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Zika Virus Infection

Risk of Spreading in Europe

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Marta Díaz-Menéndez
Tropical Medicine Department
Hospital Universitario La Paz-Carlos III
Madrid
Spain

Clara Crespillo-Andújar
Tropical Medicine Department
Hospital Universitario La Paz-Carlos III
Madrid
Spain

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Abbreviations

ADE	Antibody-dependent enhancement
CDC	Centres of Disease Control and Prevention
ChIKV	Chikungunya Virus
DENV	Dengue Virus
ELISA	Enzyme-linked immunosorbent assay
ENSO	El Niño Southern Oscillation
GBS	Guillain–Barré syndrome
JEV	Japanese encephalitis virus
ORF	Open reading frame
PAHO	Pan American Health Organization
PHEIC	Public Health Emergency of International Concern
Ro	Basic reproduction number
rRT-PCR	Real-time reverse transcription polymerase chain reaction
RT-PCR	Real-time Polymerase chain reaction
SOPV	Spondoweni virus
TBEV	Tick-borne encephalitis virus
ULV	Ultra-low volume
USA	United States of America
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme
WNV	West Nile virus
YF	Yellow fever
YFV	Yellow Fever virus
ZIKV	Zika Virus

Chapter 1
Introduction

Abstract Zika virus (ZIKV) infection is an emerging mosquito-borne disease that has recently been described in geographical areas where previously didn't exist. Two important outbreaks were described in Yap Island (2007) and in several Pacific islands (2013). In 2015 ZIKV arrived first to Brazil and later to all countries in the Americas (North, Centre and South) and Caribe. In February 2016, the World Health Organization declared ZIKV infection a Public Health Emergency of International Concern, due to the link to birth defects and neurologic abnormalities (mostly Guillain-Barre syndrome). Clinical manifestations of this infection varies from asymptomatic to self-limiting acute febrile syndrome and other symptoms similar to those caused by dengue or chikungunya. Zika is a disease of particular concern because the possibility of transmission not only by mosquito bite, but by unprotected sexual intercourse or during pregnancy. Also, there is a real risk of introduction in regions where suitable vector already exist. In this monography, scientific information available in multiple aspects of ZIKV infection (the virus, the vector, the disease or the diagnosis) is compiled. Also, specific information about the potential risk of introduction of the disease in Europe is analyzed.

Zika virus (ZIKV) is the paradigm of emergent infectious disease. It's considered emergent because it has recently been described in geographical areas where it previously did not exist. First described in Uganda in 1947 in Rhesus monkeys from Zika Forest, since then sporadic cases were described in some countries in Africa and Asia. Two important outbreaks, in Yap island (Federated States of Micronesia) in 2007 and in several Pacific Islands in 2013, with more than 35,000 persons affected warned about the complications to which ZIKV infection might be associated: congenital syndrome in fetus from mothers infected during pregnancy and neurologic abnormalities (Guillain-Barre syndrome) in adults. In March 2015 the first ZKV cases in Brazil were confirmed, and in the following months all countries in the Americas (North, Centre and South) and Caribe reported autochthonous ZIKV cases.

In February 2016 the World Health Organization declared ZIKV infection and its suspected link to birth defects (microcephaly and others) a Public Health

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Emergency of International Concern (PHEIC). The term PHEIC is defined as an extraordinary event that constitutes a public health risk to other States through the international spread of the disease and which requires a coordinated international response. This statement designates a public health crisis of potential global reach that only in 3 other circumstances has previously been declared: H1N1 flu in 2009, subsequently the resurgence of polio after its near-eradication in May 2014 and only few months later, in August 2014 in response to the outbreak of Ebola in Western Africa. The declaration of PHEIC due to ZIKV was lifted on November 18, 2016.

Some other facts make ZIKV of particular concern. First, in most cases the infection is asymptomatic, and it may go unnoticed. As ZIKV transmission occurs not only after a mosquito bite, but also after unprotected sexual intercourse, infected and viremic patients can infect others. This has particular implications if there is an intention of pregnancy. Current international guidelines recommend delaying 6 months gestation after the infection or after a stance in an endemic area for ZIKV. This recommendation can be easily followed by travellers, but cannot be accomplished by residents in an area with a current outbreak.

Another fact that hinders the management of cases and its complications is that symptoms of ZIKV infection are similar to those of dengue or chikungunya, which are transmitted by the same type of mosquito and that often co-circulate in most countries. Microbiologic diagnosis may be necessary to specify final diagnosis but they are not available in low-resources settings. Moreover, microbiologic tests are difficult to interpret not allowing an easy confirmation of the diagnosis.

Another fact that has to be taken into account is the possibility of transmission from infected travellers returning from a ZIKV endemic area who carry the pathogen into new environments or accidentally translocate vectors in transport vehicles to locations where the disease does not exist yet. Travellers can also trigger further outbreaks if they return to their non-endemic country while they are still viremic, increasing the risk of spreading to areas where suitable mosquito vectors already exist. Multiple cases of imported ZIKV has been described in ZIKV-free countries and an appropriate vector control on these countries should be done in order to minimize the risk of establishment an dissemination of the disease.

The alarm for a Zika epidemic is already being sounded internationally. This has huge potential implications on society and thus public authorities should perform surveillance and provide adequate resources in order to sustain enhanced mosquito control.

In this monography, we compile scientific information available in multiple aspects of ZIKV infection, from the virus and vector to the clinical manifestations, complications or diagnosis. There is also specific information about the potential risks of introduction ZIKV disease in Europe and conditions are analysed.

Chapter 2
The Epidemiology

Abstract ZKV was first described in Uganda in 1947. It was isolated from the blood of a Rhesus monkey from the Zika Forest, during a yellow fever study and afterwards in 1948, it was also isolated from multiple *Aedes africanus* from the same forest. Human illness was first described in 1954, in the context of yellow fever outbreak in Nigeria. Since then, very few cases had been described in literature until 2007, when a ZIKV outbreak in Yap Island (Federated States of Micronesia) affected an estimated number of 5000 patients among the population older than 3 years. In the following years, until 2012, sporadic cases were described in some countries in Africa and Asia. In 2013, a new outbreak in Pacific Islands was estimated to affect over 30,000 people and implied an increasing number of neurological complications like Guillain–Barré syndrome (GBS). Also, in 2014 the first confirmation of the potential ZKV sexual transmission (ZIKV was isolated in a semen sample) and the first vertical transmission cases were described. In March 2015, the first ZIKV cases in America during an outbreak of exanthematous illness in Brazil (Bahia) were confirmed. During which ZIKV cases have been reported in other American and Caribbean countries and territories, with a continuous geographical expansion. At the same time, an increasing number of microcephaly and other central nervous system syndromes in newborns and GBS cases in most of these countries have been reported. On 1 February 2016, World Health Organization (WHO) declared Public Health Emergency of International Concern (PHEIC), the potential link with ZIKV infection and the microcephaly and other neurologic syndromes cases. So far, 25 countries from Americas, Africa and Asia had notified confirmed autochthonous ZIKV cases and additional seven countries have reported ongoing outbreaks of ZIKV infection.

2.1 Historical Outbreaks

Zika virus (ZIKV) was first isolated in 1947, from a sample of blood of a Rhesus monkey in Zika Forest, Uganda [1]. The next year, it was described in multiple *Aedes africanus* that could infect mices, monkeys and others mammalians. At that

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time, it wasn't clear if it can produce clinical disease or latent infection in humans, given that it was demonstrated ZIKV seroprevalence of 6,1% in 99 sera tested [2].

The first ZIKV illness in humans was described in 1954 in three patients during an outbreak of yellow fever in Nigeria. One of these patients was diagnosed by isolation of the virus and the other two by a rise of serum antibodies. All of them complained of fever, but only one of them was associated with jaundice [3].

During the next 50 years (until the first important ZIKV outbreak in Micronesia in 2007), there is very limited data about this concern, despite it is widely known that ZIKV main vector (*Aedes aegypti* and *albopictus*) has a worldwide presence including Africa, Asia and America [4]. In a systematic review of the published literature about ZIKV seroprevalence from 1947 to 2007, it was found that ZKV has been endemic for years in sub-Saharan Africa and Asia with an upwards seroprevalence of 50%, and 15–40% among reproductive ages [5]. But only nine cases of clinical infections, with natural transmission and confirmed virus isolation, had been described from 1954 to 2007 [3, 6–9]. Figure 2.1 shows the seropositive identification during this period of time by a systematic review of literature [5].

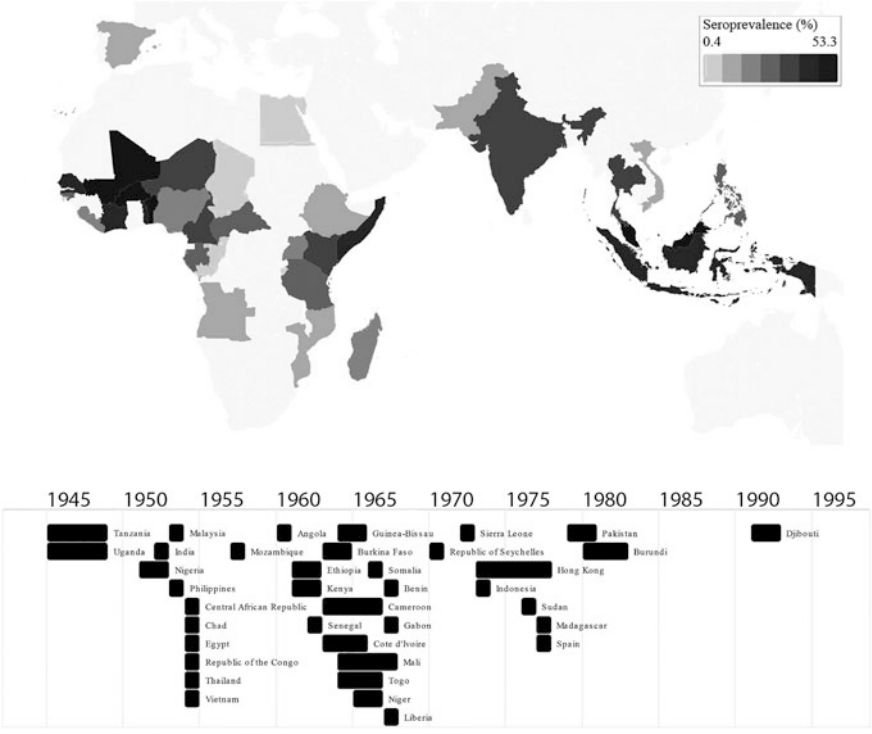

Fig. 2.1 *Above* ZIKV seroprevalence in countries up to April 2007. *Down* First years of identification of ZIKV seropositivity by country [5]

Molecular studies were carried out from 1968 to 2002 with samples of ZIKV isolated in Africa in eight countries, which map the disease as it moves during the twentieth century, from Uganda to western Africa and Asia [10].

Multiple hypotheses have been made trying to explain that period with under-reported and misdiagnosed cases of ZIKV infection. On the one hand, the high prevalence of subclinical infections and the similarity of symptoms with other arboviral infections that are endemics in the same geography could partially explain this fact [11].

Fist important outbreak of ZIKV was reported in April and May 2007, when an illness characterized by subjective fever, arthralgia, conjunctivitis and rash was estimated to affect at 73% (5000 infections) of population older than 3 years in Yap Island (Federated States of Micronesia). There were neither hospitalizations, hemorrhagic complications, nor deaths associated [12].

In 2008, while working in Senegal, two American scientists contracted ZIKV infection and one of them transmitted it to his wife, suggesting the first sexual transmitted infection of ZIKV described in an infection usually transmitted by mosquito bites [13].

Sporadic cases were described in the following years until 2013–14 in Cambodia, Cameroon, [11] Thailand [14], and characterization of strains of all cases described with a virus isolation was performed. Phylogenetic trees were constricted and two geographically lineages were identified: African and Asian. Findings in ZIKV strain isolation during the Micronesia outbreak suggested that it was initiated by a Southeast Asia strain [11, 15].

A new outbreak of ZIKV Asian lineage occurred in October 2013, affecting an estimated number of over 30,000 patients in French Polynesia throughout the archipelago. Since that date until February 2014, it was described an increase number of neurological symptoms and complications including 42 cases of Guillain–Barré syndrome (GBS) that were associated with ZIKV infections [16, 17].

During the French Polynesia outbreak, two important events were described. The first one occurred in December 2013 when a patient who had initially suffered from ZIKV infection symptoms (asthenia, fever, headache and arthralgia), presented hematospermia 8 weeks later and looked for medical care. During the semen study, real-time reverse transcription polymerase chain reaction (RT-PCR) for ZIKV was performed and resulted positive (also in urine but not in blood), adding data to the evidence that sexual transmission was possible [18]. In March 2014, two cases of pregnant women were described to have a positive reverse transcription polymerase chain reaction (RT-PCR) for ZIKV in the 38 weeks' gestation and 4 days after delivery. A positive RT-PCR in their newborns was also confirmed, becoming the first vertical or perinatal transmission described [19].

Following outbreaks took place in New Caledonia (2014) with 1400 confirmed cases, Cook Island (2014) over 900 cases, Easter Island (2014), Samoa (2015) and American Samoa (2016) [16, 20, 21].

In February 2014, the first case of autochthonous transmission of ZIKV in Eastern Island (Chile), located in the south-eastern Pacific [22], was confirmed.

ZIKV infection was notified for first time in the Americas in March 2015 in Bahia, Brazil, during an outbreak of cases presenting rash, mild fever, arthralgia and conjunctivitis, whose samples showed a negative serology of Dengue and Chikungunya virus. RT-PCR of ZIKV was performed in 21 acute-phase serum specimens, from Santa Helena Hospital in Camaçari and resulted positive in seven of them (29.2%). The phylogenetic analysis confirmed the belonging to the Asian linage and a 99% identity with a sequence from a ZIKV isolate from French Polynesia [23, 24].

There has been important speculation about how ZIKV appeared in Brazil, after Pacific Ocean outbreak. Phylogenetic studies [23] associated with a review of international event, suggested that it could have happened in August 2014 during the Va'a World Sprint Championship canoe race that took place in Rio de Janerio, Brazil. Four invited countries to the event had ZIKV circulating during 2014 [25].

On 7 May 2015, for the first time, Pan American Health Organization and WHO made an epidemiological alert about the potential spread of ZIKV infection across territories where the vectors *Aedes* were present [26].

Two months later, in 17 July 2015, it was reported by Bahia State of Brazil, an increasing number of neurological disorders that included 49 confirmed GBS in patients with history of exanthematic disease (62% of them with a previous history of ZIKV and dengue infection confirmed) [27, 28].

In October 2015, the authorities of Colombia confirmed the first case of ZIKV infection. This same month, Cabo Verde confirmed the country's first outbreak of ZKV infection with 165 suspected cases reported [22, 29].

Also, in October 2015 Brazil made a communication from Pernambuco State Health Department concerning an increasing number of cases of microcephaly since August 2015. From a total yearly average number of microcephaly per 1000 live births annually reported of 62.8 (from 2010 to 2014), the number has increased up to 1248 as for 28 November 2015 [22, 29, 30]. On 13 November 2015, Ministry of Health in Brazil reported the presence of RNA ZIKV in amniotic fluid samples from two pregnant women who had presented possible ZIKV clinical symptoms at 18' weeks and 10' weeks of gestation, respectively. Microcephaly was confirmed in their two newborns [29, 31].

Until December 2015, 18 Brazil states confirmed autochthonous ZIKV transmission. According to preliminary data, 440,000–1,300,000 cases of ZIKV infections were estimated to have happened during 2015 by the Brazilian Ministry of Health [29].

From the end of December 2015 until end of January 2016, a total of 2.016, 3.836, 99 and 147 cases were reported by El Salvador, Panama and Martinique respectively. Also Venezuela reported 192 cases of ZIKV infections. During the next months ZIKV infection spread gradually by the American continent and until 1 February 2016, 25 countries from Americas, Africa and Asia had notified confirmed

autochthonous ZIKV cases. Additional seven countries (Brazil, Colombia, Cabo Verde, Bolivarian Republic of Venezuela, El Salvador, Martinique and Panama) have also reported ongoing outbreaks of ZIKV infection [22]. Dates of cases officially reported by country, until December 2016 are illustrated in Fig. 2.2 [28].

On 1 February 2016, the World Health Organization (WHO) declared a Public Health Emergency of International Concern (PHEIC), the possible link with ZIKV infection and the microcephaly and other neurologic syndromes cases [32].

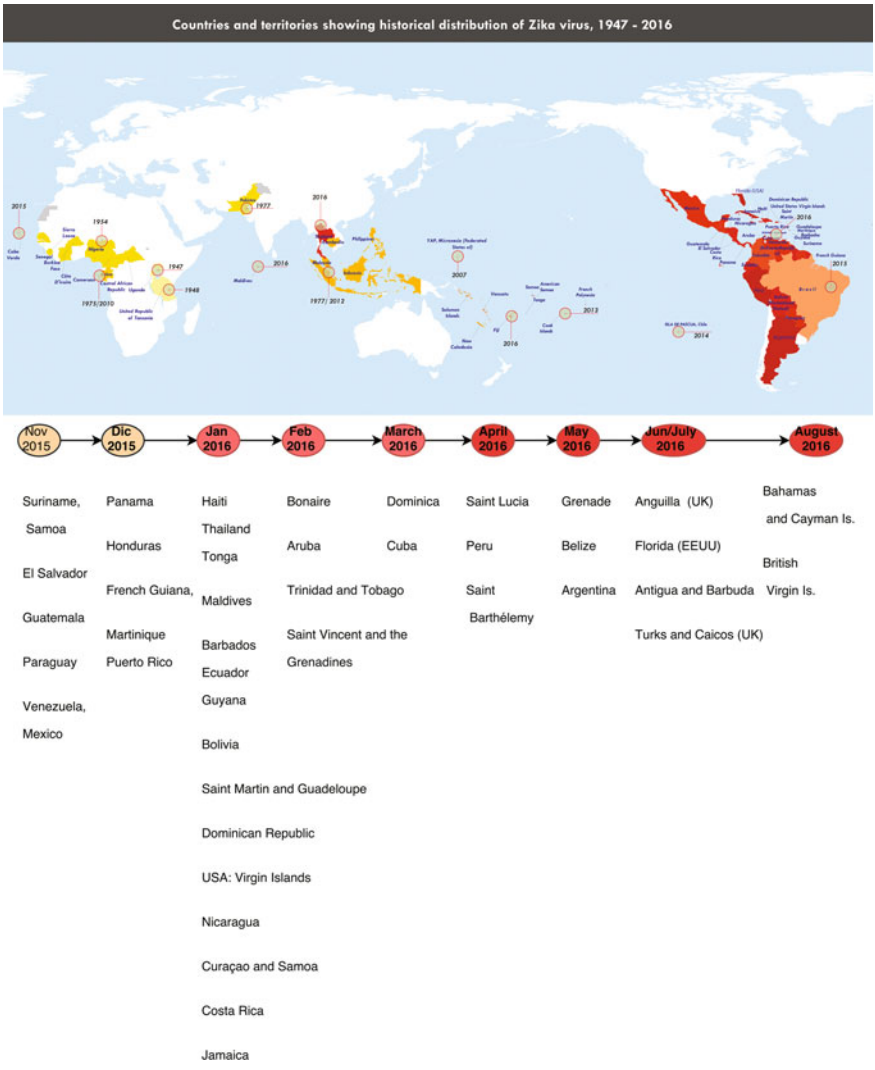

Fig. 2.2 Date of first autochthonous cases of ZIKV infection by country until August 2016 [28]

2.2 Current Outbreak

To the date of 2 September 2016, there have been four WHO meetings of the Emergency Committee under the International Health Regulations (2005) regarding microcephaly, other neurological disorders and ZIKV. Until that moment, 72 countries and territories have reported ZIKV transmission since 2007. Twenty countries have reported microcephaly and other central nervous system malformations related with ZIKV and 18 countries have reported an increased incidence of GBS. Because of the continuous viral expansion and the lack of scientific evidence, it was decided to continue considering ZIKV as a PHEIC [33, 34].

On 18 November 2016, during the fifth meeting of the Emergency Committee under the International Health Regulations (2005) regarding microcephaly, other neurological disorders and ZIKV, the PHEIC was removed because the research had already demonstrated the link between ZIKV infection and microcephaly.

Some cases have been described in Asia and Pacific, up to 20 March 2017, in India, Philippines (national), Viet Nam (national), Singapore (national), Malaysia (Petaling Jaya, Selangor), Thailand, Myanmar (Yangon) and Taiwan with only imported cases.

In Africa up to 17 January 2017, there have been described ZIKV cases in Guinea Bisau, Nigeria and Angola in which on 7 February 2017, Health officials have reported a ZIKV-related microcephaly case from Bengo province.

Finally, up to date on 7 February 2017, no additional countries or territories of the Americas have confirmed autochthonous, vector-borne transmission of ZIKV disease during the last month, but five countries have reported sexually transmitted Zika cases [35].

Up to 23 March 2017, Pan American Health Organization (PAHO) cumulative cases described a total of 20 deaths among ZIKV cases, 2807 total cases of confirmed congenital syndrome associated with ZIKV infection in America (including north, Latin American and the Caribbean and non-latin Caribbean), 551,432 suspected cases and 206,351 confirmed ZIKV infection cases [36].

In United States of America (USA), up to 29 March 2017, 5182 cases of ZIKV were reported. From them, 4886 cases happened in travellers returning from affected areas and 222 cases acquired through presumed local mosquito-borne transmission in Florida (216 patients) and Texas (6 patients). 74 cases were acquired through other routes, including sexual transmission (45 patients), congenital infection (27 patients), laboratory transmission (1 patient) and person to person through an unknown route (1 patient) [37].

Imported cases with no possibility of on going mosquito transmission have been described in Europe, from France, Spain and United Kingdom. There has been reported a total of 478 cases in Canada, up to 9 March 2017, whom 28 were pregnant women with 2 ZIKV-related abnormalities in foetuses and newborns and 3 additional sexually transmitted cases [38].

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Chapter 3
The Virus

Abstract ZIKV is a member of the family Flaviviridae, genus *Flavivirus*. It's a RNA virus with three structural proteins (capsid, pre-membrane and envelope). ZIKV has adapted to humans by losing a codon of the non-structural proteins NS1 of its genome, facilitating viral replication and increasing viral titters. ZIKV infects dermal cells and then migrates to lymph node and bloodstream, reaching different organs and tissues. There are identified three lineages: East African, West African, and Asian. Current outbreak clustered closely within the Asian lineage, and its introduction in the Americas may have taken place between May and December 2013. ZIKV is transmitted through mosquito bite, which carries the virus in its saliva, and replicate initially in dendritic cells near the site of inoculation and then spread to the blood, lymph nodes, bloodstream and finally to organs and tissues. Fetal affectation in congenital syndrome may be the result of destruction of germinal matrix in central nervous system, resulting in small brains and abnormal cortical gyration.

3.1 ZIKV Classification

ZIKV belongs to the genus *Flavivirus* within the family Flaviviridae, which includes other human pathogenic viruses such as Yellow Fever virus (YFV), Dengue virus (DENV) type 1–4, Japanese Encephalitis virus (JEV), West Nile virus (WNV), Spondoweni virus (SOPV) or tick-borne encephalitis virus (TBEV). Complete genome sequence identity and divergence analysis indicate that ZIKV have the highest identity with SPOV and the lowest with YFV [1]. SPOV and ZIKV are the only members of their clade within the mosquito-borne cluster of flaviviruses. Figure 3.1 shows the Phylogenetic relationship between ZIKV and other flaviviruses based on nucleic acid sequence of non-structural viral protein 5.

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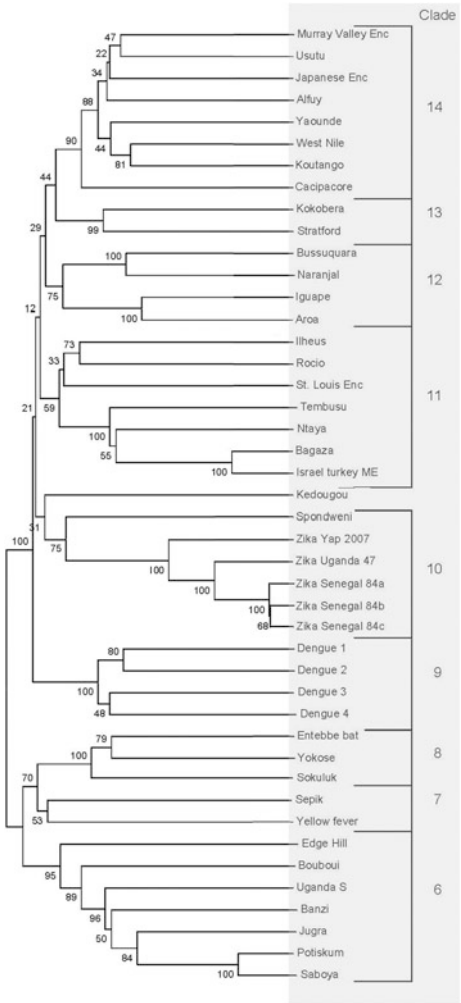

Fig. 3.1 Phylogenetic relationship between ZIKV and other flaviviruses based on nucleic acid sequence of non-structural viral protein 5. Adapted from: Lanciotti et al. [8]

3.2 ZIKV Morphology

ZIKV contains single-stranded positive sense RNA [2]. The full genome of ZIKV (the ZIKV MR 766 prototype strain) was entirely sequenced for the first time in 2007 [3]. It has a 10,794 kb genome with two flanking noncoding regions (5′ NCR and 3′ NCR). The open reading frame (ORF) encodes a single polyprotein which is cleaved into three structural proteins [capsid (C), precursor membrane (PrM) and envelope (E)] and at least seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [1]. The E protein is the major protein related to receptor binding and membrane fusion. The domain III of E protein harbours different

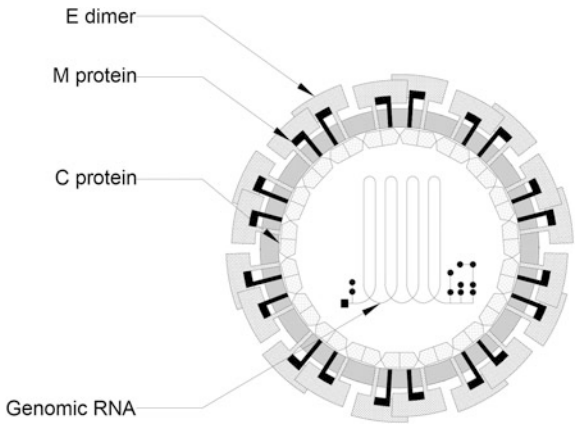

Fig. 3.2 Representation of the typical genome structure of a flavivirus

antigenic epitopes that are important targets of serological tests, neutralising antibodies, and vaccines [4].

There are 428 nucleotides located in the 3′ NCR that may be involved in translation, RNA packaging, cyclization, genome stabilization, and recognition. The 3′ NCR region is twisted into a loop and the 5′ NCR has a methylated nucleotide cap that allows translation or a genome-linked protein (VPg) [5]. Figure 3.2 represents the typical genome structure of a flavivirus.

The structure of ZIKV follows that of other flaviviruses. The virion diameter is approximately 40 nm with surface projections that measure nearly 10 nm. Hooked on the membrane there are glycoproteins arranged in an icosahedral-like symmetry, involved in adsorption to and infection of host cells. Underlying the membrane is the viral nucleocapsid approximately 25–30 nm in diameter, surrounded by a host-membrane derived lipid bilayer that contains envelope proteins E and M [6]. Figure 3.3 represents general structure of ZIKV.

Until the identification of the full length of ZIKV genome, the virus was supposed to infect primarily wild primates, causing sporadic disease in humans. Recent research supports that the virus has recently adapted to humans by losing an NS1 codon of its genome, facilitating viral replication and increasing viral titters [7].

3.3 Phylogeny of ZIKV

The first phylogenetic analysis of ZIKV was conducted after the Yap State outbreak [8]. On the basis of the full genome sequences of the ORFs, two major ZIKV lineages were described: African and Asian [9]. Later, a more comprehensive sequencing of the complete coding region of the NS5-encoding gene revealed three different ZIKV subclades: East African (prototype Uganda strain), West African (Senegal strains), and Asian (ZIKV 2007 Yap strain). It has been also postulated that viruses from East Africa moved into Asia approximately 50–100 years ago and

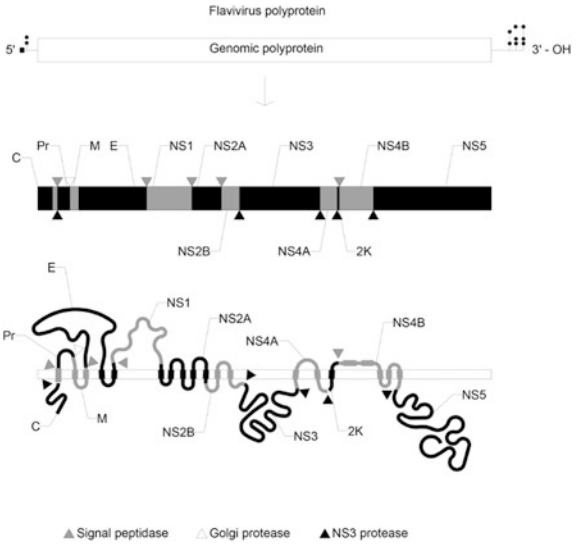

Fig. 3.3 General structure of ZIKV

evolved into a unique Asian genotype [1, 8]. The two major ZIKV epidemics in the past (Yap Island in 2007 and French Polynesia in 2013) most likely resulted from the introduction of a Asian ZIKV strain(s) [2, 9, 10].

The importance of the existence of different linages goes beyond the knowledge of the origin of the ZIKV that is currently causing the outbreak. Relevance has to do with the link to specific lineage and complications of disease, as Asian lineage ZIKV blocks the proliferation of brain stem cells and interfere their ability to develop into brain nerve cells [3, 11].

3.4 Phylogenetic Analysis of Current Outbreak

Available sequences shows that all the 2015 ZIKV isolated in American countries including Brazil [1, 12, 13], Colombia [4, 14], Puerto Rico and Guatemala [5, 15] clustered closely within the Asian lineage, sharing over 99% nucleotide identity with the French Polynesian strains [6, 15]. This finding suggests that it has spread across the Pacific Ocean to invade South America.

Regarding ZIKV affecting countries in South East of Asia (see Chap. 2), recent investigations suggest that phylogenetic analyses integrating geographical and time factors show that Southeast Asian ZIKV might not be the direct source of South American outbreaks as previously speculated. There has been described an amino acid residues on external viral proteins specifically in South American ZIKV but not found in Southeast Asian ZIKV [7, 16].

Phylogenetic and molecular clock analyses has postulated that ZIKV is likely to have emerged between 1892 and 1943 in Uganda [5, 8]. A single introduction of ZIKV into the Americas may have taken place between May and December 2013, more than 12 months before the first reports of ZIKV in Brazil [9, 13].

3.5 Pathogenic Mechanism of ZIKV

When a female *Aedes* mosquito bites an infected patient, it sucks blood containing ZIKV. To be transmitted, ZIKV must replicate in the mosquito midgut epithelial cells and later in the salivary gland cells [17]. Extrinsic incubation period lasts between 5 to10 days. After that period, ZIKV can be found in the mosquito's saliva and subsequently can infect human as the mosquito avidly sucks blood while injecting infected saliva in the host [18]. Transovarially transmission is other possible mechanism of viral maintenance [19].

Upon blood-feeding process of the mosquito, ZIKV enters and passes through the epidermal, dermal and Langerhans cells of the skin. Main potential target cells for infection are: human dermal fibroblasts, epidermal keratinocytes, and immature dendritic cells [20]. Skin fibroblasts cells have a dual function: they are initially the target cells for ZIKV infection infected but subsequently they constitute he first line of defence [21]. Multiple entry and/or adhesion factors (AXL, Tyro3, DC-SIGN, or TIM-1) favour the entry of the virus and acts as attachment factors or receptors for ZIKV [20, 21]. Viral replication activates an antiviral immune response starting apoptosis of epidermal cells, autophagy and releasing high number of viral particles. Then, virus migrates to regional lymph nodes, to the bloodstream and finally to organs and tissues including the central nervous system, the skeletal muscles, myocardium, and probably transplacentally to the fetus, leading to congenital syndrome.

In pregnant women, ZIKV primarily infects and replicates in Hofbauer cells (placental macrophages), resulting in proliferation and prominent hyperplasia of these placental cells [22]. Cytotrophoblasts are also affected, although in lesser extent. Viral replication appears to induce type-I interferon, pro-inflammatory cytokines, and antiviral gene expression. All these facts disrupts the fetoplacental barrier [23].

Fetal neurologic affectation may be initially the result of destruction of the germinal matrix, fetal principal target for ZIKV in central nervous system. Evidence from cell culture systems shows that infection of neuronal precursor cell results in cell death [24]. Damage of these cells early in development might to substantially reduce the number of neurons generated and result in small brains with abnormal or no cortical gyration [25].

For more information about repercussion of ZIKV infection in adults and fetus, see Chap. 6.

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Chapter 4
The Vector

Abstract Even if transmission has been described by other *Aedes* subgenous, the principal viral ZIKV vectors are *Ae. aegypti* and *Ae. albopictus*, which belong to the *Culicidae* subfamily. The most important morphological characteristics of these mosquitoes are the black and white pattern due to the presence of white/silver scale patches against a black background on the legs and other parts of the body. For those characteristics they are commonly known as 'Tiger Mosquito'. Both mosquitoes have adapted to survive in a variety of environments, and they prefer to live close to human settlements. *Ae. aegypti* is reported to have been ship-mediated introduced in the Mediterranean area from Africa during the eighteenth and nineteenth centuries, but its presence was eradicated possibly by elimination programmes. It is currently present in tropical and subtropical areas and it has been the main vector of American ZIKV outbreak. *Ae. albopictus* was first described in Asia and spread by commercial trades throughout tropical and subtropical areas. Unlike *Ae. aegypti*, *Ae. albopictus* can adapt to lower temperatures, due to some characteristics like embryonic diapause and overwinter in the egg stage that make it to be currently present in Europe, Middle East and North America. In order to avoid possible future ZIKV outbreak in this geographical areas, the information of people at risk, enhance surveillance, mosquitoes control and the early detection of possible autochthonous cases are a priority.

4.1 Classification of the Different Vectors

ZIKV is mainly transmitted by mosquitoes of the *Culicidae* family and *Aedes (Stegomyia)* subgenus. In this host vector, the virus breeds without affecting mosquito fitness, and remains in it all life long, being transmitted form one reservoir to another by the blood meal [1].

Ae. Africanus was the first ZIKV vector described in 1948 in Uganda [2]. Others arboviruses (Rift Valley fever virus, Yellow Fever virus, Chikungunya virus and

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Ntaya virus) have also been isolated from this mosquito that prefers monkeys to humans but also feeds on reptilians, rodent and avian species maintaining a sylvatic ZIKV cycle transmission, so sporadic human infections mainly occurred in these settings [3–5].

Subsequent ZIKV amplification studies, carried out in Africa, have described the presence of the virus in others *Aedes* subgenera as *Ae. Furcifer, Ae. luteocephalus, Ae. africanus, Ae. vittatus, Ae. taylori, Ae. dalzieli, Ae. Hirsutus, Ae. Metallicus*, and others. During a mosquito collection from April to December 2011 in south-eastern Senegal, ZIKV was detected in an *Ae. furcifer* male, strongly suggesting a possible vertical transmission of ZIKV, that could bring on a local maintenance of the virus transmission to human [6]. Further studies have demonstrated a high filial infection rates in *Ae. aegypti* and *Ae. atbopictus*, substantially higher than ratios historically measured for others flaviviruses. Although, the rate of vertical transmission (proportion of infected mosquitoes transmitting the virus to progeny) could not be determined and also the mechanism of vertical transmission is not evident, so eggs could be infected during oviposition rather than a transovarian transmission [7].

It is important to consider that the isolation of the virus in mosquito does not necessarily evidence the vector competence of transmission. Specific detection of the virus in the salivary gland of the mosquito is a prerequisite of mosquito transmission capacity.

Ae. vittatus and *Ae. luteocephalus* were found to be a potential vector in Senegal since they had viral genome in their saliva [8]. Others experimental studies have demonstrated *Ae. Aegypti* potential capacity for ZIKV transmission in Africa [9].

It is known to be a geographic variation in the oral susceptibility of mosquitoes from the same specie to different viruses [10]. In Asia, ZIKV was first isolated in an *Ae. aegypti* from Malaysia in 1966 [11], but prior to the Yap Island outbreak in 2007, limited clinical disease cases were described in literature, so also a little scientific data was described about ZIKV vectors. Because of the fist Oceanic outbreak in 2007, the study of the biological transmission of the virus was a health priority and *Aedes hensilli* was found to be the predominant specie on the island. Experimental studies of this vector were carried out and no ZIKV material was isolated in the collected mosquitoes. During the study of the vector capacity of transmission, infection rates up to 86% and dissemination rates of 23% were found, suggesting that this mosquito served as a vector during the outbreak [12].

In areas without non-human primates presence, ZIKV is transmitted in a human–mosquito–human transmission cycle, suggesting that the virus is adapted to a human reservoir host [3]. After the rural epidemic on the Yap Island, a urban epidemic took place for the first time in French Polynesia in 2013 and the main vector described was *Aedes aegypti* with a suspected secondary vector with *Aedes polynesiensis* [1]. Experimental studies have investigated the susceptibility of an Asian strain of *Aedes aegypti* to ZIKV. The virus was detected in the salivary glands of 62% of the mosquitoes on day 5 and in all of them on days 10 and 14, demonstrating the high capability of ZIKV transmission of this strain [13].

Other experimental studies have demonstrated the potential *Ae. Albopictus* capacity of ZIKV transmission in Asia (Singapore), on the day 7 after the infection, 73% of the mosquitoes exposed to the infection had virus in their saliva [14].

In May 2015, the virus was detected in Brazil and then spread through South and Central America. Recent experimental studies have proven *Ae. aegypti* and *Ae. albopictus* competence, from the Caribbean (Martinique, Guadeloupe), North America (southern United States), South America (Brazil, French Guiana), in the amplification and transmission of the Asian genotype of ZIKV. They have found geographical differences since *Ae. aegypti* populations from Guadeloupe and French Guiana exhibited a higher dissemination of the virus than other *Ae. aegypti* populations examined. Even though transmission was observed in both mosquito species at the day 14 after oral ZIKV infection [15].

Even if *Aedes sp*. is the most reported ZIKV vector in Africa, Asia, the Pacific region and the Americas, in particular, the most antropophilic subgenre, *A. aegypti*, has shown to be the principal specie in the ZIKV current outbreak, followed by *Ae. albopictus* [16].

Nevertheless, there is a small amount of available experimental data about potential ZIKV vector candidates, so it is important to further investigate the role that other species could have in the virus transmission. With this specific objective, an informatics model that predicts which are the potential species of mosquitoes that must been prioritized has a possible ZIKV vector has been developed. This model takes into account all viruses that belong to the same family as ZIKV and the mosquitoes that transmit them. The method identifies the propensity for a mosquito species to transmit any flavivirus. In total, 180 potential mosquito Zika pairs were identified as being the best candidates to be experimentally tested for Zika virus competence. From them, there were 24 *Aedes* species, nine *Culex* species, one *Psorophora* species and one *Runchomyia* species. *Ae. aegypti* and *Ae. arbopictus* result as the most highly suspected vectors of ZIKV [17].

4.2 Characteristics of the Vectors

Aedes sp belongs, like most of the mosquito species of the world, to a *Culicinae* Subfamily. *Ae. aegypti* and *Ae. albopictus* belong to the subgenus *Stegomyia.*

Regarding their capacity of adaptation, mosquitoes are capable of surviving in a variety of environments, preferring to live close to human settlements. Aquatic habitats are preferred and colonized temporary or permanent by all species of mosquitoes, so they need any type of water accumulation for the oviposition. *Ae. albopictus* has a especial adaptation capacity for climatic factors, and their eggs are more resistant to desiccation and can survive for more than a year. These characteristics have contributed to their global spread via international trade [18].

4.2.1 Morphological Characteristics of Vectors

Both *Ae. aegypti* as *Ae. albopictus* are rather small, rarely medium-sized mosquitoes, and they show a black and white pattern due to the presence of white/silver scale patches against a black background on the legs and other parts of the body. Because of this characteristic feature, they are commonly known as 'Tiger Mosquito'.

Main morphological characteristics are the following: Male palps are more than half the length of the proboscis. The female palps are up to 1/4 the length of the proboscis,

The vertex is largely covered with broad and at decumbent scales, erect forked scales are not numerous and restricted to the occiput. The scutellum has broad scales on all the lobes, and the postnotum is bare. The abdominal terga have white basal bands and often white lateral spots and the cerci are relatively short.

The differentiation between them and other indigenous or invasive mosquitoes is the presence of a median silver scale line on the scutum (dorsal part of the thorax) [18].

4.2.2 Biology and General Characteristics of Vectors

In tropical and subtropical areas, a continuous breeding takes place throughout the year, in order to maintain life cycle. In Europe, *Ae. albopictus* shows an embryonic dispause and overwinter in the eggs state, in order to maintain the cycle until the next year through the cold stations. Oviposition always takes place in small variety of natural and artificial mostly clean water containers (e.g. tree holes, water jars, flower pots, etc.). Female mosquitoes could lay between 50 and 500 eggs in the 2 or 3 days after blood meal. They use to put their eggs into the water surface. Eggs become larvae 2 days after oviposition. Larvae, is the legless stage of the mosquito, that leaves the water surface, and eat microorganism, algae, etc. It becomes in a pupae that usually lasts for about 2 days, and during this time, the metamorphosis takes place. Population takes place 8 days after ovoposition and Adults emerge from 9 to 10 days.

Males feed on plant juices as a source of carbohydrates, and female mouthparts are developed to pierce the skin of the host to obtain blood for egg maturation.

The mouth parts are extended into a proboscis which enclose by the labium when the mosquito is not feeding (Fig. 4.1).

Both mosquitoes can feed on mammalians but prefer human blood to other animal [16]. *Ae. albopictus* has been described to feed on also domestic and wild animals, reptiles, birds and amphibians [19].

Once the females find and land on the host, they will probe the skin in a few times looking for a capillary for the intake of blood. Thickness and temperature of the skin might be important probing stimuli for mosquitoes (a higher temperature

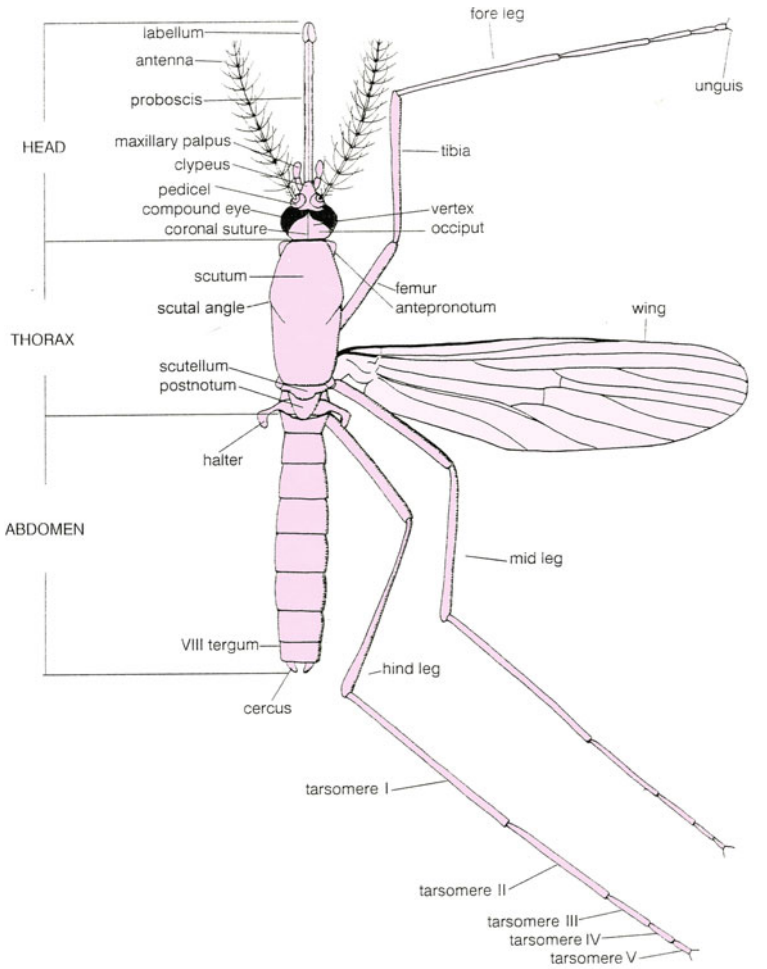

Fig. 4.1 General outline of a famele *Culicine* mosquito

should be related to a more number of blood vessels in the skin). When they have successfully punctured the skin, they inject saliva into the wound which usually contains anticoagulants, and that is the moment when ZIKV presented into the mosquito salivary glands, reach and infect the host. It usually causes an inflammatory reaction in the host at the site of the wound. Female mosquitoes can ingest blood more than three times its body weight. Blood is used for egg production rather than as a source of energy, so both sexes of mosquitoes require plant juices for the energy to fly [18].

Table 4.1 Main differences of *Ae. aegypti* and *Ae. albopictus*

Ae. aegypti	*Ae. albopictus*
27–30° are required Not winter diapause	Eggs are resistant to desiccation Low Tº adaptation, embryonic diapause and overwinter in the egg stage Eggs could survive a cold spell of −10° [21]
Blood feeding each 2–4 days During day	Feeding during dusk and night (also during daytime)
Adult rest indoors	Can also feed outside the houses
Distribution in tropical, subtropical. Range T° limited 10°	Distribution in Oriental Region and Oceania Original form Japan and China, spread into the New World by international commerce Europe
Not survive in cold winter	Could adapt at lower mean T° (5–28.5°) and lower rainfall [22]
More likely to become infected and high vectorial capacity (ability to transmit the infection) [3]	Similar vector competence [15]

Fly range is limited to less than 200 m, in both species, so the main dispersal route throughout different countries and continents is via international trade and human transports [20].

Main differences of *Ae. aegypti* and *Ae. albopictus* are described in Table 4.1.

4.3 Presence of the Vectors in the World

Aedes aegypti

It is distributed in the tropical, subtropical and warm temperature region of both hemispheres. Historically, it is believed to have been transported into the Americas and Mediterranean countries by sailing ships from Africa [19]. It is suggested to have been spread by the colonization routes and distributed in tropics, becoming highly efficient for inter-human transmission arboviruses [23].

While they are not able to survive in cold winter months, its presence is limited in certain geographical areas. It is currently distributed throughout the tropics including most of the tropical and subtropical regions in Africa, Middle East, Southeast Asia, Pacific and American continent from South and Central America to United States of America. In North America, it could be found at the 40º northern latitude in Indiana and Illinois, but they do not survive the winter months [18, 24].

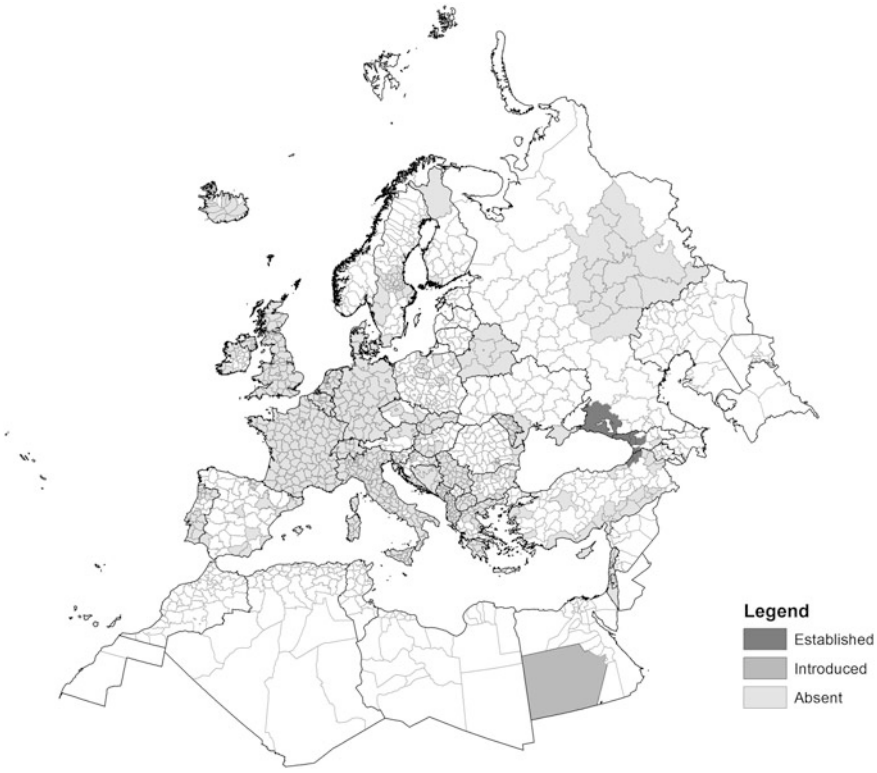

Fig. 4.2 *Aedes aegypti* distribution in Europe up to January 2017

In Europe it was established during the eighteenth century, but at the end of twentieth century its presence disappeared, probably due to elimination programmes. Present distribution in Europe is illustrated in Fig. 4.2, even if its presence is limited and not established, except for Georgia and other countries bordering the Black Sea, its distribution is expanding. In the near future, circumstances like climatic change could result in more northern and southern expansion of *Ae. aegypti* and its establishment in Europe. For more information about the risk of spreading of ZIKV in Europe see Chap. 10.

Aedes albopictus

Historically, *Ae. albopictus* was mainly distributed in the Oriental Region and Oceania. Nevertheless, during the past three decades, it has spread globally via passive transport of eggs using tyres or plants pots trough trades routes. It is already well-stabilized in Africa, American continent from the south to the North, Asia, Australia and New Zealand, Middle East and Europe [18, 22].

In Europe, it was first reported in 1979 in Albania, and in 1990 it was passively introduced in Italy. After 1999, it was spread through the Mediterranean Coast being established in countries like Greece, France and Spain [1, 19, 25].

European vector competence has been already studied, in mosquitoes collected in Germany, that were experimentally infected with ZIKV, and *Ae. albopictus* was susceptible for the human infection, but only at 27º, with transmission rates similar to an *Aedes aegypti* laboratory colony which was tested in parallel [26].

Consistently, on 3 February 2016, WHO's regional director for Europe, alerted all European countries, in which *Aedes* mosquitoes are present to control its population and enhance surveillance of the ZIKV vector. The detection of possible autochthonous ZIKV cases and information of people at risk, particularly pregnant women, about mosquito bites, is the priority [27].

Taking into account their ability of environmental adaptation and the number of imported *Ae. albopictus* population stabilized in Europe, future mosquito expansion is possible. It is suggested that Portugal, Eastern Adriatic and Turkey coast are the most likely places for establishment, among others [22].

Current *Ae. albopictus* distribution in Europe is illustrated in Fig. 4.3.

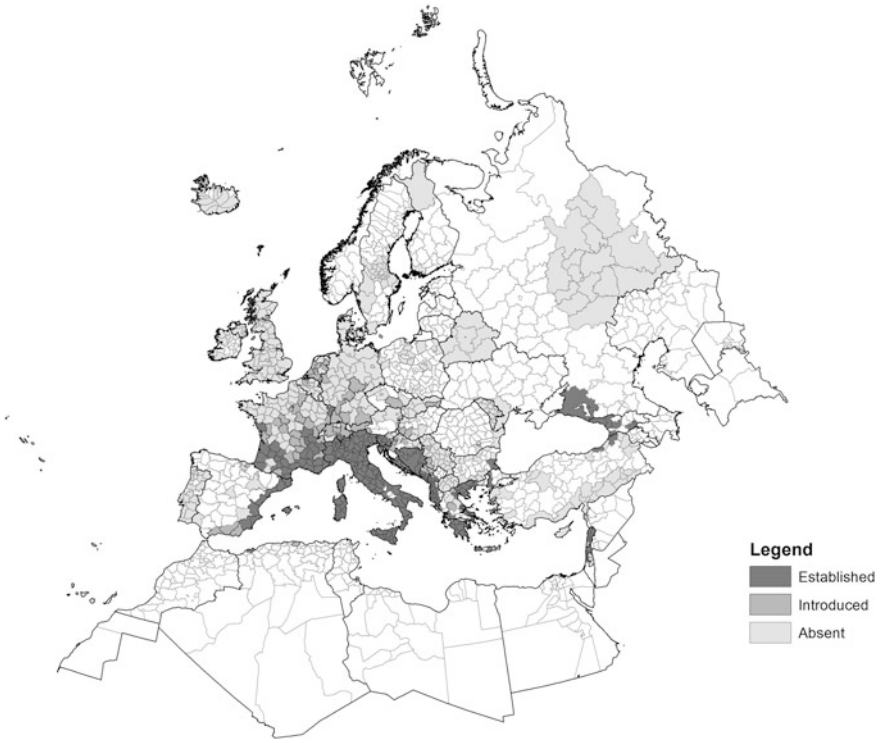

Fig. 4.3 *Aedes albopictus* distribution in Europe up to January 2017

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Chapter 5
Routes of Infection

Abstract Initial research on Zika disease defined infected mosquito bite as the only possible route of infection. Subsequent studies, most of which developed during the current epidemic, have opened the range to other mechanisms of transmission: vertical, sexual, laboratory exposition, through blood transfusion products or after monkey bite. Currently, other probable routes that need more research to be settled (breast milk, saliva or organ transplantation) have already been reported as potentially infective, but they have not been definitely documented. International heath authorities have published specific guidelines with recommendations to prevent or minimize transmission through these routes. Table 5.1 highlights the most important items in each route and summarizes the scientific evidence, with a specific link to the chapter in which more detailed information is provided.

5.1 Transmission by Mosquito Bite

Zika virus is transmitted primarily through the bite of several mosquito species, mainly *Ae. aegypti* and *Ae. Albopictus* [1]. There is information about a considerable number of mosquito species in which Zika virus strains has been isolated, including different species of *Aedes, Culex* or *Anopheles* [2] (Table 5.1).

Mosquito-mediated transmission cycle of ZIKV is initiated when a blood-feeding female *Aedes* mosquito injects the virus into human skin, affecting different cells of dermis and epidermis, goes through lymph nodes, blood stream and finally organs and tissues. The cycle of mosquito transmission ends when other mosquito bites a viremic human, sucking the infected blood. For more detailed information about pathogenic of ZIKV infection, see Sect. 3.5.

It's important to point that *Aedes* mosquito is also capable of transmitting DENV and CHiK viruses, so co-infections are relatively common findings in certain settings [3]. Co-circulation of flaviviruses in tropical and subtropical countries is very common, especially during certain periods of the year [4]. This is an important issue because these arboviriasis have similar clinical features and there is an evident lack of available tests in some settings, so clinicians may confuse the diagnosis.

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Table 5.1 Routes of transmission of Zika virus infection

Route of infection	Most important facts (reference)	Guidelines (Organization); (reference)	Extended information	References
Mosquito bite	– *Aedes* species mainly [1] – Other species of mosquito reported [2] – Frequent co-infection with other arboviriasis [4]	"*Protection against Mosquitoes, Ticks, & Other Arthropods*" (CDC) [5]	Chapter 9	[1–5]
Mother to child	– Vertical and perinatal [6] – First trimester more sensitive to damage [7, 8] – Association with fetal abnormalities [14]	"*Interim Guidelines for prevention of sexual transmission of Zika Virus*" (CDC) [15]	Chapter 6 Chapter 7 Chapter 9	[6–15]
Sexual Transmission	– From symptomatic or asymptomatic infected person [22] *Male*: presence of ZIKV in semen until 188 days after symptoms onset [18] *Female*: presence of ZIKV in cervical mucus, endocervix and genital swab [24]	"*Interim Guidance for Prevention of Sexual Transmission of Zika Virus*" (CDC) [27]	Chapter 7 Chapter 9	[16–27]
Blood transfusion	– From packet red blood cells and platelets [28, 29]	"*Guidance for Industry: Recommendations for Donor Screening, Deferral, and Product Management to Reduce the Risk of Transfusion–Transmission of Zika Virus*" (FDA) [32]		[28–32]
Other routes – Laboratory workers – Mucocutaneous	– Needle stick [35] – Presence of ZIKV in tears and sweat [37, 38]		Chapter 7 Chapter 7	[36, 37] [37–39]

(continued)

Table 5.1 (continued)

Route of infection	Most important facts (reference)	Guidelines (Organization); (reference)	Extended information	References
Potential routes – Saliva – Breast Milk – Urine – Transplantation	– Higher concentrations of ZIKV and longer persistence than in blood [40] – Other flavivirus transmitted through this via [42, 43] – Higher concentrations of ZIKV and longer persistence than in blood [46] – Other flavivirus transmitted through this via [49]	"*Infant Feeding in Areas of Zika Virus Transmission*" (WHO) [45] "Guidance for organ donation and transplantation professionals regarding the Zika virus 2016" (OPTN, PTAC, AST, ASTS) [50]	Chapter 7 Chapter 7 Chapter 7	[40, 41] [6, 42–46] [46] 47–50]

The Centres for Disease Control (CDC) provide specific guidelines to prevent mosquito bites [5].

Information regarding the different species of mosquitoes, susceptibility, and specific mechanism of transmission through the bite, should be expanded and discussed in Chap. 4.

5.2 Transmission from Mother to Child

A pregnant woman already infected with Zika virus can transmit the virus to her foetus during all trimesters of pregnancy (vertical transmission) or nearly the time of birth (perinatal transmission) [6].

Although early gestation seems to be more sensitive to the damage due to ZIKV [7, 8], infection that occurs at any trimester could also lead to fetal damage [9, 10]. The increased tendency for the development of malformations in the first trimester has been associated with the presence of high viral load of ZIKV in the pregnant's blood, which may result in miscarriage, stillbirth or intrauterine growth restriction [9, 11, 12]. The infection in the first weeks of gestation, when it's at a critical stage in brain development, can slow and disrupt normal brain development [13]. Multiple studies have linked maternal infection and the increased birth prevalence of microcephaly, cerebral microcalcifications and other malformations, supported by the presence of ZIKV in amniotic fluid samples collected in pregnant women or in blood and tissue samples of affected neonates [8]. Definitive evidence of the link has been recently published, reporting a striking magnitude of the association between microcephaly and in utero Zika virus infection [14].

ZIKV vertical transmission may lead to severe cerebral disease in the foetus that resemble lesions frequently detected in other intrauterine infections, as severe forms of congenital cytomegalovirus or lymphocytic choriomeningitis virus infections. According to the parallelism of damage due to other infections acquired during the pregnancy, microcephaly may be the result of destruction of neural progenitor cells. For more expanded information about pathogenesis of congenital ZIKV, see Sect. 5.2.1

A specific fact in pregnant is that prolonged detection of ZIKV has been reported, suggesting the possibility of alteration of immunity due to pregnancy slows viral clearance, and that both placenta and foetus can act as reservoirs of the virus [9, 10, 12].

Recent research has shown ZIKV provokes numerous pathological effects on different primary cell types and chorionic villus explants of the human placenta, which may support the possibility of placental and paraplacental routes of virus transmission [13].

The Interim Guidelines for Prevention of Vertical Transmission of ZIKV edited by CDC helps to define and reduce the risks of transmission [15].

More specific and detailed information about congenital syndrome is itemized in Chap. 6. Information about diagnosis during pregnancy can be found in Chap. 7.

5.3 Transmission Through Sexual Intercourse

Sexual transmission of ZIKV has recently been described after confirming the infection in individuals who had not travelled to endemic area while their partners have.

ZIKV transmission by sexual intercourse was first suggested in 2008 [16], and detection of a high ZIKV RNA load and replicative ZIKV in semen samples was first detected during the ZIKV outbreak in French Polynesia in 2013 [17]. Subsequently, the persistence of ZIKV in semen was documented 188 days after symptom onset [18]. Through immunohistochemical techniques, the presence of ZIKV in the head of spermatozoa was detected [19].

The duration of ZIKV in semen is usually similar to that of spermatogenesis (69-80 days), so it is postulated that infection of the spermatic progenitors and subsequent dissemination occurs during differentiation process. Hematospermia has found to be frequent in cases of ZIKV sexual transmission [20].

Semen might be infective despite vasectomy is performed, suggesting that the different structures of male urogenital tract and pre-ejaculate secretions may be a distal source of ZIKV [21]. Sexual transmission through semen occurs from subjects both symptomatic and asymptomatic [22], but ongoing research will determine the real risk for sexual transmission of Zika virus infection from asymptomatic males.

To the date, no data regarding the real risk of fetal abnormalities due to sexual transmission of ZIKV are reported. But in the cases that viral load in semen has been quantified, it reached roughly 10,000–100,000 times that of the male's blood or urine [23]. When this high viral load in semen reaches pregnant cervical and female reproductive tract, it could potentially interact in a different way as compared with the relatively lower levels of ZIKV in blood. Further investigations should clarify the impact of sexual transmitted ZIKV in the development of fetal damages.

Infective female secretions have been also described as a route of sexual transmitted infection. Cervical mucus, endocervical and genital swab are infective [24] and sexual transmitted ZIKV from a symptomatic woman to a healthy man have been published [25].

Sexual behaviours that have been linked to sexual transmission include condomless vaginal sex and anal receptive sex [26]. Semen, vaginal fluids or menstrual blood might transmit ZIKV during expose to male urethral mucosa, or undetectable abrasions in vagina, penis or rectum. However, regardless the lesions that may be in the genital tract, fluids are infectious enough so as to transmit the disease.

CDC provides the "Interim Guidelines for prevention of sexual transmission of ZIKV", that has recently included updated recommendations for both men and women, applied time intervals to the condom use and abstinence recommendations [27].

More details regarding the importance of sexual secretions in diagnosis of ZIKV infection can be consulted on Chap. 7.

5.4 Transmission Through Infected Blood Products

Transfused donated blood components have been linked to transmission of ZIKV, involving both packed red blood cells and platelets [28, 29].

There are few specific studies related to transfusional transmission of ZIKV, but they suggest low risk of transmission, and short of clinical effect after transfusion when it occurs [30].

A seroprevalence study of asymptomatic blood donors performed during the French Polynesia outbreak evidenced that 3% of donors, despite being asymptomatic, they tested positive for ZIKV [31]. This fact is extremely important, since blood donors may not be aware that they are currently infected with ZIKV, thus posing risk to transfusion safety.

Specific recommendations from FDA for donor screening, donor deferral and product management to reduce the risk of transfusion-transmitted ZIKV in areas that do not have active mosquito-borne transmission include "donation deferral for those who have had Zika virus infection (deferral for 4 weeks after symptom resolution) or symptoms suggestive of Zika virus infection during the past 4 weeks, those who have had sexual contact with a person with Zika virus infection or who has travelled to, or resided in, an area with active Zika virus transmission during the prior 3 months, and those who have travelled to areas with active transmission of Zika virus during the past 4 weeks". In endemic areas of ZIKV transmission, importation of blood components from unaffected areas is recommended if specific testing tests are not available for donors [32].

Nonetheless, certain aspects should undergo more research in order to manage blood products in a more safety way. Transmissibility studies should clarify the viral viability in blood during processing and storage of blood products or the minimum infectious dose needed to transmit the disease [30].

5.5 Other Routes of Infection

Laboratory work: During the firsts isolations of strains of ZIKV in the 60 s, one of the workers who were manipulating strains of this virus in Zika Forest, Uganda, started with symptoms and subsequently was diagnosed of ZIKV. In these reports the route of infection is not specified [33, 34]. Laboratory-acquired ZIKV infection during the current outbreak (needle stick while working with the Zika virus on an experiment in a laboratory) has been recently documented [35].

Specific recommendations for "Protecting Workers from Occupational Exposure to Zika Virus" have been suggested by CDC, for both outdoor workers, healthcare and laboratory workers [36]. More information about biosafety in laboratory, see Sect. 7.5.

Mucocutaneous Contact: A recent report describes ZIKV infection after close contact with a confirmed ZIKV patient with a very high viral load. Mucocutaneous

route seems to be feasible as different body fluids (such as sweat or tears) of patients with ZIKV disease could be infectious [37, 38].

Although the transmission of flaviviruses through intact skin or mucous membranes is very rare, they are already documented. A case of dengue virus due to mucocutaneous transmission in a health care worker has been reported, when she had a mishap managing blood from a patient with dengue virus infection. She felt blood splash onto her eyes, nose, and mouth and 10 days later she developed symptoms consistent with dengue [39].

5.6 Other Potential Mechanisms of Transmission

- **Saliva**: ZIKV has been detected in saliva, although no cases of transmission though this route have been reported. ZIKV RNA in saliva seems to exhibit highest concentrations of virus than in blood at disease onset, so this fluid can be used as an alternative specimen for acute phase molecular diagnosis, improving the initial diagnosis in the first weeks from ZIKV infection onset. Moreover, some patients can be tested positive in saliva while negative in blood [40].
  A potential person-to-person Zika virus infection through saliva, via disrupted oral mucosa or periodontal pockets as virus entry, should be considered as a possible infectious source. But this doesn´t indicate that the virus can be transmitted orally. In fact, saliva has antiviral particles and an hypotonic medium that is able to lyse enveloped virus, so orally transmission is very unlikely [41].
  Information about saliva as a diagnostic sample is provided in Chap. 7.
- **Breast milk**: as it happens with other arboviruses, infected viral particles have been detected in breast milk. But no cases of ZIKV transmission though breastfeeding have been documented exactly, unlike the case of other flavivurs as West Nile [42] nor Yellow fever [43].
  Few cases have been reported of children who were being breastfeeding while their mothers were symptomatic: most of the babies presented later with rash and all of them had ZIKV infection confirmed by positive RT-PCR. But in all cases reported, the mothers had symptoms that started during the last days of pregnancy or were in the incubation period during delivery, so perinatal transmission couldn´t be ruled out [6, 44].
  Specific recommendations for mother with breastfeeding children has been published by the PanAmerican Health Association (PAHO) [45].
- **Urine**: the detection of ZIKV in urine is so common that it has already been included as routine diagnostic test [46]. It's demonstrated that ZIKV remains detectable for longer periods in urine tan in blood after disease beginning [46], so it is an useful tool for diagnosis in cases where time has elapsed since symptoms onset.

Accidental manipulation of infective urine associated with the presence of lesions of the mucous membrane or skin wounds could lead to transmission of ZIKV, but to date no reports about this possibility has been documented.
Further information about the use of urine as a diagnostic fluid can be consulted in Chap. 6.

– **Transplantation**: ZIKV infection has been described in solid recipients and probably cases will rise due to the number of transplants performed in endemic areas and the increasing number of international travellers. Acute ZIKV infection in recipients include infectious complications and graft rejection [47].
A positive for ZIKV in blood and organ samples (brain, liver, spleen, kidney, lung, and heart) was reported in one fatal case of an immunosuppressed patient; but whether the virus could be infectious in those organs if they were transplanted or not is still unknown [48].
But similarly to what happens with other flavivirus in which transmission from an infected organ donor to transplant recipients has been documented [49], ZIKV infection after transplantation could be plausible.
In the case of transmission by this route of infection, many questions still remain unknown, for example the impact of immunosuppression on ZIKV infected recipients, the impact of ZIKV on allograft function, which organs may be affected the most or for how long infection may remain present.
A recent guidance document developed by The Organ Procurement and Transplantation Ad Hoc Disease Transmission Advisory Committee (OPTN/DTAC), the American Society of Transplantation, and the American Society of Transplant Surgeons (ASTS), suggests that "donor deferral should be considered if there is history of travel to Zika-endemic areas in the 28 days prior to donation. In the case of potential living donors with Zika infection, donation should be deferred where possible" [50].

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Chapter 6
The Disease

Abstract Approximately 20% of ZIKV infected patients show symptoms, mainly low grade fever, maculopapular rash, arthralgia (particularly in the small joints of hand and feet associated with peri-articular edema) or conjunctivitis, although multiple clinical manifestations have been described. Adults and children might show similar symptoms, but rate for symptomatic ZIKV disease among children seems to be even lower than that for adults. Antibody-dependent enhancement in response to previous Dengue Virus infection can result in an increased disease severity. ZIKV is already included in the list of infectious diseases known to cause congenital malformations in the developing foetus during pregnancy. ZIKV infection has been linked to congenital ZIKV syndrome, including microcephaly, brain abnormalities, neural tube defects, early brain/eye malformations and other consequences of central nervous system dysfunction. In adults, Guillain-Barré syndrome and other neurologic diseases have been also associated with ZIKV infection.

6.1 Clinical Manifestations

Symptoms and signs of acute ZIKV infection are very similar to other co-circulating viruses (as DENV or CHiKV). That hinders diagnosis based on clinical symptoms and requires further laboratory tests for confirmation.

Approximately 20% of individuals who become infected with ZIKV will show symptoms [1]. Outside endemic areas, the likelihood of ZIKV infection among asymptomatic individuals is close to half [2].

Incubation period between mosquito bite and onset of clinical manifestations is not clear, but it is likely to be between 2 and 14 days. Symptoms are usually mild and resolve within 2–7 days. Primary ZIKV infection elicits protective immunity, providing complete protection against reinfection with different strains derived from the primary ZIKV [3]. Severe disease requiring hospitalization is uncommon.

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6.1.1 Symptoms and Signs in Adults

Symptoms and signs of ZIKV infection starts typically with acute onset of mild fever (37.8–38.5 °C), followed, 3–5 days after, by a widely spread maculopapular rash, small arthralgia (notably hands and feet, and usually associated to peri-articular oedema), retro-orbital headache and non-purulent bilateral conjunctivitis [4, 5].

The World Health Organization released its interim case definition for suspected ZIKV infection as a person with compatible epidemiologic history presenting with rash or fever and one or more of arthralgia, arthritis or conjunctivitis [5].

Less commonly observed symptoms and signs include severe abdominal pain, nausea, diarrhoea, lymphadenopathy [6], uveitis [7], desquamating rash of the palms and soles [8] or transient hearing loss [9]. Severe thrombocytopenia during or after the course of ZIKV infection has also been reported [10]. Fatal cases appear to be rare, excluding those cases associated to complications [11–13].

It has been found a connection with more severe symptoms in patients co-infected with other infections, not only explained by the presence of Antibody-dependent enhancement (ADE) in case of co-infection with flavivirus [14]. Malaria [15] or Herpes Simplex virus Type 2 co-infection [16] has been demonstrated to increase the severity of ZIKV disease.

Recent investigations show testicular atrophy in ZIKV infected mice. High levels of viral RNA within the epididymal lumen, where sperm is stored, with also very low levels of testosterone in serum found in mice suggests that ZIKV infection in males could lead to sterility [17].

6.1.2 Symptoms and Signs in Children

Children can be infected though vertical transmission during pregnancy and delivery, in addition to the previously described routes in adults (Sect. 5.2). To rule out acute ZIKV infection acquired during delivery, the disease should be suspected in symptomatic infants during the first 2 weeks of life whose mothers have epidemiologic link suggesting possible transmission [18].

Clinical manifestations in infants and children with postnatal infection are similar to those findings observed in adults with ZIKV infection. Illness associated with Zika virus is usually mild in children. The classic symptoms that occur in adults can manifest in children atypically: arthralgia may manifest as irritability or difficulty moving or refusing to move an extremity [18]. Gastrointestinal symptoms are described to be more frequent in paediatric age, but why this occurs is unknown [19].

Attack rate for symptomatic ZIKV disease among children (<19 years of age) seems to be even lower than that for adults [1]. ZIKV related deaths in children are extremely rare and only few cases have been published [20].

Recently, there were evidenced in mice models postnatal growth restriction including microcephaly after ZIKV postnatal infection [21]. More researches are needed to completely define neurologic affectation of ZIKV infection in children after birth.

Information about postnatal affectation when foetus is infected during pregnancy, see Sect. 6.2.1.

6.1.3 Symptoms and Signs in Pregnant Woman

There are no evidences supporting that pregnancy is associated to a higher risk of acquiring ZIKV infection compared to the non-pregnant population, after *Aedes* mosquito bite [22].

Rash is the most common sign of ZIKV infection in pregnant women and it seems to be a significantly higher prevalence of lymphadenopathy and red eyes in ZIKV-infected compared with non-infected pregnant women [23]. Symptoms were observed more commonly if the women were infected within the first trimester [24–26].

Regarding the risk of transmission from nursing infected mothers, although RNA of ZIKV has been detected in breast milk, it has not been possible to culture it [27]. ZIKV transmission through breastfeeding has not been reported. CDC encourages infected mothers living in areas with on going Zika virus transmission to continue breastfeeding their infant supported by the evidence that the benefits of breastfeeding are greater than the theoretical risks of ZIKV transmission through breast milk.

6.1.4 Role of Antibody-Dependent Enhancement in Clinical Manifestations

Flavivirus closely related can result in considerable antigenic overlap. ZIKV has been shown to undergo ADE in response to anti- DENV antibodies (and also to antibodies generated by several different flaviviruses). Immunity to DENV might drive greater ZIKV replication, resulting in an increased disease severity [14, 28].

As it is documented that 53% of women giving birth in 2009–2010 in central Brazil were IgG positive for DENV, co-infection with these flavivirus is of particular concern in countries where both virus co-circulate [29]. Zika enhancement by DENV could explain the readily and strongly transmission of ZIKV in the Americas, where DENV is endemic [28].

Another fact that must be considered is if anti-dengue virus antibodies induced by tetravalent dengue virus vaccine recently licensed in Brazil, Mexico, and the Philippines might induce a more severe ZIKV disease. Mathematical models

evaluating the implication of enhancement for Zika when DENV vaccine is applied resulted in a higher and earlier peak of the outbreak of ZIKV when a high rate of DENV vaccination is observed. Also accumulated ZIKV infections may be larger for a greater rate of vaccination for DENV [30].

6.2 Complications

ZIKV infection has been linked to congenital ZIKV syndrome (including microcephaly and other birth defects) and fetal losses when women get infected during pregnancy, as well as a wide range of neurologic complications in infected adults.

6.2.1 Congenital Syndrome

Microcephaly is a neurologic abnormality usually defined as an occipitofrontal circumference more than 2 SDs below the mean or less than the third percentile based on standard growth charts for ethnicity, sex, age, and gestational age at birth [31].

An increase prevalence of microcephaly at birth in Brazil was first noted in September 2015, after the detection of ZIKV transmission in the country earlier in that same year [32]. Retrospectively, a similar increase in French Polynesia after the outbreak of 2013 was recognized [33]. Initial epidemiologic investigations found out a link between microcephaly and maternal ZIKV infection [34]. This relationship become increasingly consistent, as a consequence of the detection of RNA of ZIKV in amniotic fluid [25], placenta, umbilical cord blood, cerebral tissue, cerebrospinal fluid and serum of foetuses and newborns with microcephaly [35, 36]. But although microcephaly seems to be strongly associated, normal head circumference does not rule out ZIKV infection [26].

Both proportionate (small head circumference and small weight and height for gestational age) and disproportionate (head circumference not proportional to the other anthropometric measures for gestational age) microcephaly have been described in infants with congenital ZIKV infection [37].

Vertical transmission of ZIKV can occur throughout both symptomatic and asymptomatic infected mothers. The estimation the overall risk of any birth defect or abnormality among foetuses and infants of women infected with Zika virus during pregnancy range from 1 to 13% [33, 38, 39]. This important differences in percentages are due to different prevalence over geography, different definitions used for microcephaly, the assumptions about the proportion of pregnant women exposed, differences in study and the spectrum of clinical malformations included [40].

The greatest risk of serious fetal sequels appears to be in infection resulting early in pregnancy [32, 36]. Specifically, researchers put the risk of microcephaly at 11% when the infection occurs in the first trimester of pregnancy [39]. However,

congenital ZIKV microcephaly has been observed in the offspring of women infected as late as the final weeks of pregnancy [23].

A wide range of fetal malformations have been linked to maternal ZIK infection. Adverse pregnancy outcomes include fetal loss [23, 35, 41], slow fetal growth [23, 42], and hydrops fetalis [43].

Pathogenesis of congenital ZIKV lies on viral neurotropism, that seems to primarily target neural progenitor cells [44]. Different studies in mice and human placenta support the hypothesis that maternal infection leads to placental infection and damage. Then ZIKV should travel to the fetal brain, where it should affect neuronal progenitor cells as it was described in Sect. 3.5, slowing of brain growth [39, 45, 46]. ZIKV is also linked to intrauterine growth restriction and spontaneous miscarriage and also to a higher rate of fetal demise throughout pregnancy, including stillbirths due to placental insufficiency [47].

Differential diagnosis of congenital ZIKV syndrome still comprises the most common infections summarized in the acronym TORCH (Toxoplasma, Rubella, Other as syphilis, varicella-zoster, parvovirus B19, Cytomegalovirus and Herpes infection), with whom shares pathogenic mechanism. For that reason, some authors recommend to consider ZIKV a TORCH pathogen, and rename it to TORCHZ [48].

Prenatal abnormalities: ZIKV can slow down fetal growth in utero, leading not only to microcephaly, but also to small size and weight for gestational age. Prenatal abnormal findings include sequel related to the central nervous system, mainly ventriculomegaly (33%), microcephaly (24%), and intracranial calcifications (27%) [49]. Most frequent findings in congenital ZIKV syndrome are resumed in Box 6.1

Postnatal abnormalities: Neurologic abnormalities apparent on examination of infants with congenital ZIKV infection includes hypertonia/spasticity or hypotonia, hyperreflexia, severe irritability, congenital contractures (arthrogryposis), seizures ocular abnormalities, and sensorineural hearing loss [18, 50, 51].

Radiologic findings in children diagnosed of congenital infection includes decreased brain parenchymal volume, brain calcifications in the junction between cortical and subcortical, abnormal gyral patterns (pachygyria or polymicrogyria) in the frontal lobes, enlarged cisterna magna, abnormalities of corpus callosum (hypoplasia or hypogenesis), ventriculomegaly, delayed myelination, and hypoplasia of the cerebellum and the brainstem [50].

Ocular findings reported in infected infants include macular alterations (gross pigment mottling and/or chorioretinal atrophy) optic nerve hypoplasia, optic nerve abnormalities (disc pallor, increased optic disc cupping, hypoplasia), haemorrhagic retinopathy and abnormal retinal vasculature [18, 26].

Severe microcephaly with partially collapsed skull, thin cerebral cortices with subcortical calcifications, macular scarring and focal pigmentary retinal mottling, arthrogryposis and marked early hypertonia are identified as unique features of congenital ZIKV infection that are very infrequent in other congenital infections [52].

Most frequent findings in postnatal ZIKV infection are resumed in Box 6.1.

Box 6.1 Most frequent findings in postnatal ZIKV infection

Congenital ZIKV infection: most frequent features
Craniofacial abnormalities Cranial disproportion Cutys gyrate Craniosynostosis
Congenital contractures Arthrogryposis Clubfoot Congenital hip dysplasia
Seizures
Small for gestational age
Ocular abnormalities Microphthalmia/anophthalmia Coloboma Catarat Intraocular calcifications Chorioretinal defects (Chorioretinal atrophy, macular pallor, gross pigmentary mottling, retinal hemorrhage) Optic nerve abnormalities (atrophy and others) Vascular attenuation Glaucoma
Hearing loss
Radiological findings Intracranial Calcifications Ventriculomegaly/hydrocephaly Reduced brain volume Delayed myelination Simplified gyral patterns (e.g., polymicrogyria, pachygyria, lissencephaly) Hypogenesis of the corpus callosum Hypoplasia of the brainstem and cerebellum Enlargement of the cisterna magna Fetal brain disruption sequence (collapsed skull, overlapping sutures, prominent occipital bone, scalp rugae)
Neural Tube defects Anencephaly Acrania Encephalocele Spina bifida

No risk factors have been defined, although an increase in maternal viral load seems to be associated to a higher risk of foetus impairment [36]. Other factors related have been proposed and are low vaccination rates for yellow fever [53] and coinfection with bovine viral diarrhoea virus [54].

Guidance for the evaluation and testing of infants with possible congenital ZIKV infection has been published by different international health societies [55–57].

6.2.2 Neurologic Complications

6.2.2.1 Guillain-Barré Syndrome

Guillain-Barre syndrome (GBS) is an autoimmune disease characterized by acute inflammatory demyelinating polyradiculoneuropathy, causing acute or sub acute flaccid paralysis, triggered by certain infections. It has a 5% death rate and up to 20% of patients left with a significant disability [58].

The causality link between ZIKV infection and the development of GBS was suspected due to the unusual increase of reported cases of GBS in recent history of acute ZIKV infection during the outbreaks of French Polynesia [59, 60] and South America [61]. Clusters of GBS have been described in multiple countries during outbreaks of ZIK, but not in all those in which ZIKV is reported as epidemic. About this, Asian lineage is the strain of ZIKV associated to SGB in those cases when gene sequencing is available [62].

The World Health Organization (WHO) stated in March 2016 that the most likely explanation of available evidence from outbreaks of ZIKV and GBS is that ZIKV infection is a trigger of this neurologic disease. Anyway, the expert panel recognizes that ZIKV alone may not be sufficient to cause GBS. In that sense, past DENV virus infection seems to be an important cofactor, in which antibody-cdependent enhancement might be involved [14, 60]. Immune enhancement by pre-existing heterologous anti-DENV antibodies, has been hypothesized to increase viral replication and trigger an immunopathogenic process, leading to more severe disease and complications [63].

The presence of anti-glycolipid antibody activity (used to sustain clinical diagnosis of SGB) in the serum of nearly half of patients included in a case-control study performed during French Polynesia outbreak raised the possibility of direct viral neurotoxicity [59]. Neurological symptoms delays from 3 to 12 days from ZIKV infection, which is consistent with a post infectious autoimmune mechanism [62].

Specific guidelines for assessment and management of GBS in the context of ZIKV infection has been issued [64].

6.2.2.2 Other Neurologic Complications

A wide range of neurological complications have been recognised, including myelitis, meningoencephalitis, brain ischemia, acute encephalitis, and polyneuropathy related to ZIKV infection, revealing the potential neuropathogenic effect of ZIKV, similar to other flaviviruses [65–67].

Other different peripheral nervous syndromes among adults have been described during outbreaks in French Polynesia [60] and Brazil [68]. Although no formal link with ZIKV infection was found, there may be unknown host factors which increase the risk of developing serious neurological illness, apart from GBS [69].

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Chapter 7
Laboratory Diagnosis

Abstract ZIKV infections overlap clinical signs and symptoms with other arboviriasis, so microbiologic diagnosis may be necessary to specify final diagnosis. There are different microbiologic techniques that lead to the diagnosis on ZIKV infection. The viral genome detection in blood or urine by molecular techniques provides an accurate diagnostic result, but viremia is detectable only briefly during acute illness in these specimens. Later on, serology is the most commonly used diagnostic method for ZIKV infection. Serological cross-reactions in secondary infection with closely related flavivirus (i.e., dengue or West Nile virus) as well as vaccines to flavivirus (i.e., Yellow fever or Japanese encephalitis vaccine) are possible. Neutralising antibody detection assays can elucidate if serological positive tests are due to ZIKV or to other flavivirus. ZIKV culture is not usually available outside research laboratories. Diagnostics test are recommended to those symptomatic patients from endemic areas of ZIKV, and for all pregnant regardless they had symptoms or not. Congenital syndrome newborns also should be tested for ZIKV. Patient samples must be handled according to the biosafety guidelines in microbiological and medical laboratories.

According to case definition for ZIKV infection [1], diagnosis should be suspected in individuals with typical symptoms and signs and concordant epidemiologic exposure (stays in an area with reported ZIKV infection transmitted by mosquito, or unprotected sexual contact with a person that meets these criteria). But ZIKV outbreaks are usually associated with other arbovirus epidemics with similar clinical manifestations so microbiologic diagnosis may be necessary to specify final diagnosis. There are different microbiologic techniques that lead to the diagnosis on ZIKV infection. The choice of the laboratorial approach depends on laboratory infrastructure, technical expertise and sampling availability.

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7.1 Serological Analysis

Serology is the most commonly used diagnostic method for ZIKV infection. It comprises commercially available and/or in-house IgM/IgG enzyme-linked immunoabsorbent assay (ELISA) kits and neutralization assays.

Detection of IgM antibodies by IgM-capture ELISA (MAC-ELISA) in serum or cerebrospinal fluid are an effective method, but cross-reactivity in patients exposed to a previous flavivirus infection is observed, even in those vaccinated against Yellow fever or Japanese encephalitis [2, 3]. Even so, recent publications asses that Euroimmun ELISA, an ELISA based on ZIKV NS1-antigen, has 98–100% specificity for Anti-ZIKV ELISA (IgG, IgM) [4].

ZIKV-specific IgM may begin to appear 4–7 days after onset of symptoms, reaching its peak after two or three weeks and persists detectable for nearly 12 weeks [2]. By analogy to other closely related flavivirus, IgM antibodies against West Nile virus can be detected up to 3 months after illness onset, and more than 1 year if tested in patients with West Nile virus neuroinvasive disease [5, 6], so ZIKV IgM is likely to be detected for long periods of time. IgG antibodies appear a few days later than IgM and are assumed to persist long-life [2, 7]. Table 7.1 summarized optimal and suboptimal period for testing, and advantages/limitations of different techniques.

Due to cross-reactivity of serology with other flavivirus (both infection and/or vaccination), complementary analysis should be performed to clarify a positive IgM test. For a more reliable result, ZIKV diagnosis should be tested paired in acute and convalescent samples. Seroconversion is defined by at least a four-fold increase in paired sera, collected within 10–14 days of interval. If the patient has never been infected by a flavivirus, a minimal cross-reactivity is obtained. In case of acute flavivirus infection, it might boost cross-reactive antibodies due to previous infection or vaccination against another flavivirus [8].

However, in the case of a positive IgM for ZIKV, neutralising antibody detection assays with a plaque reduction neutralization test (PRNT) remains the gold standard for diagnosis and evaluation of tests. PRNT is the most specific test used to differentiate antibodies of close related virus. Neutralizing antibodies to ZIKV becomes detectable concurrently with IgM and consist primarily of IgG antibodies. They are expected to persist for many years after ZIKV infection and are supposed to confer prolonged, possibly lifelong, immunity. In primary flavivirus infections PRNT against ZIKV can identify the infecting virus. PRNT is not able to assess definitive diagnosis of the specific flavivirus causing acute infection in persons with a prior history of flavivirus infection [4]. International Guidelines for interpretation of serological results use a 90% cut-off value with a positive value (i.e., titter ≥ 10, that its considered the classical serum dilution onset used to determine the presence of virus-specific neutralizing antibodies) against ZIKV, together with negative PRNT (i.e., titter under 10) against other flavivirus support the diagnosis of recent infection with ZIKV. If PRNT titter is ≥ 10 for both ZIKV and DENV (or another flavivirus) a recent infection with a flavivirus can be assessed, but identification of

Table 7.1 Main characteristics of laboratory test for ZIKV diagnosis

	Specimen	Optimal period of use per current knowledge (a.c.o)	Sub-optimal period for detection per current knowledge (a.c.o)	Advantages	Limitations	References
Serology IgM IgG PRNT	Serum Serum Serum	7days–2 months 8days–years/for life 10-years/for life	4days–3 months 10-years/for life 8-years/for life	Available in most resources Available in most resources Confirmatory test to clarify positive serology	Cross-reactivity Cross-reactivity Expensive, laboratory trained personnel	[2–8]
RT-PCR	Serum/plasma Saliva Urine Semen	0–4 0–4 3–8 15–60	0–10[a] up to 29 days up to 20 days up to 188days	Provide accurate diagnosis	Only for acute phase Pregnancy: transient positivity	[2, 3, 9–18]
Viral Isolation	Serum/plasma Semen Urine	0–3 21 days 6	0–5	Provide accurate diagnosis. Only in specific laboratories	Only for acute phase	[5, 19, 20]

a.c.o after clinical onset. [a]In pregnant woman up to 10 weeks; In Whole blood up to 58 days. Adapted from European Centre for Disease Prevention and Control

Table 7.2 Interpretation of results of IgM and PRNT for suspected ZIKV infection

IgM (Elisa) for ZIV and DENV	ZIKV PRNT	DENV PNRT	Interpretation
Positive or equivocal (both tests)	≥ 10	<10	Recent ZIKV infection
Positive or equivocal (both tests)	<10	≥ 10	Recent DENV infection
Positive or equivocal (both tests)	≥ 10	≥ 10	Recent flavivirus infection; specific virus cannot be identified
Inconclusive one test AND inconclusive/negative the other	≥ 10	<10	ZIKV infection; timing cannot be determinate
Inconclusive one test AND inconclusive/negative the other	<10	≥ 10	DENV infection; timing cannot be determinate
Inconclusive one test AND Inconclusive/negative the other	≥ 10	≥ 10	Flaviviral infection; specific virus and timing cannot be determinate
Any result	<10	<10	No evidence of ZIV nor DENV infection
Positive ZIKV/negative DENV	Not available		Probable recent ZIKV infection
Positive DENV/negative ZIKV	Not available		Probable recent DENV infection
Positive DENV and ZIKV	Not available		Probable recent flavivirus infection
Inconclusive one test AND inconclusive/negative the other	Not available		Ambiguous results
Negative ZIKV/Negative DENV	Not indicated		No evidence of recent ZIKV or DENV infection

the specific infecting virus must be performed [9]. Table 7.2 resume the interpretation of results of antibody testing for suspected ZIKV infection.

Despite this benefit, PRNT is an arduous and time-consuming technique, costly and specialized infrastructure and specific laboratory trained personnel are needed due to the live virus manipulation.

In settings where PRNT is not available, positive samples for ZIKV by MAC-ELISA and negative for Dengue by MAC-ELISA may be interpreted as a presumptive recent ZIKV infection (see Table 7.2). Anyway, the diagnostic accuracy of this approach has not been validated yet [10].

"CDC Interim Guideline for Interpretation of ZIKV Antibodies Results" shows specific information that explain how to interpret combined results from ZIKV and DENV ELISA and PRNT [9].

7.2 Molecular Analysis

Acute phase diagnosis of ZIKV infection relies on conventional or real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) [11].

The viral genome detection by molecular techniques provides an accurate diagnostic result, but viremia is detectable only briefly during acute illness. Therefore, this technique is most reliable when performed within the first days of the disease, as viremia starts to drop when the rash occurs [2]. The period that viral nucleic acids remain detectable ranges for about 10 days from symptoms onset in blood, to 188 days in semen samples [3, 12, 13]. Table 7.1 summarized principal characteristics of molecular test.

Although in most patients RNA of ZIKV can only be detected in serum or blood during the symptomatic phase of the infection, whole-blood samples could be positive as long as 58 days [14]. ZIKV viremia has been described for longer periods in pregnant women (up to 10 weeks). That persistence is supposed to be a consequence of viral replication in foetus or placenta, which thus acts as a reservoir. [15, 16]. In that sense, The Interim Guidance for Care Providers Caring for Pregnant Women has been updated and extends the previous recommendation for testing of serum from <1 week after symptom onset in pregnant woman to a more extended lapse of time [17].

Currently, viral genome of ZIKV has be detected in different clinical specimens such as blood (plasma, serum), CSF, urine, saliva, breast milk, semen, vaginal secretion, amniotic fluid and tissues [2, 3, 15, 18]. It is not clear which type of clinical specimen is most appropriate for ZIKV detection. Small series of patients show that ZIKV detection in saliva may be more sensitive than detection in blood, but the presence of ZIKV in saliva and blood appeared to be equally short-lived [13]. RT-PCR in urine, semen and saliva are reported to be positive for more than 2 weeks, with the added advantage that is a non-invasive specimen [3, 19]. Highest viral loads are detected in blood: by using quantitative RT-PCR, the RNA viral load in blood ranges 7.28×10^6–9.3×10^8 copies RNA/ml, in urine ranges 2.5×10^3–8×10^6 copies RNA/ml, in semen ranges 1.1×10^7–2.9×10^7 copies RNA/ml and in breast milk ranges 2.9×10^4–2×10^6 copies RNA/ml [2, 3, 13, 20, 21].

The use of alternative samples to blood, as saliva, for testing RT-PCR when medical facilities are not available could be of particular interest. But although saliva exhibits high concentrations of ZIKV at disease onset [13] and oral swab is a non-invasive sample-collection device easy to collect in low resource settings, ZIKV RNA detection can be negative in saliva while positive in blood, so saliva should not replace blood test [13]. Isolation of viral RNA in saliva has been detected up to 29 days [22]. In that sense, urine sample is preferred for diagnosis during epidemics, due to both its persistence of ZIKV nucleic acid and its easiness to be collected.

ZIKV genome has been also detected on amniotic fluid, cord blood, CSF and placenta by RT-PCR, but the method's sensitivity on those specimens is unknown and it hasn´t been validated yet [10].

Current Guidelines recommend test for PCR until day 7 day (serum) and 21 day (urine) after onset of symptoms. Investigations at a later stage have not been standardized [23].

7.3 Viral Isolation

The virus isolation may also be used in acute samples, but it is a more laborious and time-consuming approach, also requiring more robust infrastructure, not available in most laboratories.

ZIKV culture is not usually available outside of research laboratories. Virus isolation, despite not being performed on a routine basis, can be accomplished using mosquitoes cells (such as AP-61, *Aedes pseudoscutellaris*; C6/36, *Aedes albopictus*) or mammalian cell lines (such as BHK, VERO) directly from infected mosquitoes or by inoculation into new born mice brain. Cultures are observed for cytopathic effect [7, 24]. Currently, standard laboratory methods for ZIKV are limited to few cell-lines and many assays take several days to generate meaningful result [25]. Table 7.1 summarized principal characteristics of viral isolation test.

7.4 Testing Strategies

CDC has issued strategies to test for ZIKV in different contexts [26].

Symptomatic patients:

- <14 days from onset of symptoms: rRT-PCR of serum (or whole blood) and urine for detection of ZIKV RNA should be performed. If any positive rRT-PCR results, diagnosis of ZIKV infection is established and no further testing is needed. Negative rRT-PCR results do not exclude ZIKV infection, and serum should be tested for the presence of anti-Zika. If Zika virus IgM tests results are positive, equivocal, or inconclusive, testing for PRNT should be performed.
- >14 days from onset of symptoms: ZIKV serologic testing should be performed (ZIKV IgM) and PRNT if needed to elucidate an inconclusive result.

In symptomatic patients tested at early or subacute phase, other flavivirus should be ruled out, as other arboviruses with close clinical presentations are co-circulating in ZIKV endemic areas.

Asymptomatic patients: no tests are recommended for men, children or non-pregnant women. In pregnant woman:

- <14 days from exposure: serum/urine should be tested for RT-PCR for ZIKV. If negative, a second serum specimen should be reflexed 2-12 weeks after exposure for IgM testing. If ZIKV IgM is not conclusive, PRNT should be performed. Fetal ultrasonography may also be indicated apart from laboratory test.
- >14 days from exposure: ZIKV serologic testing should be performed (ZIKV IgM) RT-PCR testing is also indicated for pregnant women tested ≥ 2 weeks after exposure and have been found to be IgM positive. PRNT test could be necessary to elucidate an inconclusive result. Fetal ultrasonography may also be indicated apart from laboratory test.

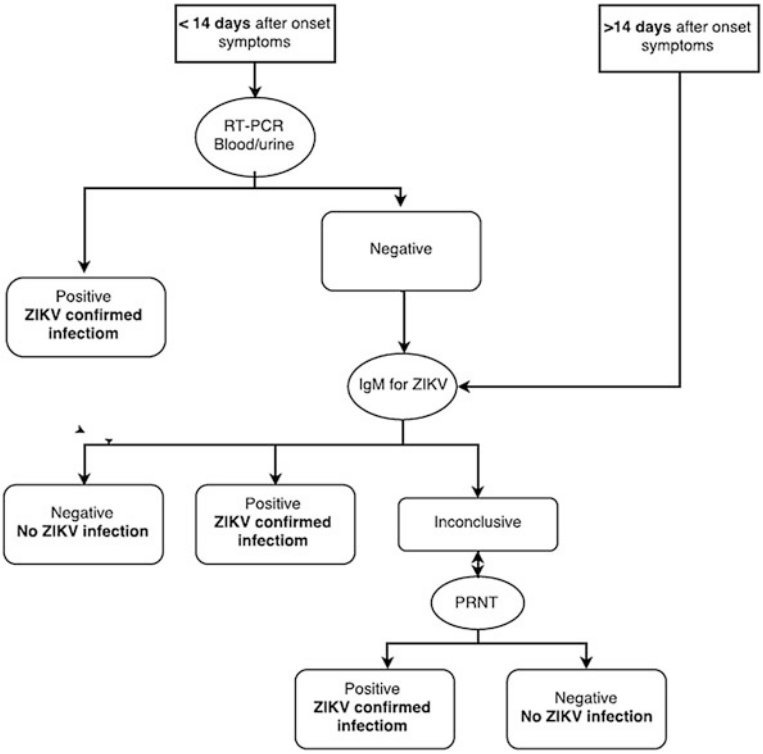

Fig. 7.1 Testing strategies for both symptomatic patients and asymptomatic pregnant women

Testing strategies for both symptomatic patients and asymptomatic pregnant women are summarized in Fig. 7.1.

Symptomatic children with postnatal infection: The diagnostic approach for these children is the same as for adults, as described above.

Congenital Syndrome: Newborns of mothers with known or suspected ZIKV infection at any time of pregnancy should be tested for ZIKV within 2 days after birth. ZIKV IgM, RT-PCR and histopathology, as appropriate should be tested in Infant serum, whole blood, placental tissue, and cerebrospinal fluid (CSF) if needed for another reason. Placental tissue should be sampled from several areas of both the fetal and maternal sides of the Umbilical cord blood is not longer recommended by CDC as it contains blood from the mother and results can be difficult to interpreter, PRNT should be performed on the infant's initial sample if it was not performed on the mother's sample. Newborns with clinical or neuroimaging findings suggestive of congenital ZIKV syndrome and a maternal epidemiologic link should be also tested.

7.5 Safety Precautions for Laboratories Working on ZIKV

As ZIKV is classified as a biological safety level 2 pathogenic agent (with exception of UK where the virus is classified as level 3), it must be handled according to the biosafety guidelines in Microbiological and Medical Laboratories [27].

The use of double gloves, a surgical mask, a laboratory coat, and eye protection when handling specimens from individuals suspected of having ZIKV infection should be included among precautions. For virus isolation through culture, the sample must be handled under Bio Safety Level 3 [28].

Other additional laboratory precautions include pre-treatment (autoclaving) of solid waste prior to disposal as medical waste, decontamination of all materials that are taken out the biosafety cabin.

Due to the link of ZIKV infection and congenital anomalies, the involvement of pregnant workers in studies with ZIKV should be minimized.

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Chapter 8
Treatment

Abstract ZIKV is a self-limited disease whose management consists of symptomatic supportive treatment with minor analgesic. Nonsteroidal anti-inflammatory drugs and aspirin should be avoided until Dengue infection could be ruled out. Multiple effective compounds have been described as having anti-ZIKV activity, but further research is needed to assess the efficacy in real infected patients. Currently, there is not an available vaccine yet. Candidate vaccines against ZIKV infection have shown promising data in efficacy in mice and non-human primates. The development of a ZIKV vaccine for humans is likely to be readily achievable within the next few years and several studies are now in Phase I.

8.1 Symptomatic Treatment

There is not specific treatment for ZIV infection. Since the infection is self-limited, management usually are rest and symptomatic treatment, including drinking fluids to prevent dehydration and administration of minor analgesics (paracetamol/acetaminophen) to reduce fever and pain.

Nonsteroidal anti-inflammatory drugs (NSAIDS) and Aspirin should be avoided unless dengue infection can be ruled out: in case of coinfection or misdiagnosis with DENV fever, these drugs may increase the risk of haemorrhage. Aspirin is not indicated in children under 10 years with any acute viral disease as it is associated with Reye syndrome [1].

The World Health Organization has published several recommendations about psychosocial needs for patients and relatives affected by ZIKV infection and its associated complications [2].

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8.2 Treatment Under Investigation

Some factors make the development of new antivirals difficult, since they must be active in central nervous system cells, cross the blood-brain-barrier and the placenta, and be safe for pregnant women and their foetuses [3–5].

Interferon [5–7], azithromycin [3, 8, 9] ribavirin [4–6, 10–13], 6-azauridine and glycyrrhizin [5, 14, 15] have demonstrated anti-flaviviral activity against different members of the flaviviral family, including ZIKV.

Oral treatment with sofosbuvir seems to protect against ZIKV-induced death in mice, but further evaluation is needed to prove its benefits as a therapy against ZIKV infection in non-human primates and ultimately humans [3].

In response to the current outbreak of ZIKV, drug-repurposing screens have recently become an alternative approach to speed-up drug development. With these procedures, new indications for current drugs may be rapidly described. Multiple effective compounds have been described as having anti-ZIKV activity using this strategy, as palonosetron, lovastatin or 5-fluoracil [5, 6].

8.3 Vaccine Against ZIKV

There are currently successful vaccines for protection against multiple flavivirus, as YFV, TBEV, JEV or DENV. Strategies for ZIKV development can be sustained in adaptation of pre-existing flavivirus vaccine platforms (e.g. inactivated or live-attenuated virus, flavivirus chimera or glycoprotein subunit technology) [8].

Different vaccines formats have shown protection on mice or non-human primates against ZIKV infection, as purified inactivated virus, plasmid DNA, adenovirus vectors or protein subunit [10–13].

The low genetic variation in ZIKV strains, allows that a single vaccine may be affective against all circulating ZIKV strains, suggesting that the development of a safe and protective ZIKV vaccine is feasible [14, 15]. But an important factor to be taken into account is whether pre-existing immunity developed before other flavivirus infection could modify immunity against ZIKV. Further investigations should be developed to determine whether protection against ZIKV with a vaccine may be weakened in areas where multiple flavivirus circulate [8].

A recently developed ZIKV vaccine candidate has shown to confer protection to mice and rhesus macaques with a single dose. This promising vaccine contains mRNAs encoding two key proteins from a ZIKV strain isolated in 2013 French Polynesia outbreak. It has demonstrated to induce high titles of antibodies against ZIKV infection. Elicited levels of antibodies reach highest point after several weeks and thereafter remain in protective levels, probably for years, without adverse events. But no data on the need of a boost, the effect of this vaccine on infections caused by other flavivirus or the efficacy in avoiding congenital ZIKV infection and disease has been reported [16].

The development of a ZIKV vaccine for humans is likely to be readily achievable in next years and several studies are now recruiting voluntaries to evaluate the safety and immunogenicity of different vaccination schedules in healthy adults.

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Chapter 9
Prevention

Abstract Prevention of infectious diseases is centrepiece for control and reduction of new cases during an outbreak. In the case of mosquito born disease, to which ZIKV belongs, prevention must be made in a transversal, multidisciplinary and simultaneously net of actions and controls. In this chapter we will approach to prevention from the different points of view. For the mosquito control, an environmental management can be carried out in order to reduce natural and artificial water containers used by the mosquito for the ovoposition, larval hatchery and mosquito live cycle. Insecticides and Biological (predators, parasites or biological competitors) or Genetic control (with technics of mosquito population reduction or replacement) can be used in order to reduce mosquito and larvae population. From another point of view, personal protection from mosquito-bite with physical barriers and repellent is essential to reduce the number of infected people and, at the same time, the mosquito source of infection. On the other hand, we will review international advice on prevention for the different mechanisms of ZIKV infection and finally a summary of the most relevant international guidelines for a better knowing and management ZIKV infection are presented.

9.1 Related to Mosquitoes

9.1.1 Environmental Management

Along with insecticides, these are the main techniques used for mosquito control. They include a wide range of measures, designed to supress mosquito population. Some important terms to environmental management for vector control are the following:

- Environmental modification: Consists of any physical transformation that is permanent or long-lasting of land, water and vegetation. Aiming to reduce the habitats of vectors.

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- Environmental manipulation: Any planned recurrent activity aimed to produce temporary unfavourable conditions for the vectors. It could be the regulation of the water level in reservoirs, vegetation removal, and water salinity.
- Modifications or manipulation of human habitat: In order to reduce man-vector-pathogen contact. Some examples are the siting of settlements away from vector sources, personal measures against vectors and provision of installations as mechanical barriers.

The environmental management of mosquitoes has several approaches in rural and urban areas.

In rural areas, agriculture and natural wetland changes could be carried out in order to descend mosquitoes proliferations in some specifically sites. For example, flooding land rice fields is known to be often involved with an overgrowth and permanent reservoir of mosquitoes, being recognized like potential sources of mosquitoes-borne diseases. In Sri Lanka a community-based ecosystem management programme was performed from 2002 to 2004. They educated rice farmers on adjusting field levels to avoid flooding and puddles and in the surveillance of drainage of water accumulations. Finally they compared mosquitoes data with a non-intervention village and they found a significant drop in *Anopheles* sp. densities in the intervention fields, but a limited impact in *Culex* and *Aedes* species [1].

Urban areas are an ideal habitat for multiple mosquitoes species so in over-populated cities they can live around human settlement, having blood meals, and a wide range of collected water in flower vases, abandoned car wheels, stuck roof gutters, etc. Surveillance and control of these facts by an appropriate community information an sensitisation are the key for the eggs, larvae and pupae control [2, 3].

WHO recommends this control in four steps: for the small water receptacles (with community clean-up campaigns for tin cans, scrap metal, etc. and cleaning home coolers), for medium to large containers that hold water for domestic use, for other large containers such as ornamental pools and for irrigation and storm water canals [4].

It is also important the surveillance and control of the potentially significant places for mosquitoes ovoposition like cemeteries (in which abound water containers and vases of flowers). Among 31 mosquito species have been found breeding in cemeteries from 16 different countries and they are considered ideal settings to perform studies in urbanized areas and mostly ideal settings to perform studies in urbanized areas and a key piece for mosquito control [5].

9.1.2 Chemical Control

9.1.2.1 Characteristics of Insecticides by Chemical Group

(a) Chlorinated Hydrocarbons

They contain carbon, chlorine and hydrogen. The Dichlorodiphenyltrichloroethane, best known as DDT, has been recognised the most useful insecticide ever

developed, but on the other hand, it is the most infamous chemical insecticide of the twentieth century [3]. It was introduced in 1939 for vector and control of others pathogens, but its wide use during the passing years caused a problem due to its indiscriminate and extended use. Because of the chemical stability, it is accumulated in the environment through food chains and in tissues of exposed organisms (including people living in treated houses). This has given rise to concern in relation to possible long term toxicity, so other alternatives were explored, as metabolic disrupters, moult inhibitors, and behaviour modifiers of insects.

Nevertheless, in 2011 WHO proposed an exception of DDT use, which had been strictly restricted for massive use and production in the Stockholm Convention on Persistent Organic Pollutants [6]. It is allowed for indoor application and use to vector-borne disease, probably for the absence of equally effective efficient alternatives to malaria control in developing countries [7].

(b) Organophosphates

Organophosphates were developed in Germany in 1932. They are the oldest and widely used is Malathion. Its fast action and low acute toxicity in humans, has made of Malathion a perfect insecticide for Ultra-low volume (ULV) application for control of adult mosquitoes. Many of ULV applications are carried out by aircraft, helicopters and also by ground equipment. Its application is calibrated to dispense droplets small enough to remain suspended in the air, but large enough to be able to kill the adult mosquito [3].

The World Health Organization (WHO) convened a group of experts in May 2016 with the objective of reviewing its potential application for ZIKV vector control, and its safety for public health. Malathion has been associated with a limited evidence in humans with elevated risk of carcinogenicity, based on observations of positive association of an increase incidence of non-Hodgkin lymphoma and aggressive cancer of the prostate. They finally concluded that Malathion is unlikely to pose carcinogenic risk to humans from diet exposure, always following use of Malathion according to good agricultural practices [8].

(c) Carbamates

Carbamates were discovered in 1951 in Switzerland. They have been used for treatments of walls and ceilings of houses in endemic mosquito areas. They are also used in ULV by aerial application. It has been described as a synergic effect with pirethroids because of their complementary methods of action.

(d) Pyrethroids

Pyrethroids are highly active synthetic insecticides and were developed between 1960 and 1970 mainly in Japan and United Kingdom. Their use led profound changes in the insecticide use as they are potent and biodegradable so they lead to a lower burden of residues in the environment [3].

The insecticides recommended by WHO belong to the class of pyrethroids with or without a synergist and organophosphorus compounds (Malathion). Since

pyrethroids have a rapid and persistent effect on mosquitoes at low doses and are safe for close contact, they are the only insecticides currently recommended for treatment of mosquito nets. It is also possible of impregnated clothes with a repellent by spray-on application or by dipping clothes in a solution at a permethrin concentration of 0.65–1 g ai/m^2, for a jacket or shirt, trousers and socks [9].

Nevertheless, poisoning is rare but can occur. There is no specific antidote and treatment is symptomatic and supportive after decontamination to prevent further absorption. Vitamin E oil preparations can be given for prolonged paraesthesia.

9.1.2.2 Mosquito Insecticide Resistance

When a mosquito population is exposed to a selected pressure from a determinate insecticide, they can become resistant and multiply in the absence of intraspecific competition, becoming a dominant population between the other mosquitoes.

So, in the endemic *Aedes* sp. areas and in ZIKV affected countries, the use of safe and efficacious insecticides against adult and larval population is essential, and for that it is necessary to monitor insecticide resistance.

Provided that insecticide resistance monitoring is an essential part of entomological surveillance, information of adult mosquito density, larval and pupal indices, ecology and habitats, and efficacy of vector control interventions, has to be collected so changes in insecticide susceptibility status should manage policy and operational decisions.

WHO has developed a guideline to ensure the description of mechanisms and geographical distribution of *Aedes* mosquito resistance in order to select appropriate insecticides for vector control [10].

It is important to take into account that mosquito population may develop "cross-resistance" and become also resistant to other insecticides in the same class, even when it has never been treated with the other insecticides. In a recent resistance study, carried out in different malaria endemic zones of western Kenya, to test insecticide susceptibility in Anopheles mosquito and they found Pyrethroids and DDT resistance but organophosphate susceptibility [11].

Other local studies have been carried out about the *Aedes* susceptibility. In Martinique, they have found a heterogeneous distribution of insecticide resistance throughout the island, suggesting that factors like environmental, agriculture practices, vector control interventions and urbanizations, may play an important role in the distribution of mosquito insecticide resistance [12].

For the monitoring of mosquito resistance to insecticide, CDC has proposed an assay to determine if particular insecticide active ingredient is useful to kill mosquito vectors. This technic is described below.

Mosquitoes have to be put into a CDC bottle bioassay and a diagnostic dose of the insecticide to be tested is introduced into the bottle. Mosquitoes must be observed during 2 h and resistance is determined by the mortality rate of mosquito dies (percentage of mosquitoes that die) [13].

There is a WHO guideline that provides recommendations on measures to protect health and safety of all persons involved in control vector. It also includes treatment and measures in case of insecticide poisoning [14].

9.1.3 Biological Control

It consists on the reduction of the mosquito population using predators (fishes, amphibians, birds, bats, etc.), parasites, biological competitors, pathogens or toxins, avoiding the adverse event to the ecosystem. It was widely used during the first half of the past century but with the discovery of synthetic insecticides it was no longer considered as an important control method. One advantage is that existing predators are conserved so they will maintain the control effort, enhancing the efficacy of the current control measures. Others benefit over the other measures is that they include no chemical contamination of environment; they could be used into some sites that are not easily treatable by other means; and are specific against target organism. Disadvantages are that they are only useful in immature stages of mosquitoes, difficulty in their application and production and their limited utility [3].

9.1.4 Genetic Control

The genetic control term is used to cover a large range of technics and strategies that basically work in two main strategies:

Mosquito population reduction through genetic manipulation:

This technic has the purpose of eliminating entire mosquito populations from particular species in a geographical place. OX513A is a transgenic strain of *Ae. aegypti* manipulated to carry a repressible, dominant, non-sex-specific, late-acting lethal genetic system, that is introduced together with a fluorescent marker. As a result of this genetic manipulation, larvae carrying this strain will develop normally, but will die before becoming a functional adult. This technology has been demonstrated to reduce *Ae. aegypti* populations in several countries in small-scale field trails, but data from epidemiological impact are still pending. A limitation of this tool is the large amount of transgenic male mosquitoes that are necessary to release in order to maintain suppression of wild *Ae. aegypti* population.

Mosquito population replacement:

The concept of creating and releasing manipulated mosquitoes that are refractory to human pathogens was first suggested in twentieth century and from that, much progress has been made in this field. Since cytoplasmic incompatibility between different sexes of the same insect was described for first time, some investigation work was necessary until the association of this phenomenon to the *Wolbachia*

pipientis, a bacterial endosymbiont of insect, was made. So, when an *Aedes* female mosquito is infected with *Wolbachia,* it will be unable to be infected with ZIKV and besides, it will transmit the bacterial infection to all its progeny, from one generation to another. If a male is not infected with *Wolbachia*, he won't be able to have offspring with an infected female, so also *Wolbachia* made a "*Wolbachia* infected mosquito" selection in the area of release. Male killing, Phartogenesis and offspring feminization are other manipulation mechanisms of the bacteria to the mosquito, that makes *Wolbachia* spread throughout the mosquito population [15]. A virulent strain of Wolbachia has been selected (wMel) to be introduced in *Ae. aegypti* populations in order to reduce the ability of the mosquitoes to transmit arboviruses to humans [16]. Laboratory results show that *Aedes aegypti* harbouring *Wolbachia* are highly resistant to ZIKV infection. This Wolbachia-harboring mosquitoes displayed lower viral prevalence and decreased disseminated ZIKV infection (from 80 to 10%) and ZIKV presented in mosquito saliva descended form 100% to 0%, suggesting that viral transmission was blocked [17]. Implementing this tool involves establishing and sustaining wMel *Wolbachia* in *Aedes* mosquito population from ZIKV endemic areas, but epidemiological studies are currently being carried out, so final results will be soon available [4].

9.1.5 Personal Protection

Personal protection, in order to avoid mosquito bites, is the key component of ZIKV protection in endemic areas. There are two important points for prevention:

Protection by physical barrier:

The use of large clothes and closed footwear, especially in areas where mosquitoes are abundant and rainy seasons in tropical and subtropical areas, minimizes skin exposure and also the mosquito bite risk.

For people living in an area where ZIKV is endemic, it is important the use of window screens, door screens, and air-conditioning in buildings to discourage day-time entry, biting and resting for *Aedes.* The use of WHO Pesticide Evaluation Scheme (WHOPES) strongly recommend long-lasting insecticidal mosquito nets for the night resting, sleeping time and the resting during the day, especially for infants, sick individuals and pregnant women.

Repellents Against Mosquitoes:

The application of repellents to exposed skin or clothing is the most common mean of personal protection against mosquito bite. There are several repellents and a variety of formulations, but WHO recommendations are the following:

- DEET (N,N-diethyl-3-methylbenzamide): It's the most common and recommended repellent. It could be found in different presentations but performed studies for concentrations above 50% do not offer a marked increase in

protection time against mosquitoes than lower concentrations. It is safe in pregnant and breastfeeding women and in children over 6 month (or two years depending on countries).
- IR3535 (3-[N-acetyl-N-butyl]-aminopropionic acid ethyl ester). It is mainly used for lice infections but it is also effective for mosquito, ticks, fly and fleas.
- Icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester). It has low skin absorption and it as effective as DEET. It is safe during pregnancy and for children older than 6 months (in some countries older than 2 years).

All of them must be used in strict accordance of the label instructions. It is important to consider that the duration of repellent protection is affected by ambient, temperature, transpiration, exposure to water, and other factors. In general the higher concentration of active ingredient provided by the fabricant, the longer duration of protection it will present.

It is also a priority that infected people with any mosquito-borne disease use all this mediums for avoiding mosquito bites and prevent further transmission and ZIKV circulation.

Special considerations in children and women:

Endemic ZIKV countries have to advice women who want to get pregnant and those already pregnant to get in contact with healthcare providers for an adequate and actualized information. Women and sexual partners who are planning a pregnancy are suggested to wait at least 6 months after a possible exposure of ZIKV [18]. Health workers are called to provide people who don't want to get pregnant the adequate contraception measures information [19].

For pregnant woman, it is essential to take special protection measures using repellent lotion and treated mosquito nets also for sleeping and rest during the day at home). It is also important to get in frequent contact with health centres to have regular check-ups. Finally, if foetus malformation occurs, health workers must give all information, support and accompaniment for a safe voluntary pregnancy interruption if it is indicated [4, 20, 21].

For children, most repellents can be used, when older than 6 months and treated mosquito nets use is advised all the possible time [4, 18].

9.1.6 Mosquito Control Measures

In the order to perform an adequate approach to the vector control, integrated strategies of all mosquito control techniques, must be considered.

Each country should adequate its measures to the mosquito, ZIKV or both presences (Table 9.1). In endemic countries, national public health authorities should implement vector control strategies and develop a risk communication strategy. Authorities should apply for personal protection measures and seek to

Table 9.1 WHO recommendation for operational strategies to be taken according to the country context [22]

Country context	Without *Aedes*	With *Aedes* but no ZIKV circulation	With *Aedes* and ZIKV transmission
Categories	A	B	C
Monitor points of entry for *Aedes* species introduction	●		
Intensify immature and/or adult entomological surveillance		●	●
Implement source reduction measures		●	●
Integrate source reduction measures with disease surveillance			●
Apply larvicides to key containers not amenable to source reduction		●	●
Conduct adult vector control in areas with ZIKV transmission or high risk, preferably using targeted residual spraying		●	●
Conduct adult vector control in high risk ZIKV transmission areas using targeted residual spraying			●
Conduct community education and mobilisation		●	●
Monitor and evaluate the quality and impact of control measures		●	●
Promote personal protection, including the appropriate use of repellents and mosquito nets		●	●

engage the community in vector control. Other strategies like mobilisation of community groups for regular door-to-door camping should be taken.

9.2 Related to Other Routes of Infection

9.2.1 Vertical Transmission and Breastfeeding

Mechanisms to prevent vertical transmission are those to avoid ZIKV infection in pregnant woman (vector control intervention, use of repellent and treated-nets and avoid unprotected sexual intercourses) are revised in Sects. 9.1 and 9.2.2.

Due to breastfeeding significant benefits for mothers and children in all countries, it is still recommended by WHO to all infants born from mothers with suspected, probable or confirmed ZIKV infection. Also those mothers who have travelled to ZIKV areas should be fed according to normal infant guidelines.

Even if it has been documented ZIKV presence in breast milk (always in women with positive RT-PCR ZIKV in blood), there are not documented ZIKV infection to infant, and this data suggested that it has more benefits than risk for infants [23, 24].

9.2.2 Related to Sexual Intercourse

The transmission of ZIKV during a sexual intercourse is explained in Chap. 5. In order to reduce the sexual ZIKV transmission it is important not to have sex and to use condoms (during the periods that are explained below) from start to finish and every time during vaginal, anal and oral sex. Not sharing sex toys can also reduce the risk of spreading ZIKV to sexual partners.

Pregnant women should not travel to endemic ZIKV areas. If a pregnant women partner has travelled to an endemic area, the use of condom is recommended during the entire pregnancy.

Centers of Disease Control and Prevention (CDC) proposed the use of condom in men after traveling a ZIKV areas during at least 6 month after the travel (if they do not have any symptoms) and, if having symptoms, for at least 6 months after the beginning of them. For woman the use of condom is proposed for at least 8 weeks after travel and 8 weeks after the beginning of symptoms if it is the case.

For people who live in an endemic ZIKV area and they or their partners are pregnant, the sexual abstinence and use of condom during all the pregnancy is proposed. If they are planning to get pregnant it will be important to discuss that plans with a healthcare provider. It they are not planning a pregnancy, the sexual abstinence or use of condom in all sexual intercourses is recommended.

9.2.3 Laboratory Accidents and Healthcare Providers

For Healthcare providers in treating ZIKV patients, standard precautions should be taken. Using adequate gloves and being aware of blood, body fluids, secretions, excretions, in contact with non-intact skin, and mucous membranes might spouse and accidental infection. Percutaneous exposure may be followed by the same procedures that usually involve contacting the occupational health office for an assessment of the exposure with consideration of all relevant pathogens including ZIKV, Human immunodeficiency virus (HIV), and hepatitis.

In the case of absence of an occupational exposure, there is no routine ZIKV testing indicated for asymptomatic healthcare personnel, so they should follow the same guidance as the general public [25].

9.2.4 Blood Supply and Transplantation

A study performed during French Polynesia ZIKV outbreak found 42 positive samples out of a total of 1505 asymptomatic blood donors studies of screening of blood donors [26].

So ZIKV outbreak may be accompanied by a risk of blood supply safety. In this context, WHO has proposed some measures for reducing risk of ZIKV transmission by transfusions [27]:

- Donors with a confirmed ZIKV infection, with a recent clinical history of ZIKV symptoms-like or who have had sex with a men confirmed of suspected of ZIKV infection, should be deferred for a period of more than 28 days.
- Blood donations may be tested for the presence of ZIKV (RNA) and ZIKV antibodies.
- The use of Pathogen reduction of blood components technology may be implemented for plasma and platelets.
- Quarantine of blood components during a period of 7–14 days and subsequently release following confirmation from the donor that they have not experienced ZIKV symptoms during the quarantine period.

In the case of transplantation, the Food and Drug Administration of U.S. Department of Health and Human Services recommendations are the following [28]:

- The living donors of cellular and tissue-based products and umbilical cord blood, should be considered ineligible for donor if they have been diagnosed of ZIKV infection in the past 6 months, they have been living in, or travelled to, an area with active ZIKV transmission within the past 6 months and also persons who have had sex within the past 6 months with a male.
- From a cadaveric donor, a person with medical diagnosis of ZIKV infection during the past 6 month will be considered ineligible.

9.3 International Guidelines for Prevention

Multiple guidelines for the prevention of ZIKV infection have been redacted, also for the prevention and management of all possible consequences of the ZIKV infection.

Some of the WHO and CDC international guidelines are presented ahead.

1. **Control of the vector**:

- Guidelines for *Aedes aegypti* and *Aedes albopictus* Surveillance and Insecticide Resistance Testing in the United States. CDC.
- Vector control operations framework for Zika virus. WHO.
- Entomological surveillance for *Aedes* spp. in the context of Zika virus. Interim guidance for entomologists. WHO.
- Monitoring and managing insecticide resistance in Aedes mosquito populations. Interim guidance for entomologists.

2. **Control of the routes of transmission**:

- Breastfeeding in the context of Zika virus transmission. Infant feeding in areas of Zika virus transmission. Summary of rapid advice guideline. 29 June 2016. WHO.
- Maintaining a safe and adequate blood supply during Zika virus outbreaks. Interim guidance. February 2016. WHO.
- Risk communication and community engagement for Zika virus prevention and control. A guidance and resource package for country offices for coordination, planning, key messages and actions. March 2016. UNICEF. World Health Organization. Pan American Health Organization. International Federation of Red Cross and Red Crescent Societies (IFRC).
- Prevention of sexual transmission of Zika virus. Interim guidance. 6 September 2016. WHO.

3. **Health workers specific information**:

- CDC Guidelines for Development of State and Local Risk-based Zika Action Plans March 8, 2016. State Actions to Consider as Risks Increase for Locally Acquired Cases of Zika. CDC.
- Protecting the health and safety of workers in emergency vector control of *Aedes* mosquitoes. Interim guidance for vector control and health workers. 10 March 2016. WHO.
- Advice for healthcare providers on medical eligibility for contraception. Medical eligibility criteria for contraceptive use. 2015. WHO.
- Health worker roles in providing safe abortion care and post-abortion contraception. 2015. WHO.
- Ensuring human rights in the provision of contraceptive information and services—Guidance and recommendations. WHO.
- Risk communication in the context of Zika virus. Interim guidance. 1 March 2016. WHO.

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Chapter 10
Risk of Globalization of the Disease in Europe

Abstract Risk of dissemination of ZIKV disease is based on multiple factors, including environmental (climate, socioeconomically, deforestation or industrialization) and travel/traveller factors. Both the disease (viremic travellers) and vector movement to mosquito-free area contributes to the introduction and establishment of autochthonous ZIKV transmission. Mass gathering events can contribute to magnify transmission due to close crowd life in a confined area. Also, multitudinary events can promote the introduction of an infectious disease to a previously naïve area when returning home. Although mathematical models estimate a low risk for introduction of ZIKV in Europe, specific European regions (mainly Portuguese Island of Madeira) account with suitable and efficient vector and opportune climate conditions to harbour the disease. Clinicians should be aware to enable early detection of autochthonous ZIKV cases. International and local guidelines can help clinicians on how to handle suspicious cases, how to confirm the infection and how to report suspected and confirmed cases. In case of autochthonous ZIKV detection, public authorities should perform surveillance and provide adequate resources to sustain enhanced mosquito control.

10.1 Factors Affecting the Risk of Spread of Vector-Borne Disease

It is known that ZIKV is primarily transmitted in tropical and subtropical regions by mosquitoes *Aedes aegypti* and *Ae. albopictus* bite, although other routes of infection have been also described (see Chap. 5). But, up to date epidemic/endemic transmission of ZIKV has been limited to tropical and subtropical regions, which suggests that ZIKV transmitted though alternatives routes to mosquito bites is not capable of maintaining on going transmission on their own in the absence of tropical/subtropical *Aedes* spp. vectors and/or a climate hot enough for ZIKV transmission by these species [1, 2].

To manage risks for endemic ZIKV infection in ZIKV-free countries some aspects should be considered.

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10.1.1 Related to Environmental Factors

Climate patterns, specially temperature and rainfall trends, but probably also changing wind patterns, have important implications on vector-borne diseases transmission. But this influence may be significantly modified by confounding non-climatic factors, including epidemiological, environmental, social, economic and demographic factors implicated [3–7].

– ***Climate facts***:

Temperature is known to modify directly vector-borne diseases in mosquito hosts. Variations of ambient temperature considerably modify insect internal temperature, as they are poikilotherms. Those variations greatly affect vector physiology and expose the pathogens they carry to ambient temperature [6, 8]. In laboratory, viral replication kinetics in mosquito-cultured cells has shown to depend on temperature, since higher temperatures lead to a more efficient viral attachment and cell infection [9, 10].

Several studies have also linked higher temperatures to shorter extrinsic incubation period, increase of female mosquito rate and faster dissemination rates [11–13].

Regarding precipitations and viral transmission dynamics, both increased and decreased rainfalls can promote the dispersal of ZIKV and other arboviriasis worldwide: although precipitations provide essential habitat for larval aquatic stage of *Aedes* lifecycle, drought can also contribute, as people increase water storage in household containers during the rainless periods, favouring larval hatcheries [14–16].

The atmospherical phenomenon known as El Niño has been linked to warm waves and drought in southern Africa and Southeast Asia, including Australia, while inundate the west coast of South America and to Central Africa [16, 17]. El Niño Southern Oscillation (ENSO) impacts on temperature conditions that caused the largest biting rates and the shortest extrinsic incubation period in 2015, which has favoured ZIKV outbreak in Latin America [18, 19]. The heather temperatures in North and Eastern South America, associated to an important drought throughout the second half of 2015 might be other of the multiple manifestations of ENSO and climate change that could have contributed to the rapid dispersal of ZIKV outside the native ecological niche [16, 20].

– ***Socioeconomic factors***:

The human population is supposed to increase from 6 billion by the end of the 20th century to around 10 billion by 2050, half of them concentrated in urban and periurban areas [21, 22]. But the increase of human density is estimated to be disproportional, because of a higher proportion of those people will concentrate in cities compared with nowadays situation. Urban populations in Africa and Asia and those in Latin America and the Caribbean are expected to increase by almost 50% [23, 24]. This is of particular concern as rapid population growth is associated to

poverty due to concentrations of population without the necessary infrastructure for the safe storage and distribution of water and drainage of wastewater. In addition, as previously mentioned, the use of inappropriate household water containers favours an optimal habitat for larval development [25, 26].

– ***Deforestation***:

Population increase implies the transformation of forestall areas in habitable areas. The consequent deforestation is expected to result in an increase in surface temperatures of up to 2 °C, with drier conditions where the land cover is reduced, which, again, favours the spread of vector-borne diseases as ZIKV [27, 28].

– ***Industrialization***:

Industrial activity contributes to nearly 33% of atmospheric concentration of CO2 and 50% of methane. This cumulus of gases has been linked to an increase of ambient temperature. Also, CO_2 and methane emissions can increment plant foliage. These facts would impact in vector-born insects as the increase of temperatures and density biomass would provide more favourable microclimates for insect vectors [29, 30].

10.1.2 Related to the Travels and Travellers

The increasing numbers of people travelling worldwide is the result of a more economical mass transport and liberalization of international trade. International travellers (9.9 million in 2015) flying from Brazilian airports (ZIKV-affected area) to North America, Europe and Asia represents 65, 27 and 5%, respectively [31, 32]. These data exemplify the magnitude of the problem.

There are a number of different situations related to international movement of people and materials that directly impact on the distribution and incidence of vector-borne diseases. Travellers can either be a carrier of pathogens into new environments or accidentally translocate vectors in transport vehicles. People can also trigger further outbreaks if they become infected by vector-borne diseases during the travel and return to their non-endemic country while they are still viraemic, thereby increasing the risk of spreading to areas where suitable mosquito vectors already exist [32, 33].

However, it should not be forgotten that other routes of ZIKV infection than mosquito-bite have been reported (see Chap. 5), so the spread of ZIKV via viremic travellers to areas without the *Aedes* mosquitoes is equally of concern.

To asses the acquisition risk for the disease, it´s important to identify the type of stay of travellers in endemic areas for ZIKV, because it will also entail a different risk of transmission once the traveller returns to his/her country of origin: many travellers may go to holiday resorts or other locations where the risk of infection is dramatically lower compared to that of the resident population. In that sense,

immigrants returning home to visit their family (also known as VFR o "visiting friends and relatives") represent a high risk group for mosquito exposure [34, 35].

An increased risk of introduction and establishment of autochthonous ZIKV transmission has been linked with viremic travellers returning from a ZIKV endemic country traveling by air transportation and with the commercial transport of mosquito larvae by trucks, ships or aircraft. Although rapid identification of subclinical and/or viremic patients is nearly impossible, a special emphasis should be placed on fumigation at ports and border crossing points and use of larvicides and insecticides must be highlight [36–38].

10.1.3 Introduction of Mosquito Vector-Born in Naive Areas

A modification in the geographical distribution of mosquitos has an important impact on the exposure of naive hosts to that disease. The introduction of an exotic vector or pathogen in an area where it did not previously exist, requires the existence of certain factors that assess whether the pathogen or vector is able to persist in that new environment or not, and how susceptible the local population to the disease is [4, 39, 40].

When a vector moves to another location and changes the suitability of the environment, it can modify the development, survival, and reproductive rates of vectors and pathogens, affecting the intensity of disease transmission and exposure of the population to that disease.

On the other hand, sensitivity and susceptibility to vector-born diseases by population is influenced both by genetic or acquired immune developed in response to previous exposure. In the specific case of ZIKV, naive, non-immune population are especially susceptible to epidemics of acute disease, and that is one of the reasons why this disease has spread so rapidly [4, 41].

For more accurate information about mosquito dynamics, see Chap. 4.

10.2 Risk Due to Mass Gathering Events

The World Health Organization (WHO) defines a mass gathering as "an organized or unplanned event where the number of people attending is sufficient to strain the planning and response resources of the community, state or nation hosting the event" [1, 2]. Mass gathering events meets theoretical ideal conditions for the transmission of infections between people from remote and widespread geographical locations, with potentially different immune responses.

Pilgrimage, sports events, outdoor shows, musical festivals, political or cultural events and other celebrations that congregate crowds in a confined area increases the risk of a range of infectious diseases [3–7]. Travellers to these crowded events

cannot only introduce an infectious disease to a previously naïve area, but can also magnify transmission at the people meeting and further propagate transmission after their return home. This statement has been fulfilled on multiple occasions, as was the case of the transmission of 2009 H1N1 pandemic afterward a large Easter holiday gathering in Iztapalapa, Mexico [6, 8]. Similarly, crowded congregation of population has been associated to the spreading of the Great Pandemic in 1918 and the Asian Flu Pandemic in 1957, creating a global disease outbreak [9, 10].

The paradigm of mass gathering event at risk is the annual Islamic pilgrimage to Mecca and Medina in Saudi Arabia (Hajj), which congregates approximately three million Muslim pilgrims arriving from different parts of the world to Mecca. The outbreak of meningitis in 2000–2001 after the Hajj demonstrates the importance of this type of mass events in the transmission of infectious diseases [11–13]. Other example that shows the possibility of dissemination of infectious diseases during a mass gathering event was the congregation in Taizé, Germany, for Christian pilgrimage in 2006, that was followed by primary measles cases identified among unvaccinated persons [14–16].

On the other hand, there are examples in which concentrations of people are not followed by an amplifying of a pre-existing disease: the outbreak of Middle East Respiratory Syndrome coronavirus (MERS-CoV) was at its peak in 2013 just when pilgrimage to Saudi Arabia was started. No cases of this respiratory infection disease were identified during Hajj and Umrah (a minor pilgrimage) during this year, even though some cases were identified during the following years [16, 17]. So probably other different factors must also be present to provide a favourable environment for the dissemination of infectious diseases.

Because of ZIKV outbreak in Brazil and its risk of dissemination, all the alarms were focused on the Olympic games of 2016, held in Rio. Previously, health authorities in Brazil were aware of other vector-borne risk and had already celebrated multiple mass gathering events with no evidence of significant international spread. As an example, during the 2014 FIFA Football World Cup in Brazil, mathematical models estimated 33 cases of DENV (range 3–59 cases) among foreign tourists, of nearly 600,000 of expected visitors [18, 19]. So far, only 3 cases of DENV were reported associated to this sport event [16, 20]. It's postulated that the low numbers of cases finally diagnosed of DENV were the result of a year with an unusually low incidence in Brazil, probably due to a severe drought in 2014, reducing the number of expected cases in visitors. Also, the typical lower temperatures of the months in which the event was held (June and July), contributed to this small amount of cases [21, 22].

Predictions of the risk of acquiring ZIKV during Olympic and Paralympic Games in Rio 2016 were initially difficult to asses. The lack of knowledge of real incidence of the disease, due mainly because of asymptomatic cases were not reported, made accurate estimations difficult to weigh. Based on forecast of between 500,000 and 1,500,000 ZIKV infections (both symptomatic and asymptomatic) [23, 24] the calculated risk during the period of the celebration (between August and September) ranged from 9×10^{-6} to 3×10^{-5}, which represents a risk 15 times lower than that of DENV for that period [25, 26].

Likewise, other mathematical models predicted travel-associated ZIKV risk in visitors to Olympic games in 2016, anticipating between 6 and 80 cases of both asymptomatic and symptomatic cases of total ZIKV infections among travellers attending the Olympics, with between 1 and 16 of them expected to be symptomatic [27, 28].

But by the end of Rio Olympic Games, no cases of ZIKV infection involving spectators, athletes or anyone associated with the Olympics were achieved [29, 30]. The lack of cases associated to this mass gathering event was probably because visitors' exposure level to mosquitos was lower than that of residents (i.e. adequate use of repellents, mosquito net). Also, tourist were likely to confine their stays to areas close to the Olympic Stadium and other mainly touristic, which were previously fumigated and free of mosquito and larvae, avoiding areas on the outskirts of the city, where mosquito density was higher [31, 32].

Other multitudinous event in Brazil is its annual Carnival. During the previous festivities of Carnival 2016 held in February, more than 1 million of visitors stayed at Rio de Janeiro, which represents twice the number expected for the Olympic Games. At that time, risk of ZIKV was highest, as cases reported were at their peak and most of activities were outdoors, increasing the exposure of visitors to mosquitoes. The resulting risk calculated for ZIKV infection for tourists was 36 per million tourists [32, 33]. But after Carnival celebration in 2016, there was no a significant increase of diagnosis over the following months [34, 35].

2017 carnival represents a new opportunity for ZIKV of spread. Although a decline in cases of Zika infection has been reported in Brazil, tourists and visitors are still posed to risk of ZIKV infection.

10.3 Specific Risk of Globalization in Europe

In Europe, multiple imported cases of ZIKV have been previously reported [36–38]. This is of particular concern, as *Aedes* vectors are known to be present, as previously described (Chap. 4). Therefore, returning ZIKV-viremic travellers to European countries may become a source of local transmission in the presence of this suitable vector. Previously, *Aedes* mosquito was implicated in local transmission of CHiKV and DENV in Mediterranean countries [4, 39, 40]. The species have also been responsible for outbreaks of yellow fever in Italy in 1804 [4, 41]. The existence of previous autochthonous arboviriasis transmitted by the same vector, make the infection by ZIKV also feasible in Western Europe at least seasonally, when competent vector species are present. The arrival of summer in North Hemisphere is linked to a peak in mosquito *Aedes* population and a most efficient viral replication, so from June to September climatic influence could also favour the establishment of ZIKV disease in Europe [42].

Different mathematical models have assessed the risk of ZIKV arrival, establishment, and autochthonous in Europe. Estimations of imported cases of ZIKV have been made based on published estimations of 500,000–1,500,000 infections in

Brazil (both asymptomatic and symptomatic) during 2015 [23]. Models predicts between 508 and 1.778 imported cases, particularly in France, Portugal, and Italy. Of these, approximately 20% would likely be symptomatic so it´s expected between 116 and 355 symptomatic ZIKV infections into all European countries in 2016 [38].

Once ZIKV arrives, a competent vector must be present to maintain the disease. The competence of European *Aedes* mosquitoes (both *Ae. aegypti* and *Ae. albopictus*) as an efficient vector for the currently circulating Asian genotype of ZIKV has also been assessed. Despite the fact that *Ae. albopictus* plays a central role in the transmission of other arboviruses in Europe, it's suggested that this mosquito is not good enough to maintain local transmission of ZIKV compared to CHIKV and maybe, DENV [43], concluding that there is low risk for ZIKV expansion in most parts of Europe, with the possible exception of the warmest regions bordering the Mediterranean coastline, and specially Madeira. In this specific autonomous region of Portugal two main factors are present: the presence of *Ae. Aegypti* (which is considered the most competent vector for ZIKV), introduced in 2005 [44] and the close contact with Brazil, with whom it maintains active commercial exchanges and people shares the same language [43].

Although the amount of travellers arriving from the Americas to Madeira is low compared to other cities in continental Europe, the presence of *Ae. Aegypty*, an extended season associated to high vectorial activity and the hazardous outbreak of DENV fever in 2012 [45], stress the potential for autochthonous transmission of ZIKV in that Portuguese location [42].

Other factor that must be considered is the numbers of travellers returning to Europe from areas in the Americas with known ZIKV activity during the period of higher vectorial capacity. It is estimated than between 475,000 and 715,000 travellers will arrive from endemic areas for ZIKV to the main central European capitals from May to October, of the total of population at risk of nearly 800 million central Europeans. In July and August, (when temperatures and vectorial capacity in Europe are peaking), 15,7% of this population might reside in areas with known occurrences of *Ae. albopictus* (i.e. 241 million of centre Europeans). European countries with a large percentage of their population living in areas where basic reproduction number (Ro) is over 1 in August include Albania (83% of population at risk), Italy (78%), Croatia (44%), France (20%), Greece (25%), Montenegro (39%), Slovenia (28%) and Spain (19%) [42], so health authorities of those specific locations should be aware of current risk.

Other mosquito species as *Culex*, which is present in central Europe that are able to transmit other arboviruses, has proved not to have vector competence for ZIKV [46].

Taking all these facts under consideration and adding that the average temperatures of s of most areas of Europe the possibility of large outbreaks of ZIKV in most areas of Europe, at least for the immediate future, seems to be irrelevant.

Imported cases of Zika virus have been documented in Europe and are expected to continue, not only because the high proportion of travellers arriving from the most affected regions to Europe. Sexual transmission of Zika has been also reported in European citizens without a previous travel to endemic region [47, 48].

There are documented precedents of containing an arbovirus disease outbreak in Europe. In 2014, after an outbreak of CHiKV in Montpellier, a successful integrated prevention and response programme was performed. Although the absence of immediate mosquito control treatment near the primary case's residence (because the vector was initially not identified) and a lack of awareness of this disease among health professionals facilitated the spread of the infection up to 12 cases. However, there was a quick response following the alert. Subsequently after the detection of the cases, French authorities focused on epidemiological and Entomological investigations and stressed vector control treatments, which played a part in prompt containing the outbreak [49].

EU countries must be prepared for this new threat. This requires capability to detect and diagnose cases early, perform systematic and regular surveillance, and adapt resources to sustain enhanced mosquito control. Clinicians should be aware to enable early detection of ZIKV cases and there must be sufficient and validated laboratory capacity for virus detection, virus identification and serological testing. International and local guidelines can help clinicians on how to handle suspicious cases, how to confirm the infection and how to report suspected and confirmed cases. In case of autochthonous ZIKV detection, public authorities should perform surveillance and provide adequate resources to sustain enhanced mosquito control. Also, information should be promptly circulated to all health professionals, public health services and other sectors. Failure to do so could lead to the possibility of spreading more extensively, resulting in greater costs for vector control and health care for infected people [50].

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