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Review

Zika virus infection or the future of infectious diseases☆

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ABSTRACT

Zika virus belongs to the *Flaviridae*, an extended phylogenetic family containing dengue or yellow fever, viruses whose shared main vector are *Aedes aegypti* mosquitoes. The virus originally came from Central African simian reservoirs and, from there, expanded rapidly across the Pacific to South America. The disease is an example of exantematic fever usually mild. Mortality is very low and mainly limited to secondary Guillain–Barré or foetal microcephaly cases.

Diagnostic confirmation requires a RT-PCR in blood up to the 5th day from the onset or in urine up to the 10–14th day. Specific IgM are identifiable from the 5th symptomatic day. Clinically, a suspected case should comply with: (a) a journey to epidemic areas; (b) a clinically compatible appearance with fever and skin rash, and (c) a generally normal blood count/basic biochemistry.

There is some evidence that causally relates Zika virus infection with foetal microcephaly. While waiting for definitive data, all pregnant women coming from Central or South America should be tested for Zika virus.

Infección por el virus Zika o el futuro de las enfermedades infecciosas

Palabras clave:
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RESUMEN

El virus Zika es un *Flavivirus* filogenéticamente cercano al de la fiebre amarilla o del dengue, cuyo vector principal es el mosquito *Aedes aegypti*. El virus procede de un reservorio simiano africano y ha protagonizado una expansión fulminante a través del Pacífico hasta Sudamérica. Provoca una enfermedad leve caracterizada por fiebre con exantema. La mortalidad se circunscribe a casos de Guillain-Barré y de malformación encefálica fetal con microcefalia.

Un caso sospechoso será aquel con: a) antecedente epidemiológico de desplazamiento a zona endémica; b) cuadro pseudogripal con exantema, y c) hemograma/bioquímica levemente alteradas o normales.

La confirmación diagnóstica requiere identificar al virus por RT-PCR en sangre (hasta el quinto día sintomático), orina (hasta el día 10-14) o IgM específicas a partir del quinto día. Existe alguna evidencia que da soporte a la relación causa-efecto con la microcefalia fetal. A la espera de datos definitivos, las mujeres embarazadas procedentes de Centro y Sudamérica deben ser testadas para descartar la infección.

In previous editorials[1] the authors highlighted the advisability of promoting and extending knowledge of viruses transmitted by mosquitoes–the arbovirus–due to its potential for expansion in Europe, especially in the Mediterranean ecosystem of the Iberian Peninsula, colonized by the vector-mosquito *Aedes albopictus* (*A. albopictus*) or *tiger mosquito*. The Zika virus (ZIKV), beyond its proven ability to become endemic at a tremendous speed, it monopolizes covers in the media, which present it as a new infectious threat to Europe. We will have to have strong and well-informed opinions so that before the eventual moment of truth–the real possibility of an autochthonous outbreak–health professionals may be able to prevent that panic, even though momentarily, interferes with rational decision making.[2] The way knowledge was

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Table 1
Arbovirus identified in the European Union and with possibility of transmission by *Aedes* spp.

Family	Virus	Transmission	Disease	Cases in EU	Cases in Spain
Togaviridae	Chikungunya	U, S, R	SF	Autochthonous	Imported
	Sindbis	R	SF	Autochthonous	No
	Mayaro	R	SF	Imported	No
	Eastern encephalitis	R	SF, ME	Imported	No
Flaviviridae	Dengue	U, S, R	SF, HF	Autochthonous	Imported
	Yellow fever	U, S, R	SF, HF	Imported	Imported
	Usutu	R	SF	Autochthonous	No
	West Nile	U, S, R	SF, ME	Autochthonous	Autochthonous
	Zika	U, S, R	SF, ME	Imported	Imported
Bunyaviridae	Batai	R	SF	Autochthonous	No
	Tahyna	R	SF, ME	Autochthonous	No

HF: haemorrhagic fever; SF: systemic fever; ME: meningoencephalitis; R: rural; S: suburban; U: urban; EU: European Union.

handled in the early days of the unfortunate Ebola virus infection should not be repeated.

So we have reviewed the available evidence on the Zika arbovirus; hopefully it will be useful.

Phylogeny of the virus

The ZIKV belongs to the *Flaviviridae* family, flavivirus genus, and, therefore, is related–and is antigenically close–to other flaviviruses such as yellow fever, Japanese encephalitis, dengue fever and West Nile. They are all enveloped viruses and are characterized by single-stranded RNA and icosahedral capsid.[3] Instead, it is phylogenetically far from the chikungunya virus, which belongs to the *Togaviridae*.[4] This, as we shall see, must be taken into account during the diagnostic process. A summary of the various arbovirus classified by families and their clinical and epidemiological characteristics is shown in Table 1.

Genetic studies have allowed us to distinguish 2 lineages–but only one serotype–of ZIKV: African and Asian. It is a situation similar to that presented by the chikungunya virus, which proves that the spread to Asia through animal cycles or perhaps humans with low clinical expression is old. As with the American epidemic of chikungunya (first cases in San Martin, Lesser Antilles, December 2013), the invasive lineage is Asian.[5] The spacing between the start of the 2 American pandemics of zika and chikungunya fevers only represents a 17-month lapse. There is no evidence for the existence of mechanisms of synergy between the two virus, neither at host nor at vector level. Chances are that the human and commercial traffic between emerging countries of the Western Pacific and South America have entered virus and vector just as it happened in Europe (chikungunya epidemic transmitted by *A. albopictus*, Italy, 2007).[6]

With regard to its geographical origin, first isolation took place in Uganda, so that ultimately we are most likely dealing with a pathogen from the jungle biotope of Equatorial Africa, which has taken advantage of the human invasion of its space to spread. It is quite possible that local ecological factors such as deforestation and climate change, act as accelerators of the epidemic because they promote breeding of *Aedes* vector mosquitos. It's not the only one; it merely follows the path previously travelled by HIV, chikungunya virus and possibly, yellow fever.[7]

Reservoirs and vectors

The virus was first isolated in 1947, when American researchers identified it in Rhesus macaques (*Macaca mulatta*) in the Zika Forest (Uganda) where the presence of yellow fever virus was studied.[8] The moment of its description gave us a sample of its hard traceability, as some of the macaques came from India. In any case, it is more than plausible that has a more or less extensive simian reservoir with an African wild life cycle. The first human case was described in Nigeria – 1954, and from there, surprisingly, there are isolated case reports in a multitude of countries Egypt, Indonesia, Gabon, Malaysia, Philippines, Vietnam, Tanzania and others who did not activate any alarm but obviously translated a global level expansion.[9,10]

This point is interesting because it draws a parallel with the spread of the yellow fever virus in the XVIII–XIX centuries and the chikungunya virus in the early XXI century: Origin in an Old World jungle-like ecosystem, presence of isolated cases for some time, first in Africa and then in Asia, major epidemics in the American biogeographic system and outbreaks in Europe related to these epidemics. Yellow fever was a scourge to the port cities of Europe (Barcelona, 1821) or North America (New York, 1791). At the time they were controlled thanks to improved surveillance of persons and commercial goods–inspections, quarantines–as well as through the implementation of measures against the *Aedes aegypti* (*A. aegypti*) vector, which, at that time, was common in Western Europe.[11] It was not until much later that the massive use of attenuated virus vaccine sent the disease back to its original animal reservoir. Take the following into account.

The expansion of ZIKV took place in parallel to its main mosquito vectors: *A. albopictus* spread from Southeast Asia, and *A. aegypti* re-expanding to large tropical and temperate zones of the world. *A. aegypti* is a much more efficient vector regarding establishing itself in human cycles; therefore, countries which densely colonized by this arthropod are at increased risk of epidemics. To complicate matters further, the simultaneous transmission of dengue, chikungunya and zika is demonstrably possible. Imagine the challenges and questions faced by our colleagues in South America.[12,13]

Although the element that increased transmissibility of the chikungunya virus (genetic mutation) was identified, the factor or combination of factors that underlie the pandemic conversion of the ZIKV is unknown. There are signs that point to the existence of an invasive spread from Micronesia–Yap island, 2007–and the Pacific–French Polynesia and Easter Island, 2014–vectorized by endemic species of *Aedes* (*A. polynesiensis*) until virus and *A. aegypti* came into contact, possibly after an import in Brazil. The coincidence with the expansion of chikungunya virus and its little manifest clinical signs and symptoms kept it quiet until suspicions surfaced when it was linked with neonatal microcephaly cases occurred in Brazil, already in 2015.[14]

As for its current extension, zika fever cases were reported in epidemic form in all the countries of Central and continental South America except Peru, Chile, Argentina and Uruguay, as well as many of the Caribbean island countries. A map of the current distribution is shown in Fig. 1. Do not forget that in its "minor" epidemic form or as isolated cases, it is possible to isolate it in most tropical–subtropical areas of Africa, Asia and Oceania, especially in the South Pacific (Samoa) plus the Cape Verde focus, belonging to

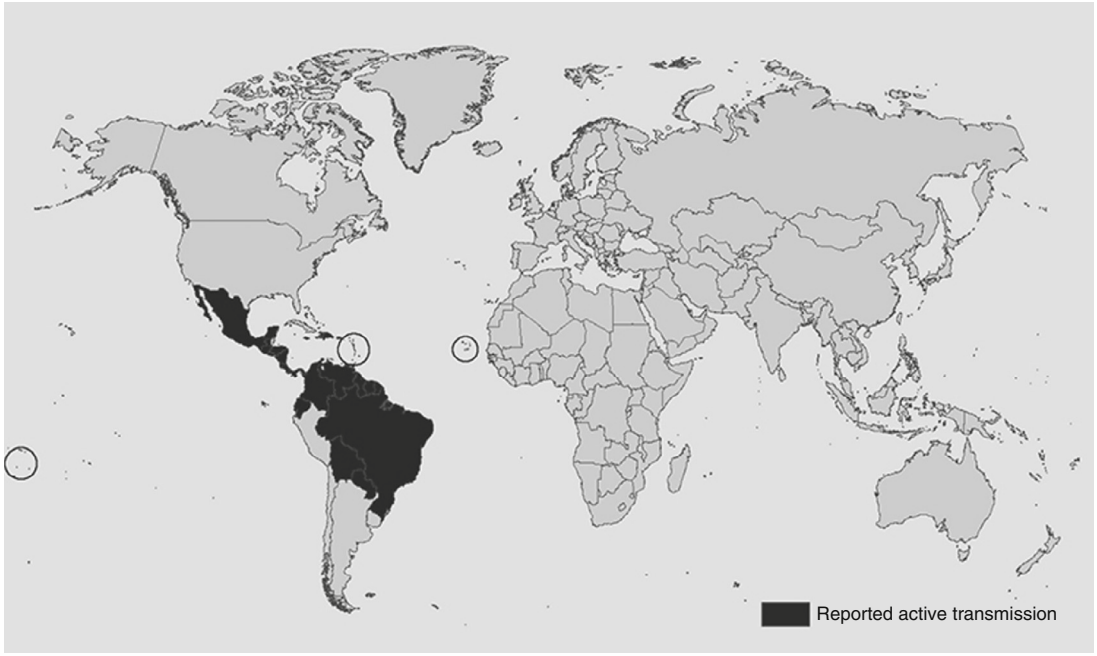

Fig. 1. Distribution of Zika virus in the world on February 2016.
Source: WHO. Available in: http://www.cdc.gov/zika/geo/active-countries.html.

an isolated ecosystem (Macaronesia). At mnemonic level, it can be considered that their distribution is superimposed to dengue.[15]

Transmission

A ZIKV epidemic is only possible if there is: (a) a competent vector-mosquito in high densities and with marked anthropophilia in its eating habits, and (b) a large susceptible population. This is, to date, the situation in South America. In Mediterranean Europe, the chances of developing a similar pandemic are remote due to the limited vector capabilities of *A. albopictus*. However, the occurrence of secondary cases is possible from imported index cases during the summer periods with higher mosquito densities. Note that Catalunya, the Andalusian Costa del Sol, the French Riviera and Italy's Romagna–which also have a very high traffic airport (Milan) and 2 high traffic ones (Barcelona and Rome)–should be considered areas of special epidemiological surveillance, not only due to the possibility of occurrence of cases per se, but because between all of them they can have more than 100 million visits of tourists per year, not counting a great number of internal journeys (domestic tourism). They said regions may, therefore, *act as a first order platform for the expansion of the ZIKV to the rest of the EU*.[16,17]

Fortunately, transmission from person to person without a vector is, in general, marginal in epidemiological terms. However, it is true that the application of *polymerase chain reaction* (PCR) techniques identifies viral genetic material in tears, semen, urine; almost any fluid from individuals in the acute viremic phase. As in the case of ebola virus, there may be some circumstantial risk of sexual transmission, limited in this case to the viremic phase, although the epidemiological significance of positive PCR–semen positive of one of the 3 cases tested at 10 weeks of the clinical onset–is not fully known. There are 3 documented cases of sexual transmission.[18] Blood transfusions should be considered a possible transmission route and the adequacy of its screening is under debate during epidemic periods if the possibility to do it exists, technically speaking. As with chikungunya, it is reasonable to consider the possibility of transmission through transplants, although it is a subject in its infancy, and should be further investigated.[19,20]

The virus has been isolated from amniotic fluid, placenta and tissues both from live births and abortions. It is, therefore, capable of inducing a vertical mother–neonate transmission, either through the placental barrier, or during labour.[21]

Pathogenesis and clinical features

As with all arboviruses, after inoculation, a replication phase initiates in the histiocytes of the dermis, which then invade the regional lymph nodes and eventually the bloodstream. It incubates for a period ranging from 3 to 12 days, so time periods over 15 days without clinical symptoms counting from an eventual transmission rule out the disease, key data with regard to diagnostic approach in travellers. Asymptomatic infections are common and it is estimated that only one in four infected develop clinical symptoms.[22,23] Moreover, symptomatically, it presents as moderate fever with arthralgia and sometimes arthritis. In a variable percentage of patients–although probably high–it develops conjunctivitis and maculopapular rash between the second and third day of fever. This does not persist beyond 3–5 days and the rash disappears soon after. Abnormalities in blood count, coagulation and biochemistry are unspecific, if any.[24] A list of semiotic characteristics of dengue, chikungunya and zika fevers is shown in Table 2. Altogether it is a self-limiting and benign disease; complications are extremely rare, with the exception of its relationship with neurological disorders in adults (Guillain–Barre syndrome) or foetuses (malformation of the brain with secondary microcephaly).[25,26]

With regard to Guillain–Barre syndrome, apparently a small percentage of patients may develop it, in varying degrees, at the end of the disease, reflecting immune involvement against myelin components. Guillain–Barre syndrome is primarily responsible for the mortality attributable to ZIKV. The exact mechanism that triggers paralysis is unknown, but epidemiological data that relate to the outbreak of ZIKV are consistent in at least 4 countries (Brazil, Salvador, Colombia and French Polynesia). We would therefore face a similar behaviour to that described in connection with West Nile virus infection, its close relative. We can conclude, therefore, that travellers affected by polyneuropathy with flaccid postinfectious

Table 2
Clinical differences between dengue and chikungunya fever.

	Chikungunya	Dengue	Zika
Subclinical cases, %	20	80	70–80
Fever	Common (70–80%)	Present (>95%)	Common
Myalgia	Present	Common	Common
Arthritis	Common	Rare	Rare
Conjunctivitis	Common	Rare	Very common
Exanthema	Common (50–60%)	Less common (50%)	Very common (90%?)
Neutropenia	Rare	Common	Rare
Lymphopenia	Common	Rare	Rare
Thrombocytopenia	Rare	Common	Rare
Haemorrhages	No	Potential	No
Hemoconcentration	No	Common	No

paralysis or other neurological disorders travelling from the Holarctic ecosystem (North America, Europe, Siberia, China and Japan) West Nile should be suspected, in patients travelling from the neotropical ecosystem (Center and South America) Zika should be suspected, and if the patient has not travelled, the possibility of infection by an autochthonous Toscana *Bunyavirus*[27,28] will need to be considered.

Pathological processes by which ZIKV can damage the foetal brain are unknown; to date, the relationship continues being epidemiological. Experts voices advise caution. Alarmism must be avoided because there may be other concurrent causal factors.[29] It is not a frequent complication and affects very unevenly the large pandemic area, with a concentration of cases–more than 4000–in the eastern part of Brazil (especially the states of Pernambuco and Bahia, with incidences of up to one case per 1000 live births).[30,31] While new data becomes available, pregnant women, especially in their first trimester, should not travel to endemic areas under the premise of prudence. In addition, a strict ultrasonographic monitoring of pregnant women travelling from these areas should be performed plus the screening of all those who relate a history of fever either during their stay or up to 30 days following their return to Europe would be indicated. 70% of ZIKV infection cases are asymptomatic and, therefore, there are arguments in favour of universal screening of healthy pregnant women travelling from risk countries. However, the foetal risk associated with these subclinical infections is unknown, therefore, compliance with the usual gynaecological ultrasound controls is, so far, the cornerstone for proper control of the occurrence of foetal malformations. Although the *Centres for Disease Control and Prevention* issued opinions initially against screening of asymptomatic pregnant women, in just two weeks it went on to recommend the opposite, which seems to be the established recommendation in Europe.[32–34]

Diagnosis

Diagnosis is based on clinical suspicion. Its short incubation period (3–12 days) makes the diagnosis of zika fever very unlikely in patients where no travel to endemic areas is identified in the last 15 days. The determination of specific IgM antibodies is the main confirmation test, given the evanescent period of viraemia, at least in blood.[35] Thus, it is useful to consider a basic time schedule of appearance and disappearance of antigens and antibodies (Fig. 2).

In serum samples taken during the first 5–7 days, viral RNA can be detected by PCR techniques with *reverse transcription* (RT-PCR). At present there are no other direct microbiological diagnostic tests such as antigen detection. Due to the rapid disappearance of viraemia, a negative result of the blood RT-PCR does not exclude ZIKV infection if the sample is collected beyond 5–7 days of the onset of symptoms. In urine, RT-PCR may persist positive until 10–14 days and is considered a good performance test; however, similar to the one performed in blood, its negativity does not exclude infection, and serology should be performed.[36] Specific IgM antibodies are usually detectable from 4 to 5 days of clinical progression by *enzyme-linked immunosorbent assay* –ELISA or indirect immunofluorescence techniques, although there are records of late positives–as in all viral diseases–that would force a second determination if there is a well-founded diagnostic suspicion. Remember the old principle that clinical symptoms dictate and declare the case if suspected, even in the absence of confirmation.[37]

Is there serological cross-reactivity with other flaviviruses of its extended family? Yes, there is. False positives may occur in patients who have had a previous flavivirus infection–dengue fever, being the most frequent–and those vaccinated against yellow fever or Japanese encephalitis. Against this background, confirmation by serological testing becomes important, by means of plaque reduction neutralization test if the case requires it; it is a laborious test; that is, if the resources are limited, severe cases and in pregnant women should be prioritized. IgG antibody detection is of little use. The usual multiplication by 4 of the serological titre between 2 samples separated by at least 2 weeks entail a diagnosis of seroconversion and a high probability of recent infection. Again, it may be useful in asymptomatic pregnant women with a history of fever during their stay in an epidemic area, but little else.

Given that in most cases we will need to guide the patient without the possibility of serological confirmation until after a few days, the clinical diagnosis can be based on compliance with the following conditions: (a) epidemiological history (travelling to endemic area in last 15 days or close contact with an imported index case); (b) clinical symptoms consistent with the appearance of rash, and (c) blood count and basic biochemistry slightly abnormal or normal. It is worth remembering that patients travelling from areas with high malaria transmission – mainly Sub-Saharan Africa–should undergo a thick film with thin blood smear.

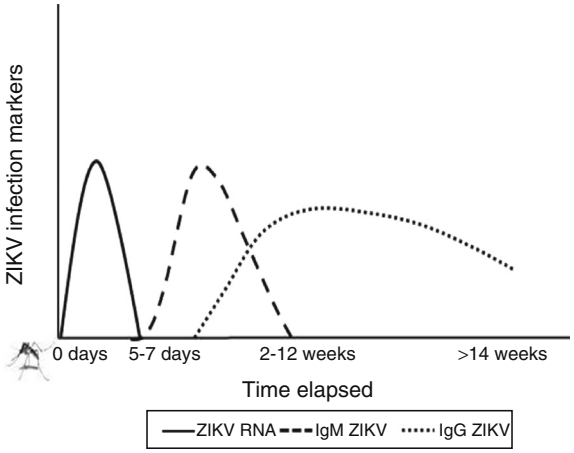

Fig. 2. Evolution of diagnostic markers during Zika virus (ZIKV) infection.

IgG dependent immunity is probably protective, long term, against new reinfections.

Prevention and treatment

The basic preventive measures are those that are intended to prevent contact with the vector, whether environmental (massive fumigations, enhancing ecological competitors or predators, eliminating breeding areas) or personal (use of mosquito nets on doors and windows or covering beds, repellents). Personalized recommendations vary according to sex:

(a) Pregnant women are recommended not to travel to endemic areas and postpone pregnancy until 30 days after their return to Europe, twice the maximum incubation period. In the case of having suffered some febrile episode during their stay and up to 4 weeks after their return, it is recommended to delay pregnancy until 6 months.
(b) It is recommended that men travelling from endemic areas have relations with a condom until the end of pregnancy if their partner is in that situation. Any wanted pregnancy should be delayed by using condoms for at least 4 weeks if during their travelling have not had any febrile episode and up to 6 months if they have experienced it, according to the very limited data available.[38]

The *A. aegypti* vector is a mosquito who does not like travelling; lives and dies mainly in a specific peridomestic area, i.e. the rate of expansion of the epidemic probably depends more on the mobility of sick people than on the mobility of virus-infected vectors. Whether the movement of sick people in territories with *A. albopictus* should be limited is beyond the scope of this review, but it would not hurt to recommend *resting at home* until the–short–period of viraemia has passed.

The main prevention research channel is the development of vaccines. There are safe and effective vaccines against other similar flavivirus, such as yellow fever, Japanese encephalitis and recently, dengue.[39] The existence of a single serotype should simplify the process, but a specific vaccine should not be expected to be on the market before 2 years, if everything goes well.[40]

We have no direct action antiviral treatment or any treatment that may limit its transmission; fever can be treated with acetaminophen to start with, and NSAIDs can be reserved for patients with joint involvement, provided dengue had been ruled out, otherwise the risk of bleeding may be enhanced.

Conclusions

It is difficult to be categorical with respect to a prediction about the risk of establishment of human cycles of the virus within the European Union. However, *A. albopictus* is considered a mediocre vector with respect to viral transmissibility. Most likely there is no risk in the period from November to May due to the low population density of the mosquito. In warm periods the risk will be marked by population growth, besides the outstanding anthropophilia of this mosquito.[41] Epidemiologists should coordinate local actions aimed at maintaining environmental health and eliminating breeding sites; this is the basis. Healthcare professionals should strive to understand the disease and *incorporate* it in their schemes in order to identify suspected cases *as early as possible*, and monitor and recommend that such patients take anti-vector measures, while awaiting definitive diagnostic confirmation with diagnostic tests that microbiologists will need to assess, acquire and validate. These techniques should be also appropriate to potentially conduct large-scale screening programmes in healthy pregnant women. All this is easy to say but difficult to implement, as well as expensive. Impossible to implement (and fund) in its full extent especially without the support of area epidemiologists and, above all, family doctors.[42]

OK, but what about those of us who work with infectious diseases? Then we could apply the following to ourselves: *the future arrived today*–if it did not come yesterday–and we must assume that imported diseases are and will be a steadily increasing part of our activity, whether called MERS-CoV, chikungunya, ebola or zika. We need to accept it and adapt, standardize and formalize the new knowledge that their treatment requires in a more cross-functional way, counting on both healthcare levels and speeding up diagnostic methods. Otherwise, we are condemned to a perpetual reactive attitude in which doctors are not always the ones who take control of the countermeasures to be applied.

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