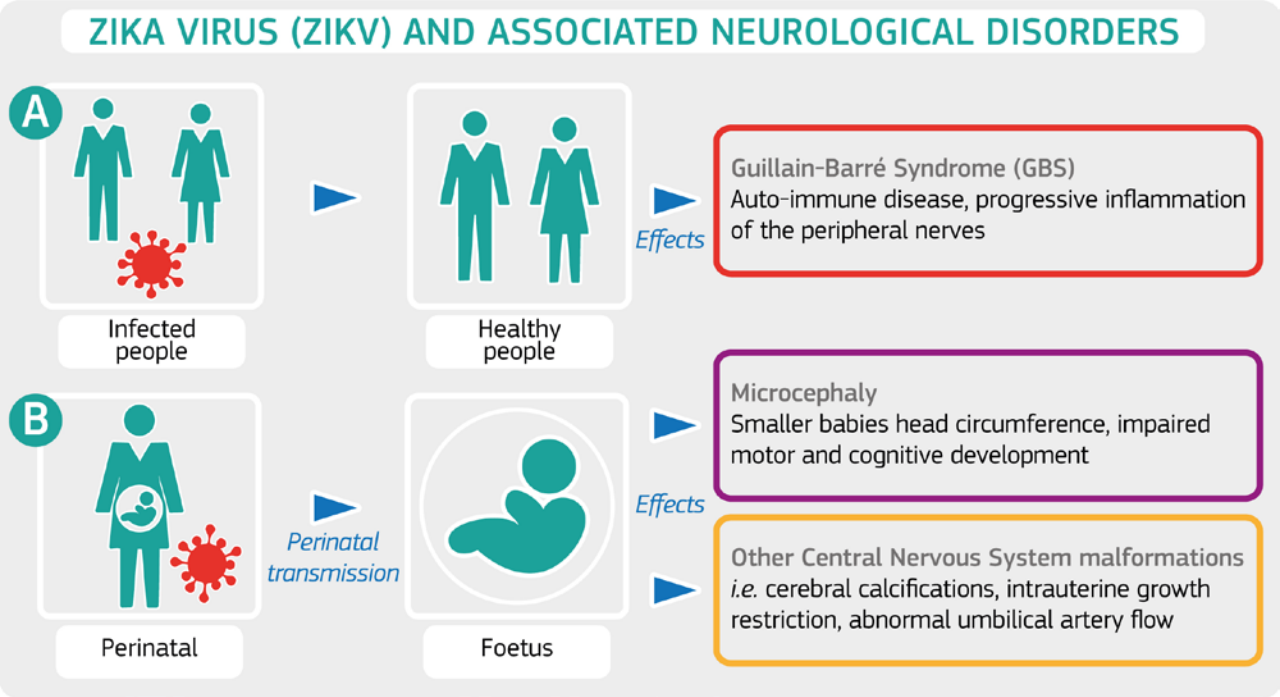

Figure 13. Zika virus (ZIKV) and associated neurological disorders (Source: JRC)
(A) Transmission of Zika virus (ZIKV) from infected persons to healthy persons can induce the Guillain-Barré Syndrome, a neurological disorder characterised by a progressive inflammation of the peripheral nerves. (B) The neonatal transmission of ZIKV from women to newborns is associated with a negative impact on infants' health. Neurological disorders observed in newborns, including microcephaly, tested positive for ZIKV. Other central nervous system malformations have been also suggested.

5.3 Zika virus and media misinformation

The outbreak of Zika virus (ZIKV) has been closely followed by the media. Information about ZIKV is readily available on the internet, but is often not supported by reliable scientific references. In Table 3 we have compared a list of relevant media news items with scientific findings, in order to give an overview of the veracity or falsity of news released to the public.

Table 3. Information on the outbreak of Zika virus (ZIKV): "media versus science" – comparison of media information and scientific knowledge, based on peer-reviewed publications

Media news	***Peer-reviewed papers***
The spread of Zika virus (ZIKV) may be associated with the El Niño regime, increasing global temperatures and global travel[c].	Malone et al. (2016) has raised the hypothesis that the El Niño weather pattern may have favoured the spread of mosquitoes in many areas, coupled with cargo and cruise shipping routes, indicating the potential for the additional spread of Zika virus (ZIKV) during 2016 to many regions of the Americas[125]. *The information spread by the media on this topic was correct.*
The introduction of Zika virus (ZIKV) into Brazil is related to the World Cup held in Brazil in 2014[d].	Musso (2015) stated that Zika virus (ZIKV) strain found in the Brazilian outbreak is correlated with the Polynesian one. The timing of the Va'a World Sprint Championships in Rio de Janeiro, in which Polynesia competed, fits with the beginning of the outbreak[126]. Pacific countries infected with ZIKV did not compete in the 2014 World Cup soccer competition. *The information spread by the media on this topic was incorrect.*
The United States of America wants to start vaccine trials on humans by the end of 2016[c]. In the USA, Human vaccine trials started in August 2016[e].	Fourteen vaccine developers in countries including France, the USA, Brazil, India and Austria are currently working on the development of a vaccine against Zika virus (ZIKV), but a timeline for its

	distribution cannot be predicted[56]. The National Institute of Allergy and Infectious Diseases has established strategies to test the vaccine candidates[127]. Preliminary trials started in August 2016[b,f]. *The information spread by the media on this topic was correct.*
A possible association of Zika virus (ZIKV) infection with birth malformations and neurological syndromes has been publicised, stating that doctors were able to isolate the virus in some children suffering from microcephaly. The Centers for Disease Control and Prevention (CDC) reported that "some samples" from children born with microcephaly tested positive for ZIKV, while "several" did not[d]. A link was established between ZIKV and microcephaly[g].	Different scientific papers supported the hypothesis that the virus could be transmitted from a pregnant woman to the newborn, but there are not sufficient data to confirm that Zika virus (ZIKV) is the causative agent of microcephaly[102,114-119,128]. Nevertheless, World Health Organization (WHO) has accepted the correlation between the two events[h]. *The information spread by the media on this topic was correct.*
Infection of the foetus is believed to have higher prevalence during the first gestation semester[c].	A scientific study has provided strong statistical support for the association between Zika virus (ZIKV) infection and microcephaly, by establishing that the period of risk was likely to include the first trimester of pregnancy. Further analyses are necessary to prove this relationship[129]. *The information spread by the media on this topic was not fully correct*.

Four out of five infected people show no symptoms[c].	It is estimated that 80% of Zika virus (ZIKV) infections are asymptomatic[97]. *The information spread by the media on this topic was correct.*
Transmission is mainly through mosquito bites, although sexual transmission has also been reported[i,j]. Zika virus (ZIKV) can also be transmitted through blood transfusions[k].	Zika virus (ZIKV) is primarily transmitted to people through the bite of an infected mosquito. Other routes of transmission that have been proposed include sexual and perinatal transmission. ZIKV may also be spread through blood transfusion[105]. *The information spread by the media on this topic was not fully correct.*
Genetically modified mosquitoes could be the cause of Zika virus (ZIKV) outbreak[l,m].	The potential use of genetically modified mosquitoes for pest control has been demonstrated to work in containing the disease, although it requires that a great number of mosquitoes be introduced into the environment[130]. There is no evidence that genetically modified mosquitoes cause Zika[n]. Other containment methods have been attempted, such as using molecular interference (iRNA) to induce mosquito death or infection of wild mosquitoes with a bacteria that can reduce the transmission efficacy[131-132]. *The information spread by the media on this topic was not correct.*
The pandemic potential of this virus is high, given global sport activities, such as the Olympic games, and the increase in global travel[c].	By 4 April 2017, the European Centre for Disease Prevention and Control (ECDC) had reported 2 142 imported infection cases in European citizens returning from affected countries[100].

	The information spread by the media on this topic was correct.
Labs will soon have a test that can detect all three viruses borne by the Aedes mosquito – Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) viruses. The test, however, will be effective only during the initial infection period of five days[o].	Immunoassays based on Ig detection are available for Dengue (DENV) and Zika (ZIKV) viruses, but the cross-reactivity of the test is too high to be able to discriminate the causative virus[90,133]. The Centers for Disease Control and Prevention (CDC) released a qPCR-based emergency test for the detection of ZIKV, DENV and Chikungunya virus (CHIKV)[p]. *The information spread by the media on this topic was correct.*
Doctors' hypothesize that pesticides used for pest control in Brazil could be responsible for foetus malformations that had recently been linked to Zika virus (ZIKV)[q,r,s].	Based on a European Food Safety Authority (EFSA) report, pyriproxyfen has low toxicity effects on mammals, including humans[134]. No reliable scientific sources were found to correlate pyriproxyphen with microcephaly. Similarly, malathaion was found to have low toxicity by oral route, no acute toxicity via dermal exposure and potential genotoxicity[135]. *The information spread by the media on this topic was not scientifically supported.*

Further information on misleading media news can be found on the World Health Organization (WHO) website[t].

6 Mosquito control strategies

In ecosystems, mosquito abundance is controlled by natural predators such as birds and fish, and by natural events such as hurricanes, droughts and cold spells. Urban areas provide a positive breeding environment for mosquitoes, given that natural predation is not significant and there is high availability of breeding sites. In these situations, the risk of infection is higher and human health is threatened. Mosquito-control strategies are therefore needed. There are currently five main methods of mosquito control i) extermination through the use of insecticides; ii) biomass reduction through the use of traps; iii) reduction of biomass through genetic modifications; iv) land reclamation in order to fight or prevent the spread of mosquitoes; v) control of water spaces (places) where the eggs and larvae can survive.

Most common insecticides used for mosquito control are chemical-based compounds that are applied by nebulisation/fumigation. Unfortunately, they raise (eco) toxicological issues, as with the extensive use of Dichlorodiphenyltrichloroethane (DDT)[136]. The use of insecticides was found to be very successful in eradicating diseases such as malaria from many regions[136]. Most recently, in Brazil, attempts to control the spread of Zika virus (ZIKV) have been made through mosquito-eradication campaigns using pesticides such as pyriproxyfen and malathion. Pyriproxyfen is an insecticide that is used for pest control on tomato and cotton crops, as it suppresses embryogenesis and inhibits metamorphosis and reproduction. The media recently speculated about its effects on the incidence of microcephaly cases[q]. Based on a European Food Safety Authority (EFSA) report, this chemical is considered to be very toxic to aquatic organisms, but no major effects were found for other organisms, including humans[134]. Nevertheless, it must be noted that human risk was not evaluated due to the low toxicity profile found for model mammals. Malathion, another pest control agent, was shown to have low toxicity for rats through oral exposure and no acute toxicity via dermal or inhalation exposure[137]. However, there is strong evidence that malathion may be an endocrine disruptor and have genotoxic properties[138]. This chemical is currently under revision in Europe.

Attempts at biological control based on biocides have been reported. *Bacillus thuringiensis* var. israelensis (Bti), a group of toxin-producing bacteria, has been used for mosquito control. This bacteria produce toxin crystals that act as insecticides and have no toxicity for humans. Unfortunately, the use of Bti was shown to have an indirect impact on non-target organisms; for example, it was found to reduce bird breeding behaviour as a result of the decrease in food availability[139].

Sterile male mosquitoes have been introduced into the field in order to reduce offspring. The potential of these organisms for biomass control has been demonstrated, although a great number of mosquitoes must be introduced in the environment, which limits the size of the treatable area[130]. Interference RNA (iRNA) technology is also promising as it is target-specific and can be used on fumigation campaigns, in the same way as insecticides. The technology works by disrupting the expression of any gene of interest in a specific manner by using double-stranded RNA (dsRNA). The dsRNA targeting key genes involved in apoptosis can be topically applied to mosquitoes and used as molecular insecticides to control their spread. However, mass production of iRNA products is currently not possible, and specific formulations should be developed to ensure the efficient delivery of the dsRNA into mosquitoes[131].

At the moment, mosquito traps and nets are the safest and most available method of mosquito control. Mosquito traps, for adult stages, mimic the scent of warm-blooded animals, thereby attracting female mosquitoes. This is an effective pest-control mechanism for relatively small areas, but it has limited success in open field areas. The same applies for oviposition traps, which are artificial breeding containers impregnated with insecticides. Oviposition traps are attractive to gravid mosquito females which become compromised upon contact with the insecticide. There are other effective insecticide products that are safe for the environment (e.g. vegetable oils). In habitational areas, the use of traps together with the active reduction of potential breeding sites (such as fountains, ponds or water-filled containers) can effectively reduce mosquito abundance.

Despite many attempts to reduce the negative effects of mosquitoes on humans, it would be unwise to remove them completely from the ecosystem as they are part of the food chain. For example, mosquitos are fed on by bird species living in the Arctic tundra, and are also part of the diet of dragonflies, spiders, frogs and fish[140]. In the absence of mosquitoes, many plants would lose their pollinators[140]. Mosquitoes are therefore part of the food web for some species, and permanently wiping out these insects could have adverse effects in nature, and consequently on humans.

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List of abbreviations and definitions

Bti	Bacillus thuringiensis var. israelensis
CDC	Centers for Disease Control and Prevention
CHIK	Chikungunya
CHIKV	Chikungunya virus
CNS	Central Nervous System
DDT	Dichlorodiphenyltrichloroethane
DEN	Dengue
DENV	Dengue virus
DHF	Dengue Haemorrhagic Fever
dsRNA	Double-stranded RNA
ECDC	European Centre for Disease Prevention and Control
ECSA	East/Central/South African
EFAS	European Flood Awareness System
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked ImmunoSorbent Assay
GBS	Guillain-Barré Syndrome
HABs	Harmful Algal Blooms
hNPCs	Human Cortical Neural Progenitor Cells
iRNA	interference RNA
JRC	Joint Research Centre
qPCR	quantitative Polymerase Chain Reaction
RNA	ribonucleic acid
TBE	Tick-borne encephalitis
TBEV	Tick-borne encephalitis virus
WHO	World Health Organization
WN	West Nile
WNF	West Nile fever
WNV	West Nile virus
YFV	Yellow Fever virus
ZIK	Zika
ZIKV	Zika virus

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