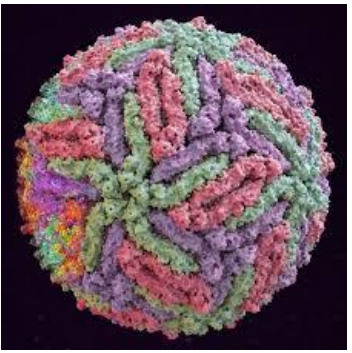
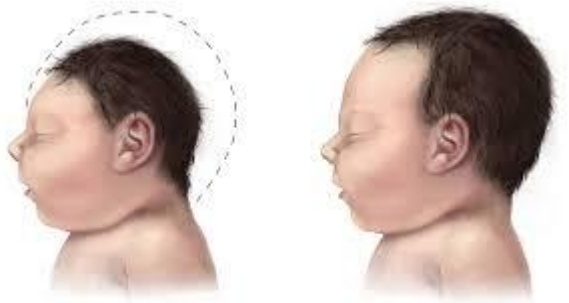
Il virus Zika (ZIKV)



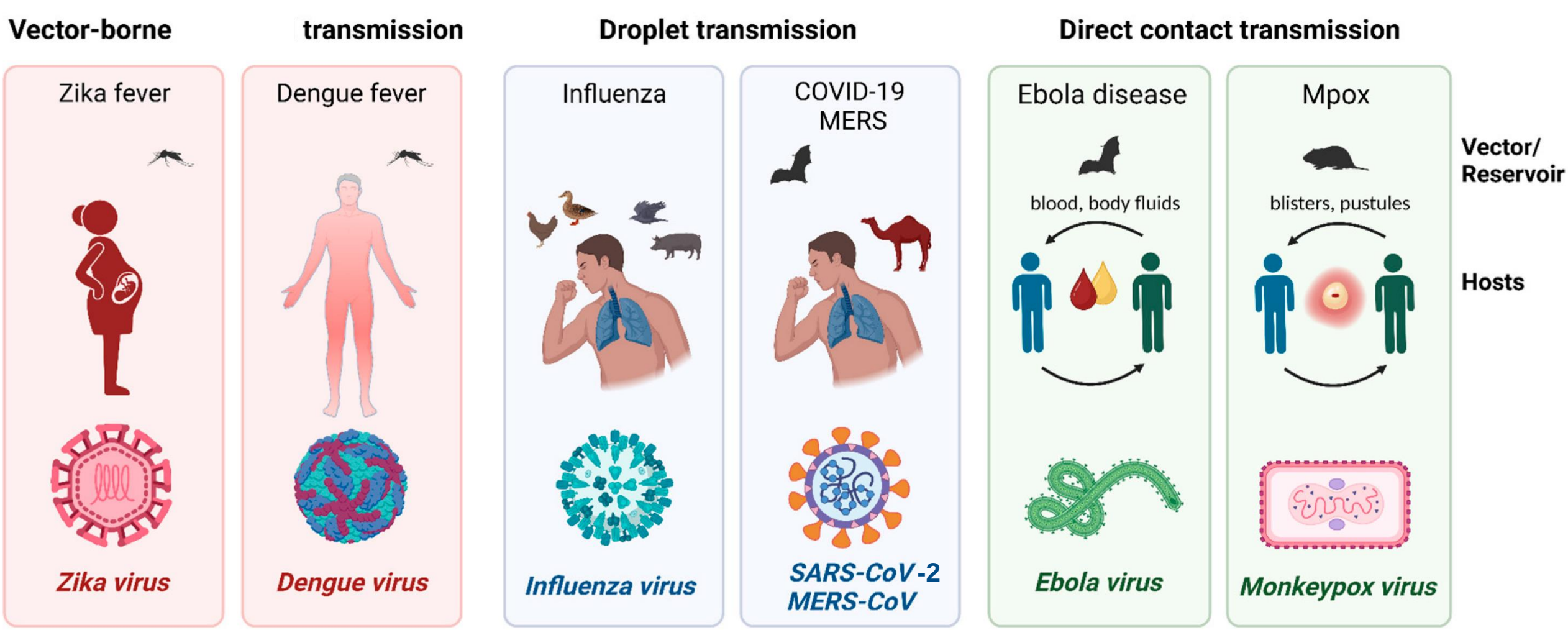


Emerging and re-emerging viruses

Key players among the different pathogens that have caused recent epidemics

Emerging viruses are defined as those causing new human infections that had never been encountered earlier

reemerging viruses are defined as those causing infections after lying quiescent for many years or even decades… (new population, changes in viral biology, etc.)



A vector is an organism, often an arthropod, that transmits an infectious disease from one individual to another. They can be actively involved in the pathogen's lifecycle or simply carry the pathogen

A reservoir host is a species in which the pathogen endemically circulates and is considered to have coevolved with

Flavivirus (ssRNA+ genome)

ARthropod-**BO**rne virus (Arbovirus)

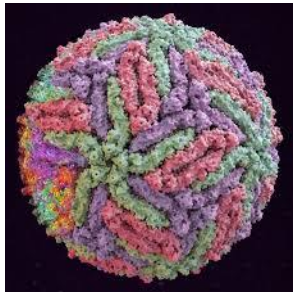
Family: Flaviviridae
Genus: Orthoflavivirus
Species: orthoflavivirus zikaense

Principal vector	Transmitted virus
AEDES 	Dengue (DENV) Yellow Fever (YFV) Zika (ZIKV)
CULEX 	West Nile (WNV) Japanese Encephalitis (JEV)
	Tick-borne Encephalitis (TBEV) Alkhurma (AHFV)

- 1648 : first description of Yellow fever
- 17th–21th century– outbreaks of YF and Dengue
- 1927: isolation of YFV
- 1932 YFV vaccine by Max Theiler (Nobel prize in 1951)
- 1943: Isolation of DENV
- From 1970's dramatic increase in severe dengue cases Before 1970, only 9 countries had experienced severe dengue epidemics. The disease is now endemic in more than 100 countries.

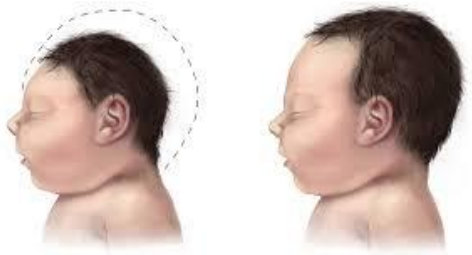
Not all the mosquitoes are the same. Different mosquitoes spread different viruses and bites at different times of the day.

Where and when we met Zika



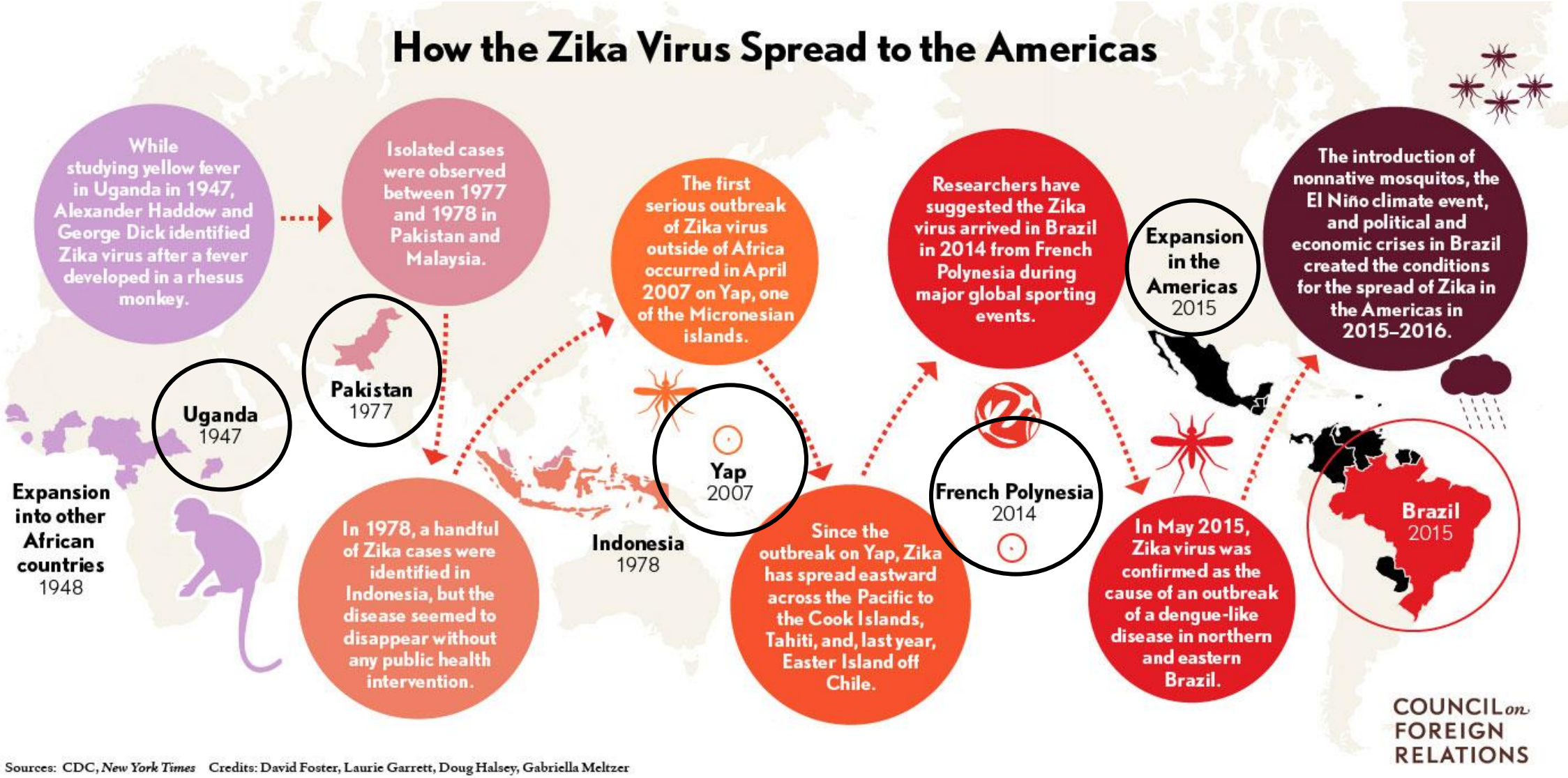
Who is Zika

How it is transmitted



Which organs it targets

Where and when we met Zika...



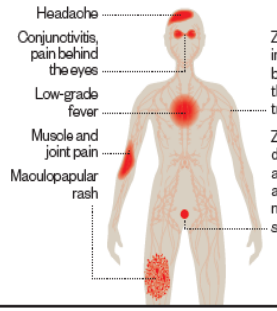
FEATURE

ORIGINS OF THE ZIKA OUTBREAK AND ITS POTENTIAL THREAT

Researchers are working to understand more about the Zika virus – how it is transmitted, its link to microcephaly and Guillain-Barŕe syndrome, and whether a vaccine can be developed. By Dawn Connelly.

TRANSMISSION, SYMPTOMS AND DIAGNOSIS

The main route of transmission for Zika virus is through the bite of an infected mosquito, although other routes have been reported. Symptoms develop 2–10 days after infection and are usually mild, lasting up to a week. There is no specific treatment.



Zika virus is present in blood and may be transmitted through blood transfusions.

Zika virus has been detected in semen at least two months after infection and may be transmitted sexually.

SEXUAL TRANSMISSION

Public health England advises men returning from Zika transmission zones who have experienced symptoms to use a condom for six months, or one month if no symptoms.

DIAGNOSIS

Acute infection is identified through polymerase chain reaction in specialist labs. Serological tests may indicate anti-Zika antibodies but can cross react with other flaviviruses. Treat with rest, fluids and paracetamol. Do not give aspirin in case dengue is present.

Public Health England has advised women returning from Zika transmission areas to avoid becoming pregnant for 28 days.

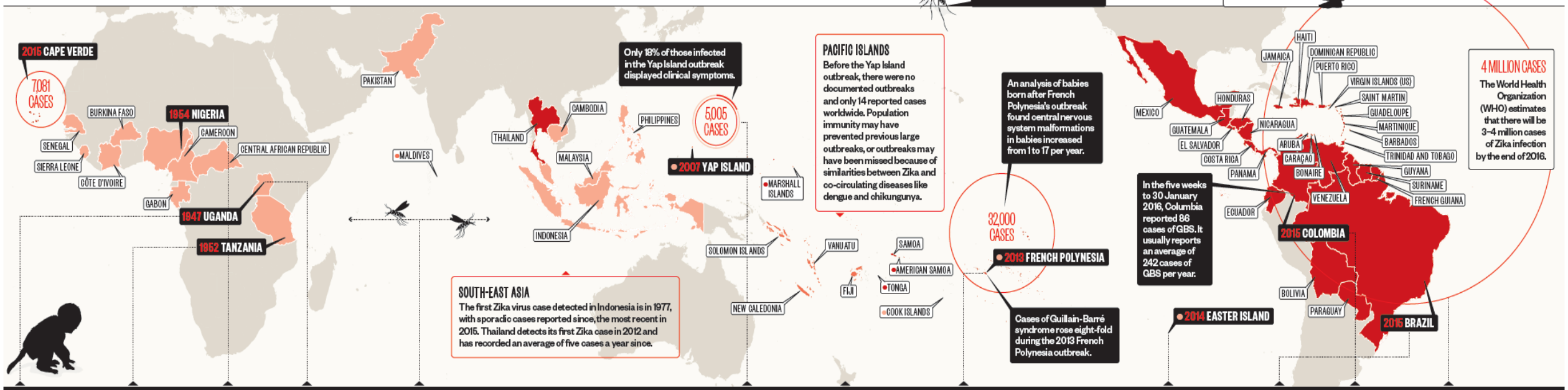
Zika virus can be transmitted from mother to child during pregnancy.

EXPLOSION OF CASES

Zika virus is a flavivirus related to yellow fever, dengue, West Nile and Japanese encephalitis. It is mainly transmitted by mosquitoes and was first identified in Africa over 60 years ago.

Zika transmission as of 22 February 2016
- Reported in the past two months
- Reported in the past nine months
- Cases or transmission before 2015

The *A. aegypti* mosquito can only fly 400 metres in its lifetime, so people, rather than mosquitoes, spread the disease.

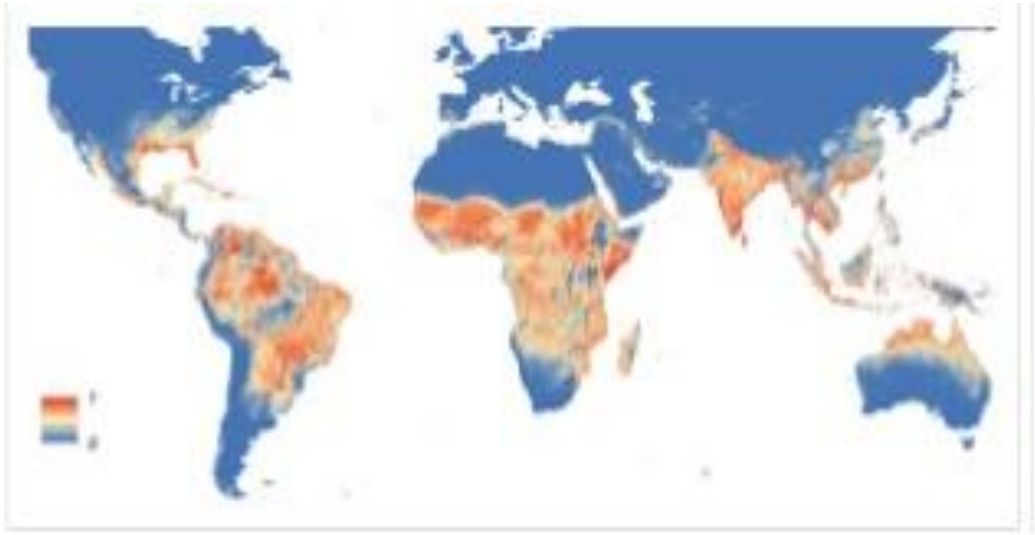


1947	1952	1954	1964	1960s–1980s	2007	2012	2013–2014	2014	2015	2015	2016
The Zika virus is first identified in Zika Forest in Uganda in 1947 in a captive, sentinel rhesus monkey.	Zika virus antibodies are subsequently detected in humans in Uganda and Tanzania.	Zika virus is isolated from a young girl in Nigeria in 1954.	A researcher infected with Zika provides the first proof that Zika causes human infection.	Zika virus is detected in mosquitoes and rhesus monkeys across equatorial Africa and Asia. Sporadic cases are reported in humans but these are rare and mild.	The first large outbreak of Zika virus disease is in Yap Island, Micronesia, where it is estimated to have affected 5,005 people, which represents 73% of residents age three years and older.	Two distinct lineages of Zika virus are identified — African and Asian.	The next major outbreak happens in French Polynesia in 2013–2014, affecting an estimated 32,000 people. Outbreaks are also reported in the Cook Islands and New Caledonia.	The virus then spreads to Oceania, arriving in The Americas at Easter Island by 2014.	**May:** Brazil reports local transmission of Zika virus.	**October:** Columbia and Cape Verde report local transmission of Zika.	**February:** As evidence of a link between Zika and birth defects mounts, the WHO declares a public health emergency.

Zika vectors

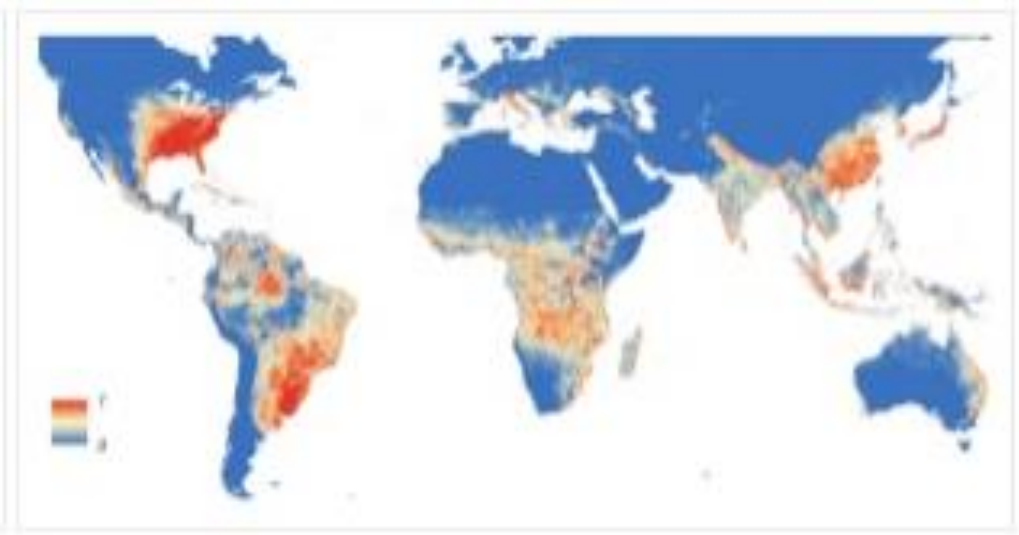
Aedes aegypti

Originated in sub-Saharan Africa, spread throughout the tropics centuries ago



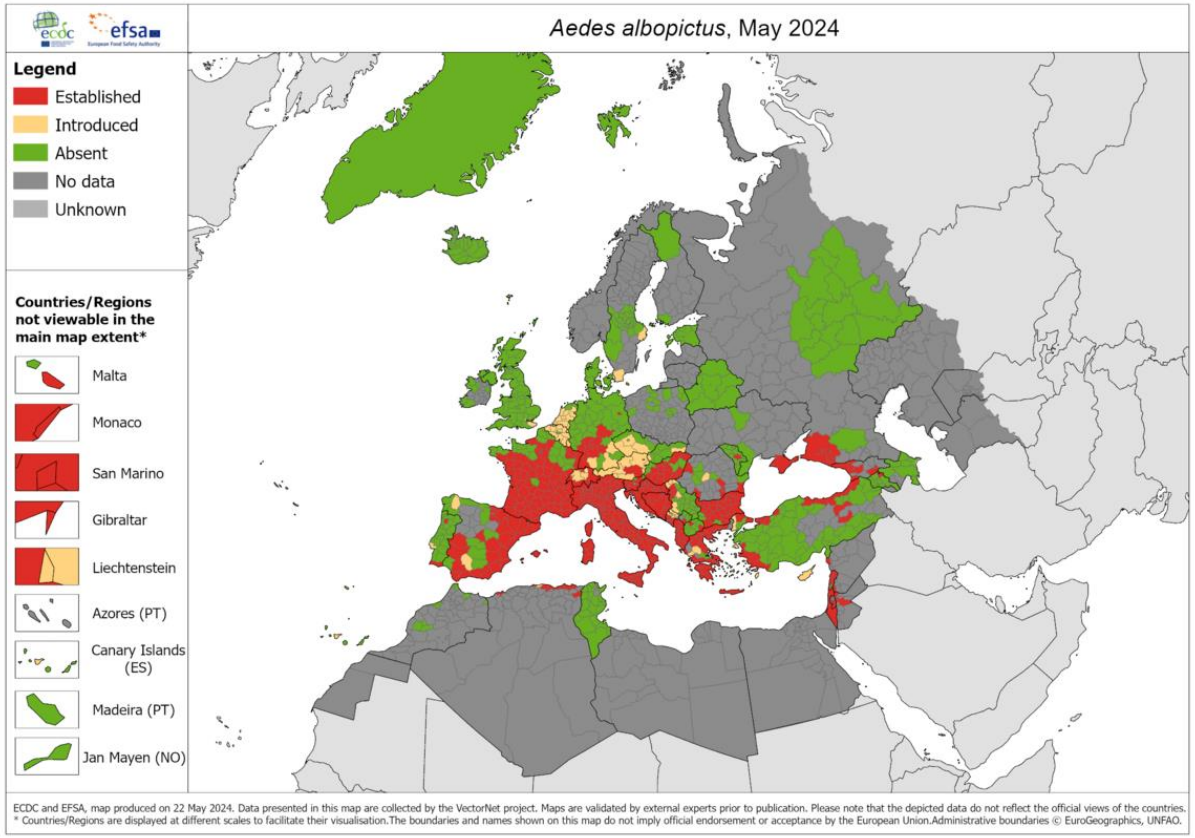
Aedes albopictus

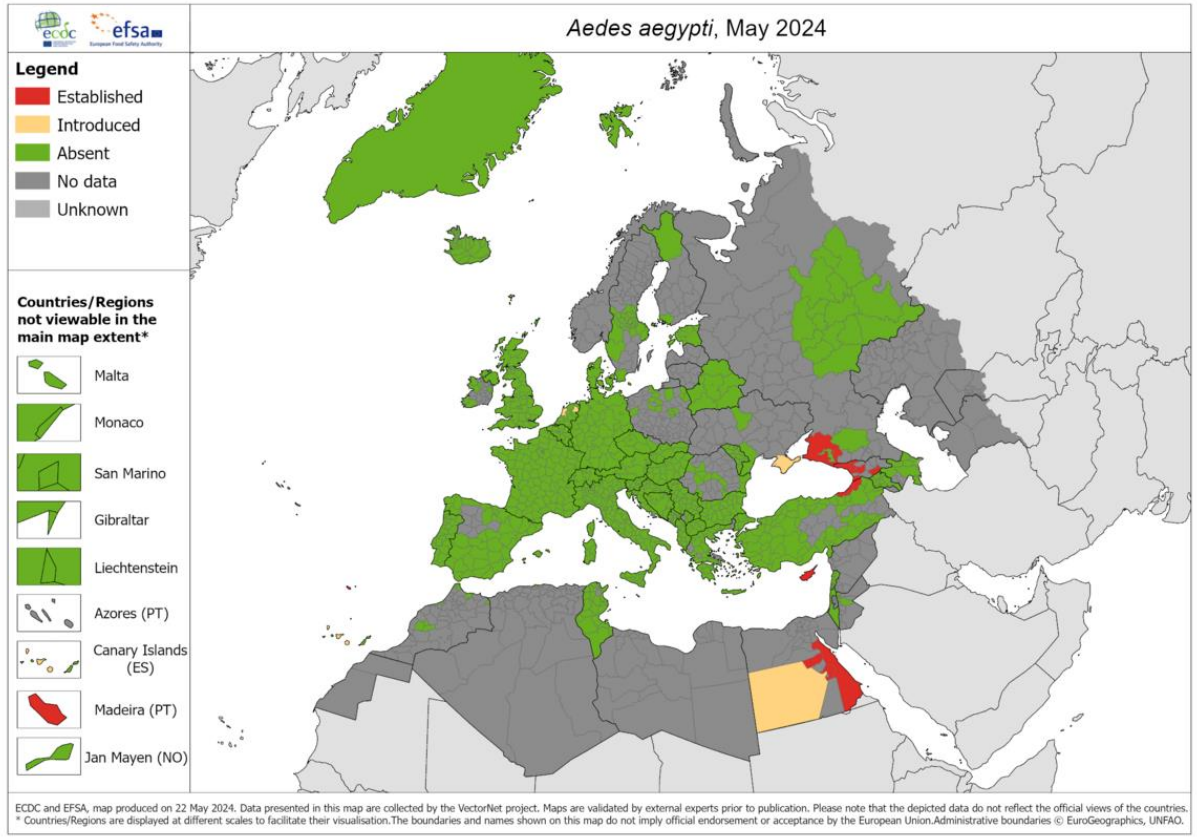
Originated in Asia, spread to the Americas, Africa and Europe beginning in 1985



Ae. albopictus was susceptible to ZIKV infection (**infection rate**: **10%**), and the virus could disseminate and was secreted in the mosquito's saliva (**dissemination rate: 29%; transmission rate: 29%)** after an extrinsic incubation period of 11 days. **The observed vector competence was lower than that of an Ae. aegypti tested in parallel. (experimental infection!)**

Aedes in Europe:





Since the previous update (October 2023), there were changes in 12 regions.

Since the previous update (October 2023), the mosquito has been recorded as newly introduced into Gran Canaria (Spain).

World Health Organization

COUNTRIES AND TERRITORIES WITH CURRENT OR PREVIOUS ZIKA VIRUS TRANSMISSION

DATA AS OF MAY 2024

Zika virus and Aedes aegypti distribution

Countries and territories with current or previous Zika virus transmission,[1] by WHO regional office

WHO Regional Office	Country / territory	Total
AFRO	Angola; Burkina Faso; Burundi; Cabo Verde; Cameroon; Central African Republic; Côte d'Ivoire; Ethiopia; Gabon; Guinea; Guinea-Bissau; Kenya; Mali; Nigeria; Senegal; Uganda	16
AMRO/PAHO	Anguilla; Antigua and Barbuda; Argentina; Aruba; Bahamas; Barbados; Belize; Bolivia (Plurinational State of); Bonaire, Sint Eustatius and Saba; Brazil; British Virgin Islands; Cayman Islands; Colombia; Costa Rica; Cuba; Curaçao; Dominica; Dominican Republic; Ecuador; El Salvador; French Guiana; Grenada; Guadeloupe; Guatemala; Guyana; Haiti; Honduras; Easter Island– Chile; Jamaica; Martinique; Mexico; Montserrat; Nicaragua; Panama; Paraguay; Peru; Puerto Rico; Saint Barthélemy; Saint Kitts and Nevis; Saint Lucia; Saint Martin; Saint Vincent and the Grenadines; Saint Maarten; Suriname; Trinidad and Tobago; Turks and Caicos; United States of America; United States Virgin Islands; Venezuela (Bolivarian Repub	49
SEARO	Bangladesh; India; Indonesia; Maldives; Myanmar; Sri Lanka; Thail	
WPRO	American Samoa; Cambodia; Cook Islands; Fiji; French Polynesia; Democratic Republic; Marshall Islands; Malaysia; Micronesia (Fede New Caledonia; Palau; Papua New Guinea; Philippines; Samoa; Si Solomon Islands; Tonga; Vanuatu; Viet Nam	
EURO	France (Var department)	
Total		

Countries and territories with established *Aedes aegypti* mosquito vectors, but no known cases of Zika virus transmission, by WHO regional office

WHO Regional Office	Country / territory	Total
AFRO	Benin; Botswana; Chad; Comoros; Congo; Democratic Republic of the Congo; Equatorial Guinea; Eritrea; Gambia; Ghana; Liberia; Madagascar; Malawi; Mauritania; Mauritius; Mayotte; Mozambique; Namibia; Niger; Réunion; Rwanda; Sao Tome and Principe; Seychelles; Sierra Leone; South Africa; South Sudan; Togo; United Republic of Tanzania; Zambia; Zimbabwe	30
AMRO/PAHO	Uruguay	1
EMRO	Afghanistan; Djibouti; Egypt; Oman; Pakistan; Saudi Arabia; Somalia; Sudan; Yemen	9
EURO	Cyprus; Georgia; Região Autónoma da Madeira – Portugal; Russian Federation; Turkey	5
SEARO	Bhutan; Nepal; Timor-Leste	3
WPRO	Australia; Brunei Darussalam; China; Christmas Island; Guam; Kiribati; Nauru; Niue; Northern Mariana Islands (Commonwealth of the); Tokelau; Tuvalu; Wallis and Futuna	12
		60

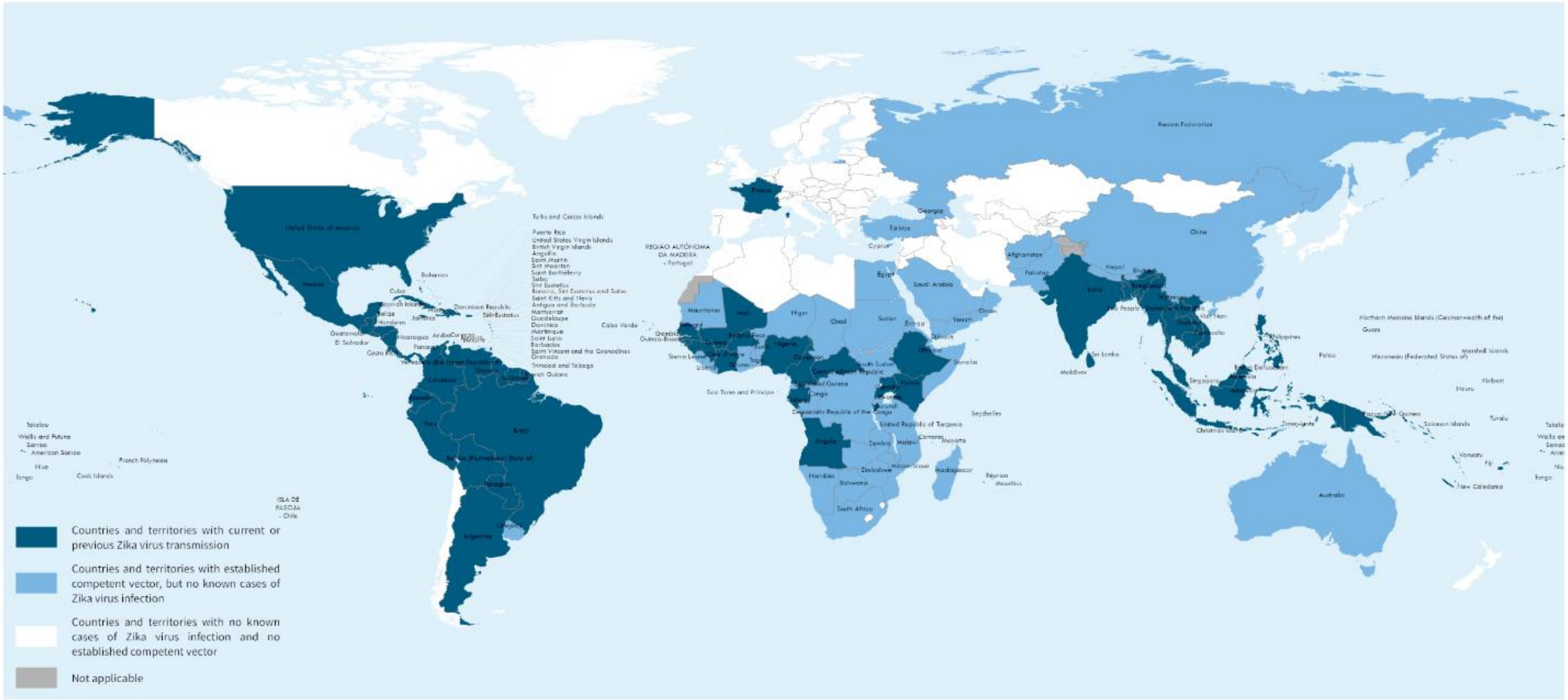
RAPID COMMUNICATION

Vector-borne transmission of Zika virus in Europe, southern France, August 2019

Sandra Giron[1], Florian Franke[1], Anne Decoppet[2], Bernard Cadiou[3], Thierry Travaglini[3], Laurence Thirion[4], Guillaume Durand[4,5], Charles Jeannin[3], Grégory L'Ambert[3], Gilda Grard[4,5], Harold Noël[6], Nelly Fournet[6], Michelle Auzet-Caillaud[2], Christine Zandotti[5], Samer Aboukaïs[2], Pascal Chaud[1], Saby Guedj[7], Lakri Hamouda[7], Xavier Naudot[8], Anne Ovize[8], Clément Lazarus[9], Henriette de Valk[6], Marie-Claire Paty[6], Isabelle Leparc-Goffart[4,5]

Countries and territories with current or previous Zika virus transmission

(as of 27/05/2024)



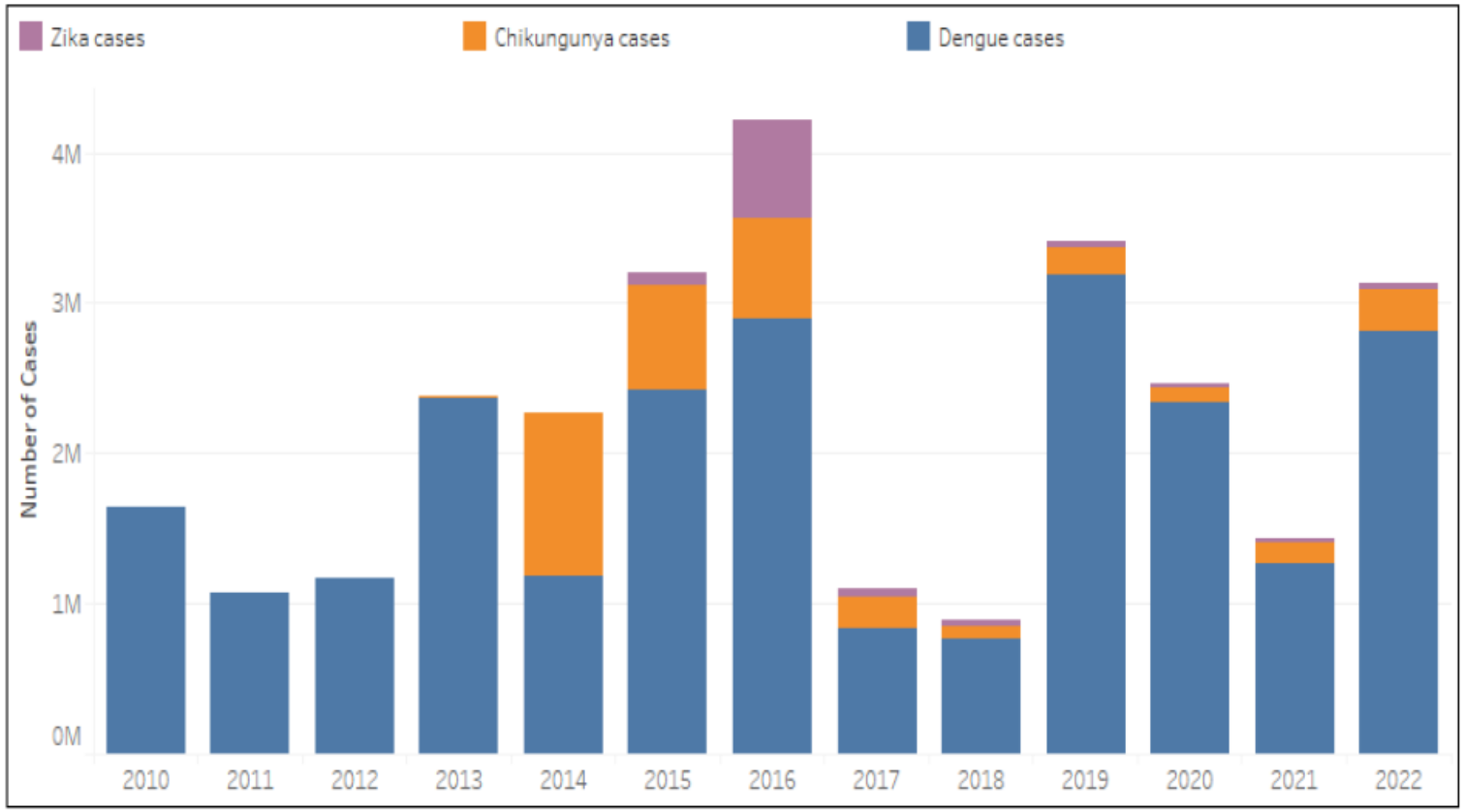
The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: WHO Health Emergencies Programme
Map Date: 29 May 2024

World Health Organization

The thirteen-year pattern of circulation of these arboviruses is depicted in (Figure 1)., which shows that the introduction of chikungunya in December of 2013 is clearly observed in 2014. Similarly, following the introduction of Zika in 2015, there was widespread circulation of the virus in 2015. Nevertheless, dengue circulation has continued to predominate.

Figure 1. *Distribution of reported cases of dengue, chikungunya, and Zika by year. Region of The Americas. 2010-2022*



Source: *Data entered into the Health Information Platform for The Americas (PLISA, PAHO / WHO) by the Ministries and Institutes of Health of the countries and territories of the Region. Available at: https://www.paho.org/plisa*

2022

DENGUE
2,815,920 cases
283.85 cases x 100,000 Pop.
4,610 severe dengue (0.2%)
1,290 deaths
0.046% case fatality rate (CFR)
Nicaragua is he country with the highest cumulative incidence

CHIKUNGUNYA
273,841 cases
28.67 cases x 100,000 Pop.
87 deaths
0.032 % case fatality rate (CFR)
Belize is he country with the highest cumulative incidence

ZIKA
40,528 cases
4.24 cases x 100,000 Pop.
2 death
0.005 % case fatality rate (CFR)
Belize is he country with the highest cumulative incidence

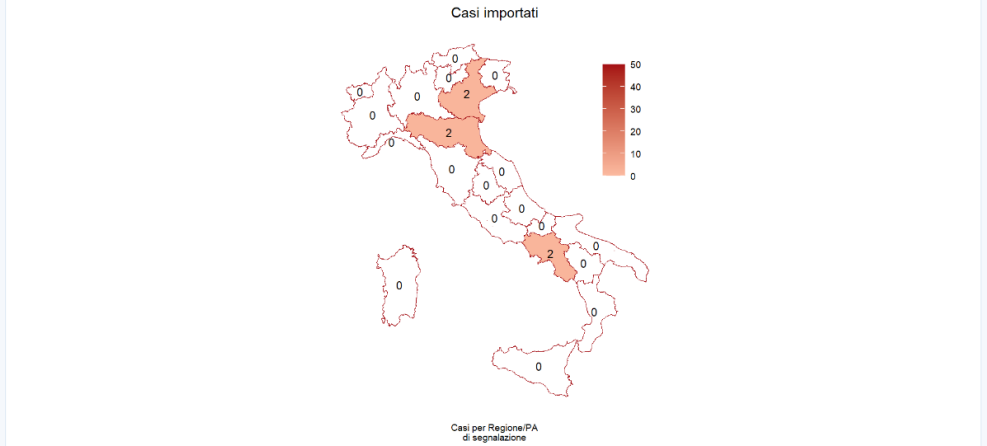
Zika virus in Italy

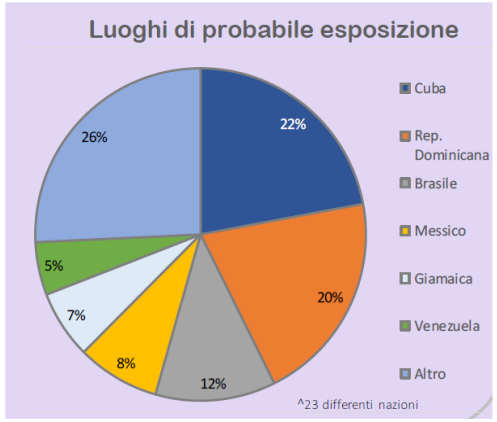
Oltre 140 casi importati, soprattutto nel 2016
Il solo caso definito come autoctono dovuto a trasmissione verticale.

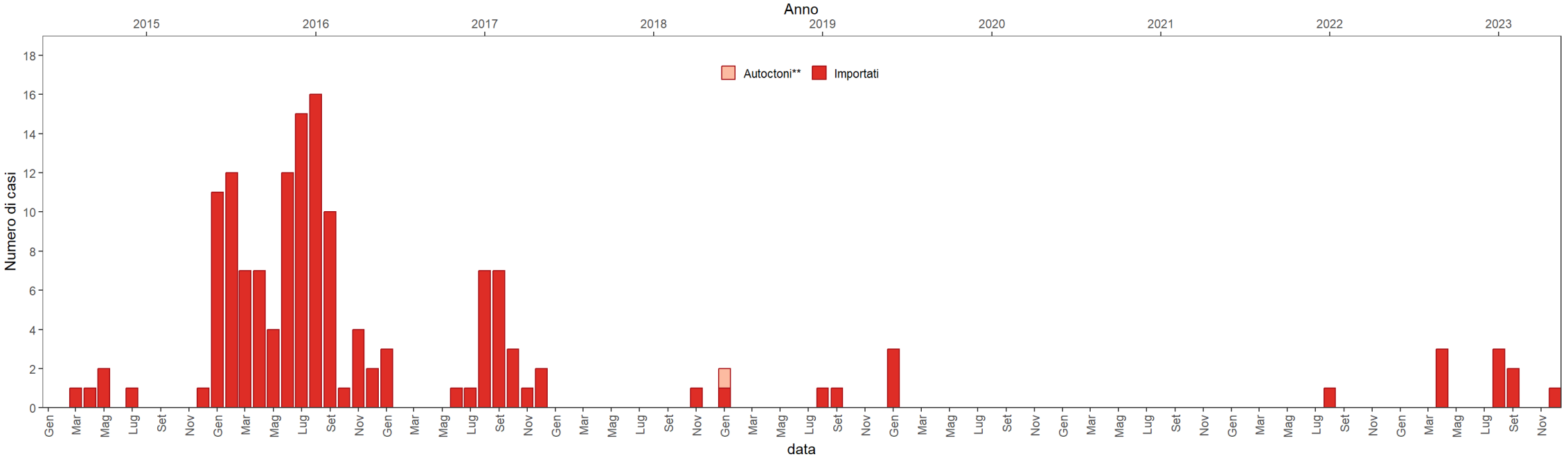
6	50% | 50%	49 anni	0	0 casi | 6 casi
Casi*	Maschi | Femmine*	Età mediana*	Decessi*	Autoctoni | Importati*

*Dati in fase di consolidamento

Casi per Regione/PA*







**Trasmissione materno-fetale*

Symptoms and treatment

The incubation period is around 3-14 days

Incubation period

infection → symptoms

- few days to a week

These symptoms are mild and usually last for 2-7 days. (4 out of 5 infected « asymptomatic »)

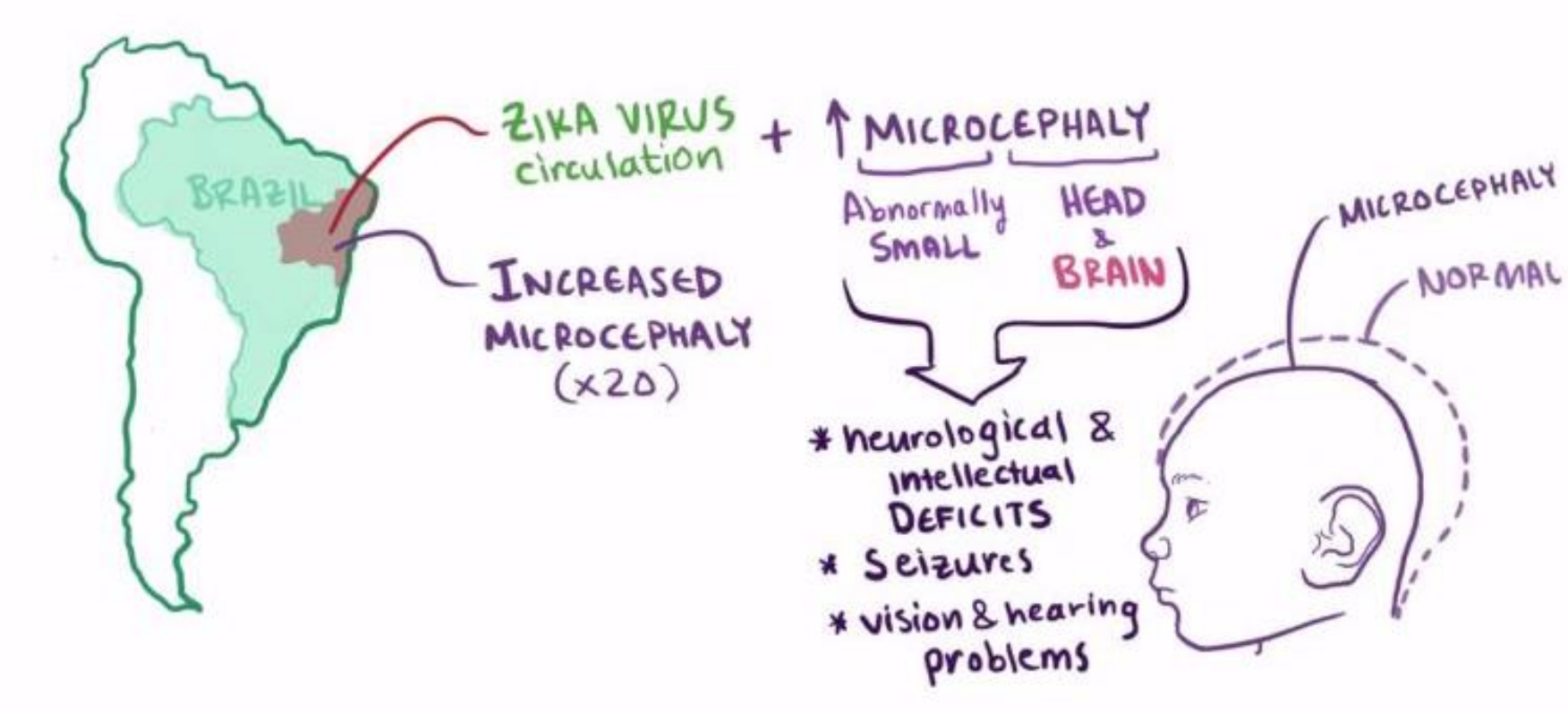
TREATMENT

* plenty of rest
* fluids
* Medicine (Acetaminophen)
  ↳ reduce pain + fever

There is no specific treatment or vaccine currently available.

Association with neurological disorders:

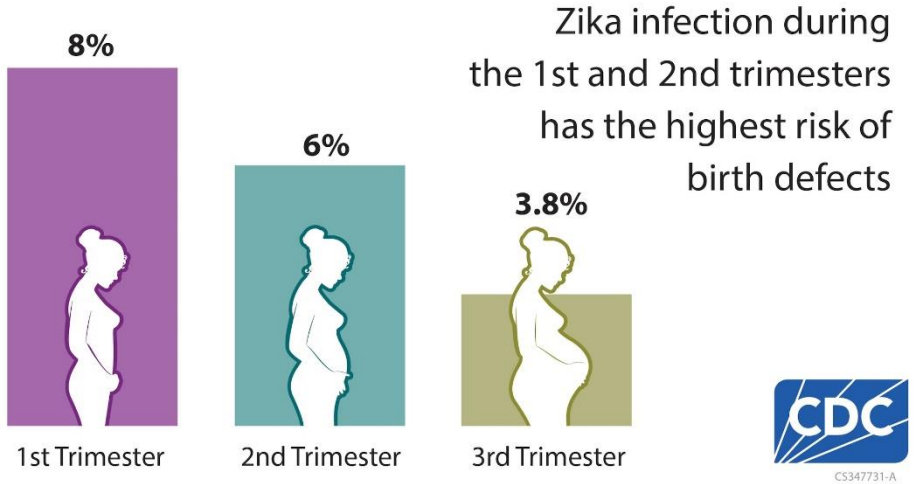
- **Guillain-Barré syndrome:** a rise in the incidence first reported in 2013 outbreak in French Polynesia
- **Microcephaly:** in Brazil, French Polynesia and other Latin american countries



Congenital zika syndrome (CZS)

Zika virus infection during pregnancy can cause birth defects of the brain or eye. These birth defects can occur alone or with developmental problems in a particular pattern called congenital Zika syndrome. The following conditions can occur in a baby with congenital Zika virus infection:

- Smaller than expected head size, called microcephaly
- Problems with brain development
- Feeding problems, such as difficulty swallowing
- Hearing loss and vision problems
- Seizures
- Decreased joint movement, called contractures
- Stiff muscles, making it difficult to move



Not all babies born with congenital Zika syndrome will have all of these conditions. Some infants who do not have microcephaly at birth may develop it later. In addition, some babies might look healthy at birth but can develop long-term health problems as they grow.

Laboratory testing for Zika virus and dengue virus infections

Interim guidance

14 July 2022

World Health Organization

Sample: Blood (Saliva, Urine, and Semen)

RT-PCR: It is useful in the first week after the onset of symptoms (maybe longer in whole blood specimens)

Serology Test: to detect antibodies, useful usually after five days (beware of cross reactivity with other Flavivirus such as Dengue or Yellow fever).

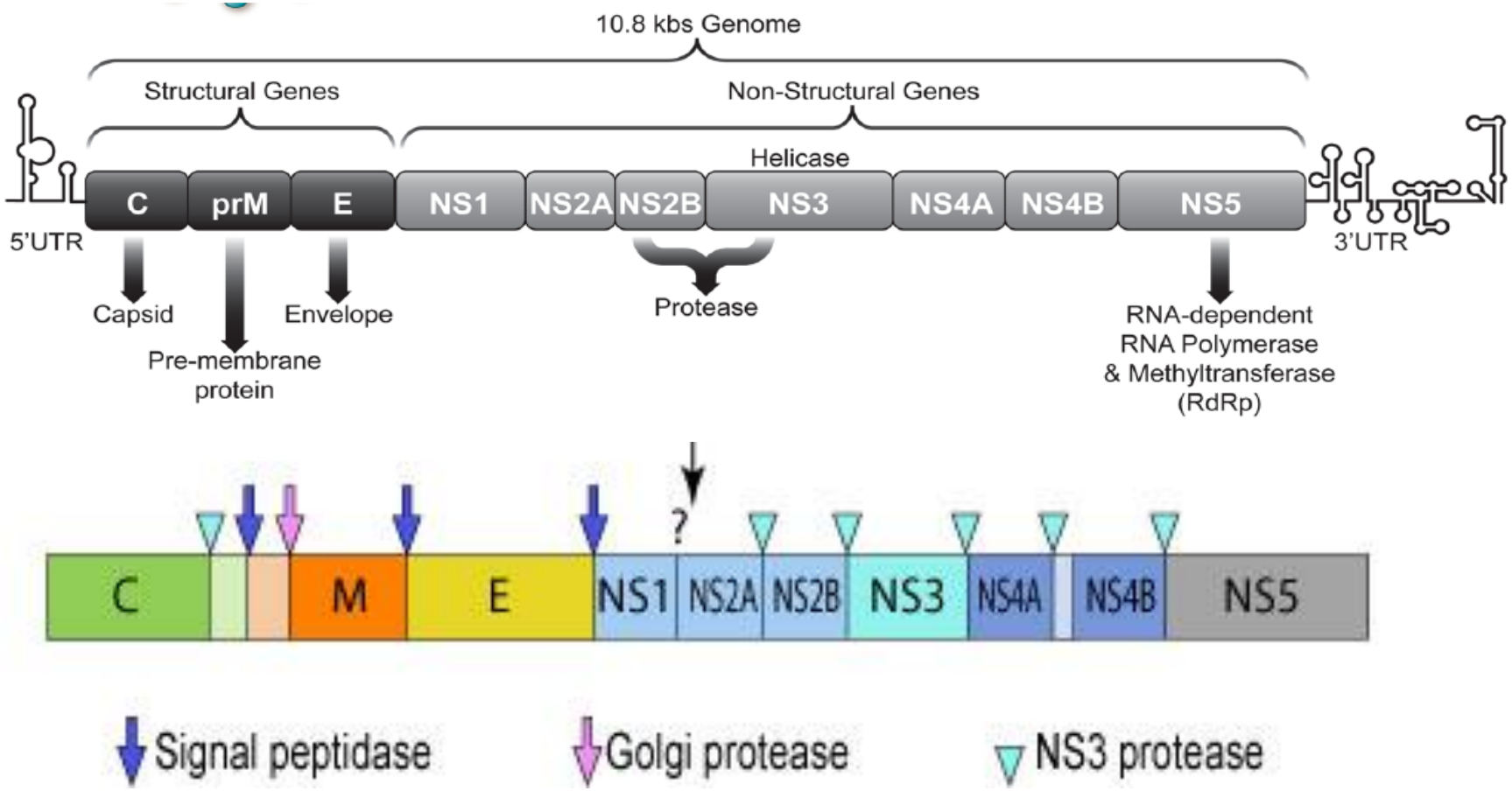
Differential diagnosis:

Yellow Fever, Dengue, Chikungunya, Oropouche, Malaria, Richettsiosi, Leptospirosis, Parvovirus B19, Ross River virus, Enteroviruses, COVID-19…

	Zika (ZIKV)
Criteri clinici	Una persona che presenta esantema cutaneo, con o senza febbre **e** almeno uno dei seguenti segni o sintomi: • artralgia, • mialgia, • congiuntivite non purulenta/iperemia.
Criteri di laboratorio[1]	Test di laboratorio per caso probabile: • rilevamento di anticorpi IgM specifici per ZIKV nel siero Test di laboratorio per caso confermato (almeno uno dei seguenti): • identificazione dell'acido nucleico di ZIKV da un campione clinico; • identificazione dell'antigene del ZIKV in un campione clinico; • isolamento del ZIKV da un campione clinico; • identificazione di anticorpi IgM specifici verso il ZIKV in 1 o più campioni di siero e conferma mediante test di neutralizzazione; • sieroconversione o aumento di quattro volte del titolo di anticorpi specifici per ZIKV in due campioni successivi di siero e conferma mediante test di neutralizzazione.
Criteri epidemiologici	- Anamnesi riportante un'esposizione in un'area con trasmissione di ZIKV nelle due settimane precedenti l'insorgenza dei sintomi, o - Contatti sessuali con un caso confermato di infezione da ZIKV nei 3 mesi (uomo), o 2 mesi (donna) precedenti - Contatti sessuali con una persona che abbia soggiornato in un'area con trasmissione da ZIKV nei 3 mesi (uomo) o 2 mesi (donna) precedenti
Classificazione	
Classificazione – Caso possibile	Persona che soddisfa il criterio clinico ed epidemiologico.
Classificazione - Caso probabile	Qualsiasi persona che soddisfi sia i criteri di caso possibile che i criteri di laboratorio per caso probabile.
Classificazione - Caso confermato	Qualsiasi persona che soddisfi i criteri di laboratorio per caso confermato.

Zika Virus structure and life cycle

Envelope, 50 nm, ssRNA+, 10genes, 11 kb



The ZIKV genome consists of 10,794 nucleotides in a single-stranded positive-sense RNA that encodes a polyprotein of 3,424 amino acids and 10 proteins crucial for the viral life cycle. ZIKV RNA has two untranslated regions (UTRs) and a single open reading frame (ORF).

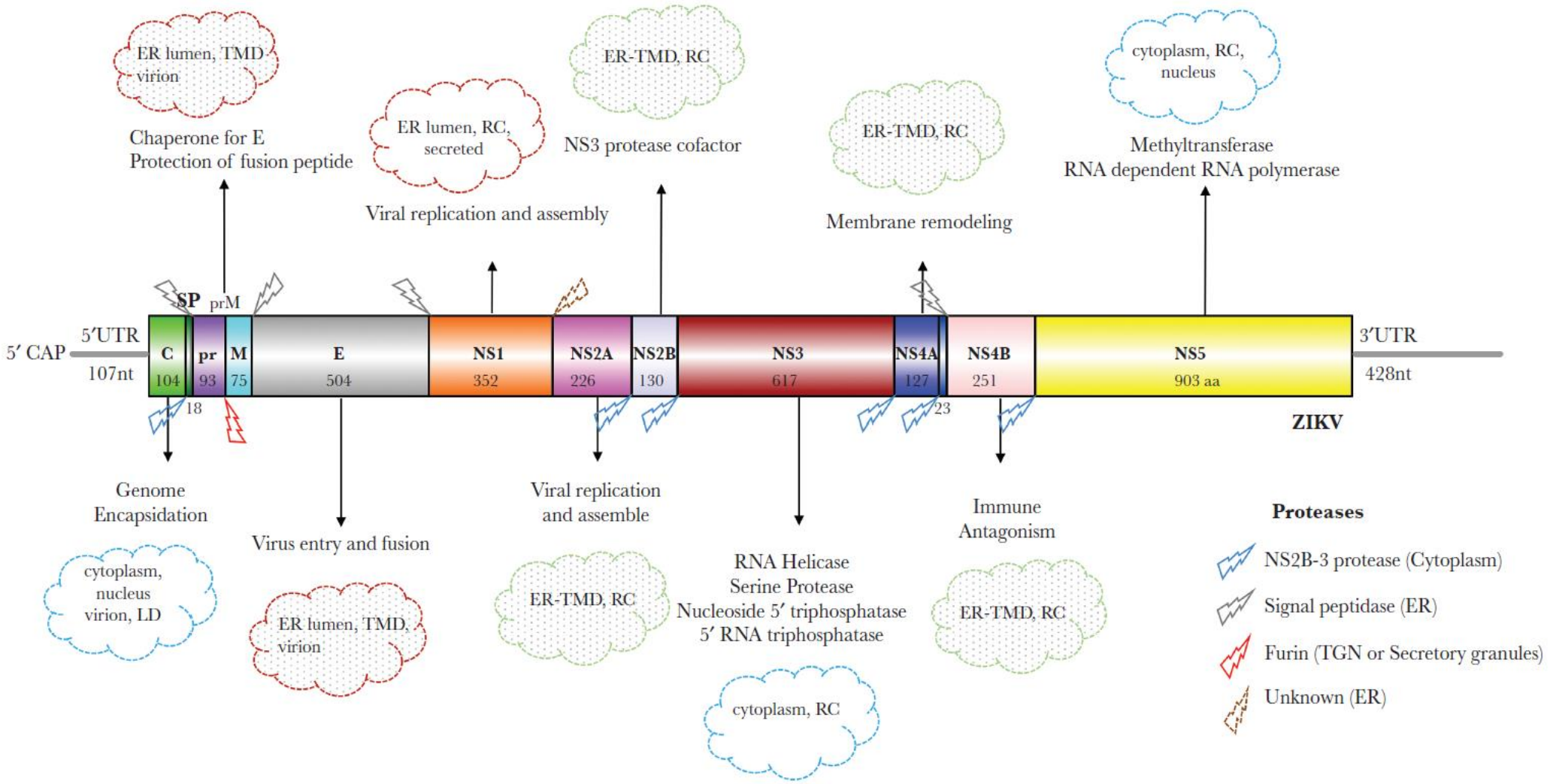
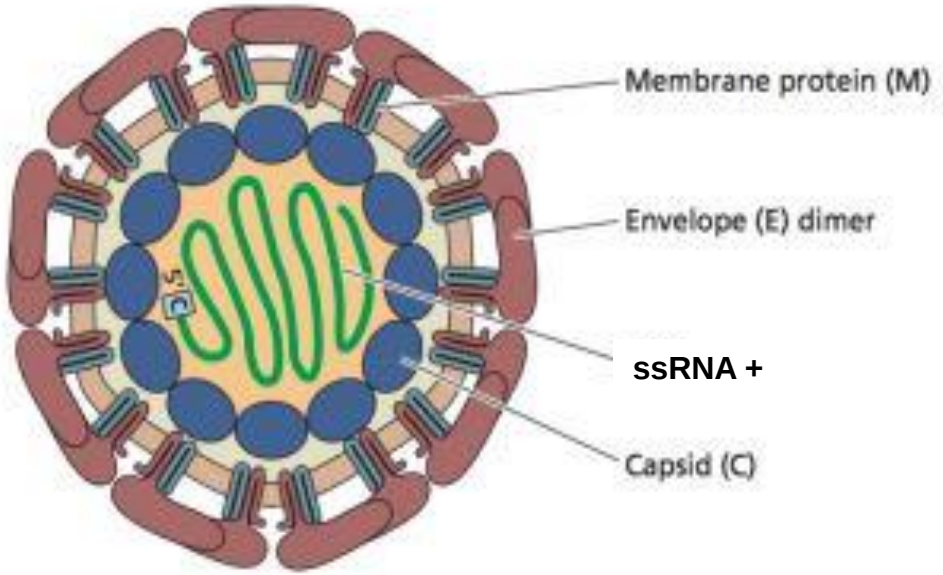


Figure 1. Architecture of Zika virus (ZIKV) genomic ribonucleic acid (RNA), translation, and cleavage of ZIKV polyprotein, function, and localization of ZIKV proteins. The ZIKV genomic RNA is capped but it lacks a poly A tail. The gray lines represent 5'- and 3'-untranslated regions (UTR). The viral RNA codes for a polyprotein that is cotranslationally cleaved to yield 10 proteins. The 3 structural proteins (C, prM/M, and E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) are displayed with the length of each protein (number of amino acids). The cleavage profile of the polyprotein, the proteases involved, as well as the role and the subcellular location of individual proteins are provided in the figure. Information shown in the figure is true for flaviviruses in general, but many aspects need to be specifically verified for ZIKV. The figure does not provide an exhaustive list of the functions of each protein. The viral proteins interact with several host proteins, which expands their functional repertoire. aa, amino acid; ER, endoplasmic reticulum; LD, lipid droplet; nt, nucleotide; RC, replication complex; SP, signal peptide; TGN, Trans-Golgi Network; TMD, transmembrane domain.

Zika Virus structure and life cycle

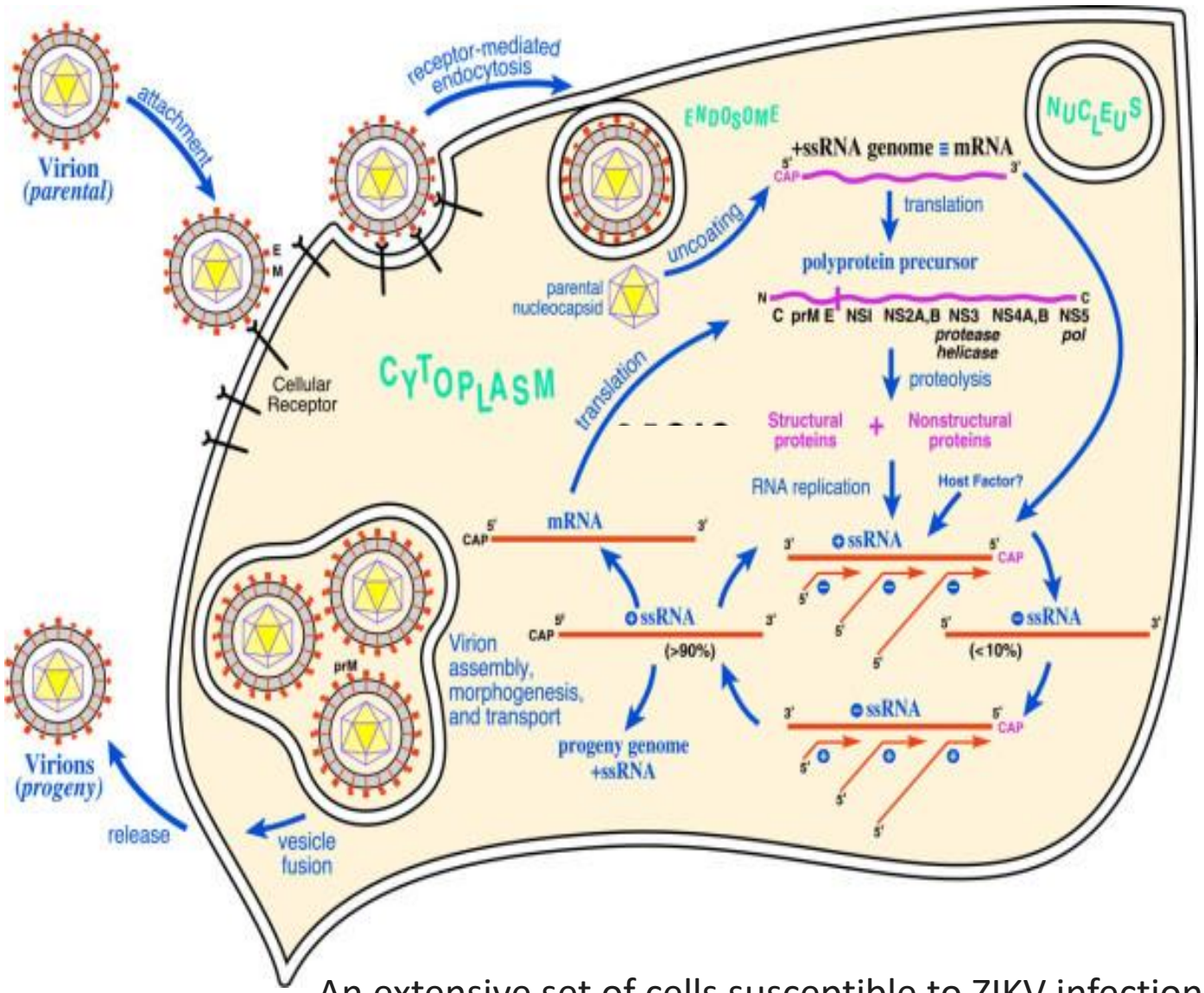
Envelope, 50 nm, ssRNA+, 10genes, 11 kb



Putative receptors: DC-SIGN, AXL, Tyro-3

Viral envelope protein E intermediates membrane fusion by receptor-mediated endocytosis

As the capsid breaks apart in the cytoplasm, there is a release of the viral RNA. The positive-sense RNA genome is translated by the host ribosomes attached to the ER (viral factories), which results in a polyprotein processed by proteolytic cleavage.



An extensive set of cells susceptible to ZIKV infection

Zika Virus structure and life cycle

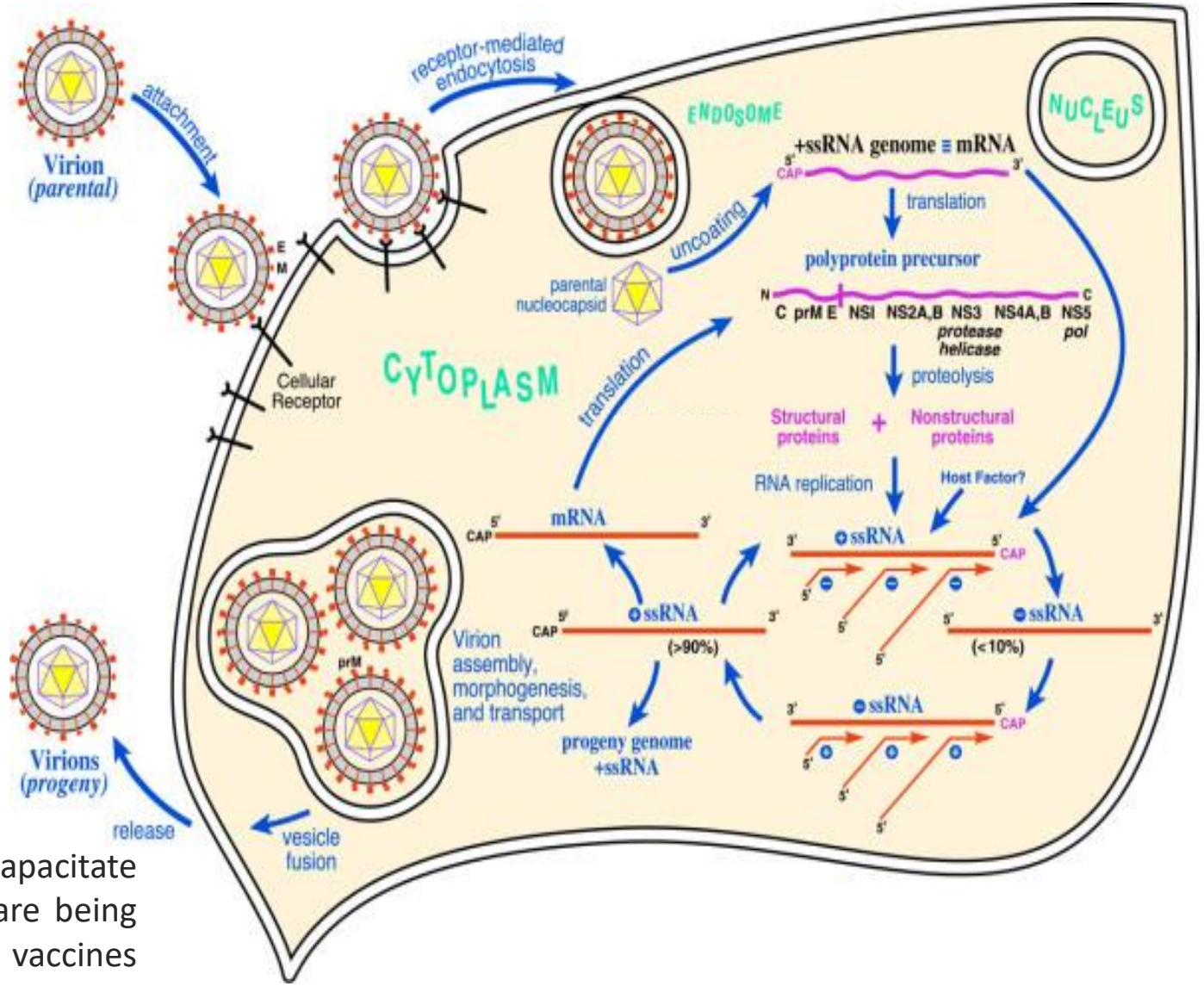
ZIKV virion assembly involves:

(i) prM interaction with E protein in the endoplasmic reticulum;

(ii) encapsulation of the RNA genome with C protein and coverage with a lipid bilayer containing a prM-E protein complex to form immature virions;

(iii) cleavage of prM protein into M protein by furin or furin-like protease in the *trans*-Golgi network before release of mature virions

Prior to virion release from the cell, cleavage of prM to M protein results in release of the *pr* peptide, allowing rearrangement of E proteins into homodimers and facilitating virion maturation



Inhibiting the function of prM during the viral lifecycle may incapacitate ZIKV infectivity and pathogenicity. ZIKV prM and E proteins are being used as antibody-activating epitopes in most of the ZIKV vaccines currently undergoing clinical trials

Zika Virus genotypes

The first Zika phylogenetic study was conducted after the epidemics in Yap Island 2007 (Lanciotti et al. 2008)

Phylogenetic analyses of ZIKV genetic diversity identified two major ZIKV lineages referred to as the African lineage and the Asian lineage, respectively.
Strikingly, all ZIKV strains responsible for human large outbreaks to date belong to the Asian lineage

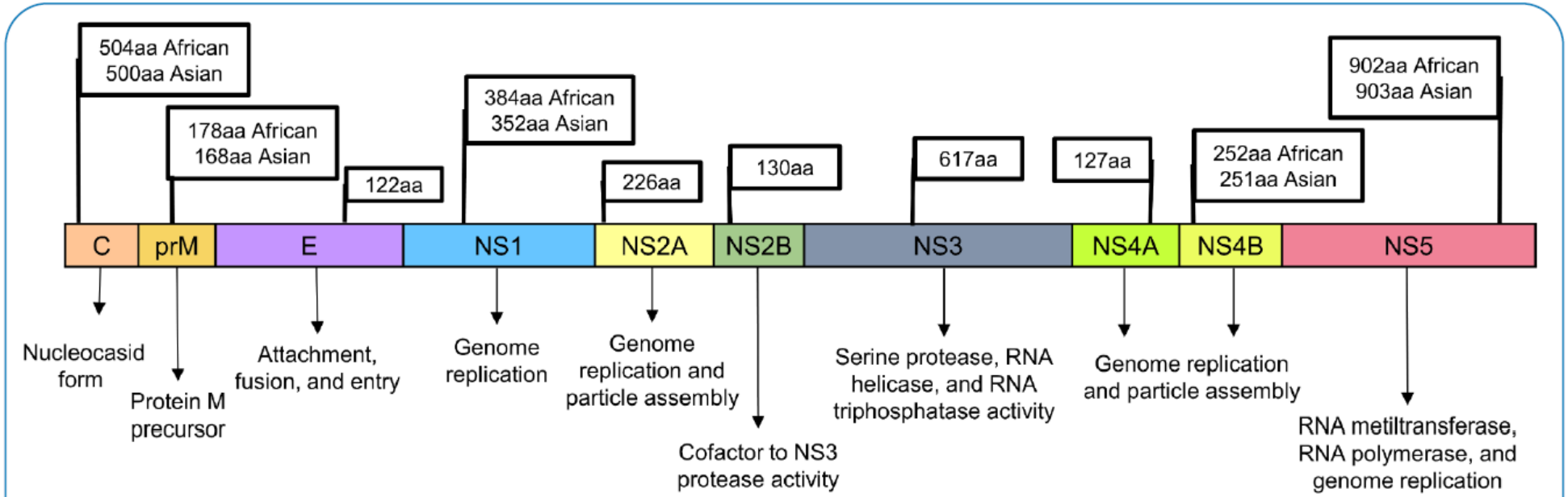
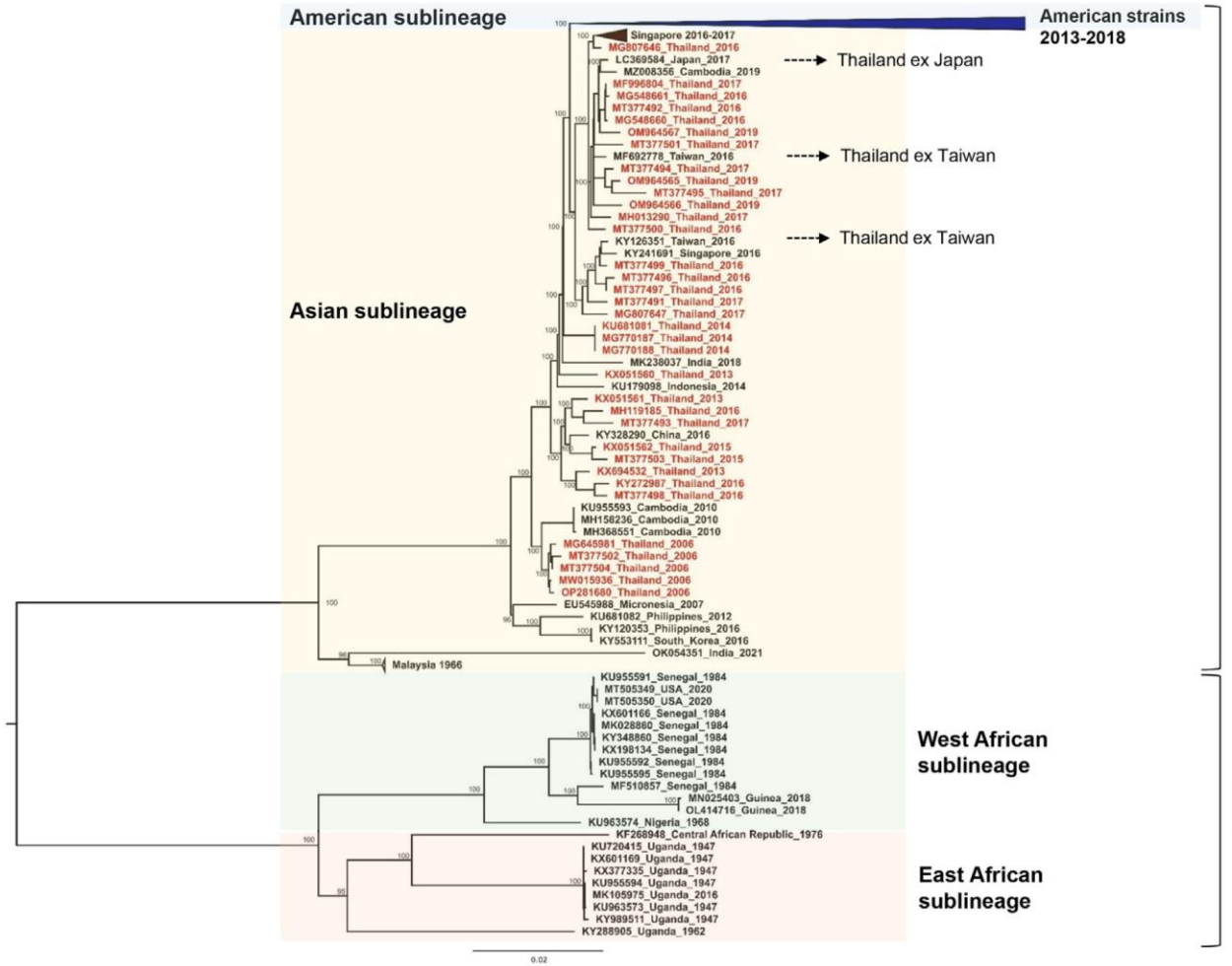
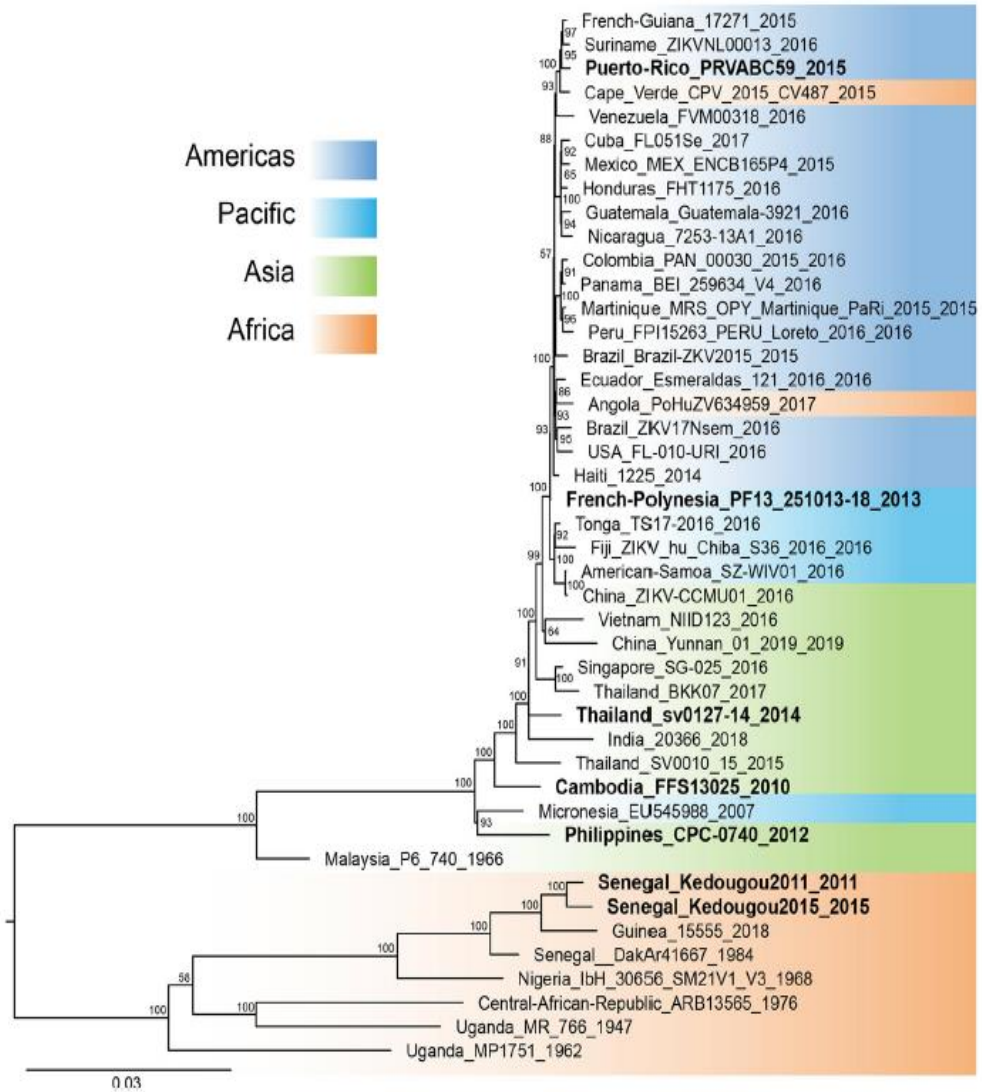


FIGURE 1: ZIKV polyprotein from the African- and Asian-ZIKV genotypes, structural proteins (C, prM, and E) and nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) as well as their sizes, in amino acids (aa), and functions in the viral cycle.

Zika Virus genotypes





Zika virus likely originated in Africa and spread East, circulating within Africa prior to 1950; Asia by 1966; Pacific islands by 2007; and the Americas by 2015.Over this period, the epidemiological properties of the virus appeared to change dramatically from modest, likely endemic circulation with mild symptoms (subject to reporting bias), towards epidemic cycles infecting up to 50% of affected communities with major neurological complications.

Asian zika strains had two independent disseminations from Southeast Asia into the Pacific region: the first in Yap Island and the second in French Polynesia
Later, all of the Asian lineage strains isolated from American countries (e.g. Colombia, Brazil, Puerto Rico, Guatemala) showed >99%nucleotide identity, temporal and phylogenetic closeness with the French Polynesia strains (Lanciotti et al 2016)

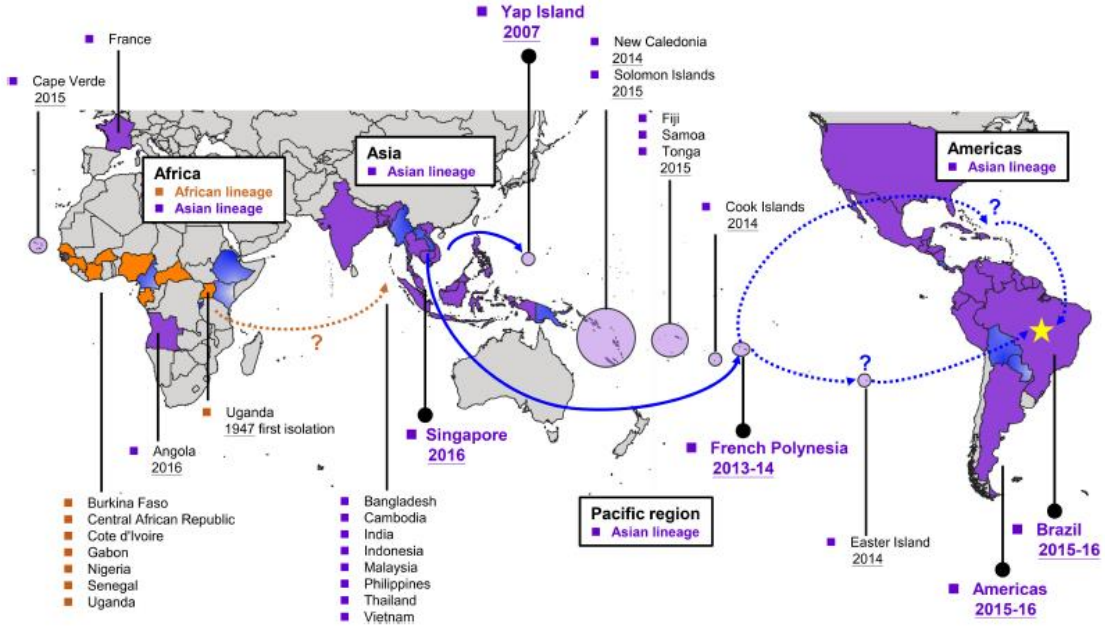


FIGURE 1 | Global dissemination of Zika virus (ZIKV). ZIKV originated from Africa and was then introduced to Asia. Thereafter, ZIKV continuously evolved in Asia and began to cause outbreaks in the Pacific region from 2007. The 2013–2014 French Polynesia strain became the common ancestor worldwide and resulted in recent ZIKV outbreaks in the Americas. According to phylogenetic analysis, the African lineage viruses were circulated mainly in African countries, whereas those circulated in Asia, the Pacific regions, and the Americas were Asian lineage viruses. Countries or areas that have locally acquired ZIKV cases are colored with orange (African lineage circulation), purple (Asian lineage circulation), or gradient blue (the circulating lineage has not been confirmed).

French Polynesian strains were suggested to simoultaneously disseminate into Fiji, Samoa, Tonga, to the Easter Island, Cook Ilands, New Caledonia, and the Americas

In the Americas thousands of CZS reported...

The accumulated mutations contribute to neuropathogenicity and increased transmissibility observed?

Studies on the phenotypic differences between African and Asian strains gave counterintuitive results:

In vitro: historical African strains exhibit higher growth rates and induce higher rates of cellular apoptosis than the Asian strains
in vivo models: historical African strains have higher virulence than the Asian strains in mice
Mosquitoes are more susceptible to the African strains than the Asian strains

The Asian strains, responsible for the ZIKV outbreaks in the Pacific and the Americas, were less virulent with lower vector competence than the African strains.

That is also true when analysing recent african strains

Recent African strains of Zika virus display higher transmissibility and fetal pathogenicity than Asian strains

Fabien Aubry[1,16], Sofie Jacobs[2,16], Maïlis Darmuzey[3,16], Sebastian Lequime[4,5], Leen Delang[2], Albin Fontaine[6,7,8], Natapong Jupatanakul[1,9], Elliott F. Miot[1], Stéphanie Dabo[1], Caroline Manet[10], Xavier Montagutelli[10], Artem Baidaliuk[1,11], Fabiana Gámbaro[11], Etienne Simon-Lorière[11], Maxime Gilsoul[3], Claudia M. Romero-Vivas[12], Van-Mai Cao-Lormeau[13], Richard G. Jarman[14], Cheikh T. Diagne[15], Oumar Faye[15], Ousmane Faye[15], Amadou A. Sall[15], Johan Neyts[2], Laurent Nguyen[3], Suzanne J. F. Kaptein[2]✉ & Louis Lambrechts[1]✉

Here, we experimentally compare seven low-passage ZIKV strains representing the recently circulating viral genetic diversity. We find that recent African ZIKV strains display higher transmissibility in mosquitoes and higher lethality in both adult and fetal mice than their Asian counterparts. We emphasize the high epidemic potential of African ZIKV strains and suggest that they could more easily go unnoticed by public health surveillance systems than Asian strains due to their propensity to cause fetal loss rather than birth defects.

More recent studies have focused on phenotypic comparison of the ancestral (Cambodia 2010) strain and different contemporary Asian strains isolated after 2010 showing that the contemporary strain exhibits higher neurovirulence, causing microcephaly in the mouse model, enhancing the viral infectivity in mosquitoes, and possessing superior fitness than the ancestral strain in vivo

TABLE 1 | Potential factors contributing to the recent emergence of Zika virus (ZIKV).

Potential factors		Description	References
Pathogen			
Mutation	prM-S139N	prM-S139N mutant causes a more severe microcephalic phenotype with a thinner cortex, more robust brain cell apoptosis, and more NPC differentiation disruption in mice	Yuan et al., 2017
Mutation	NS1-A982V	NS1-A982V mutation enhances ZIKV transmission in a mosquito-mouse-mosquito transmission cycle	Liu et al., 2017
		NS1-A982V mutation of ZIKV enhances the inhibition of interferon-beta production	Xia et al., 2018
Mutation	E-V763M	E-V763M mutation increases ZIKV replication, neurovirulence in neonatal mice, and maternal-to-fetal transmission	Shan et al., 2020
Mutation	C-T106A, prM-V123A, NS1-A982V, and NS5-M3392V	ZIKV with C-T106A, prM-V123A, NS1-A982V, and NS5-M3392V mutations has a fitness advantage	Liu et al., 2021
Host			
Genetics	Host genome background	The pathogenesis of discordant and dizygotic twins from ZIKV-infected mothers was compared, and host genetics was found to substantially affect the severity of a ZIKV infection, even when infected with the same strain	Caires-Junior et al., 2018
Immunity	Preexisting anti-flavivirus immunity	Previous DENV immunity had no or cross-protection impact against ZIKV infection	Gordon et al., 2019; Rodriguez-Barraquer et al., 2019; Carvalho et al., 2020
Environment			
Temperature	Climate change	Elevated temperatures can expand the geographic vector range, decrease the extrinsic incubation period of the pathogen, and increase the female mosquito biting rate	Morin et al., 2013; Paz and Semenza, 2016

prM-S139N, S139N mutation in the pre-membrane (prM) coding region; NS1-A982V, A982V mutation in the non-structural protein 1 (NS1) coding region; E-V763M, V763M mutation in the envelope (E) coding region; C-T106A, T106A mutation in the capsid (C) coding region; prM-V123A, V123A mutation in the pre-membrane (prM) coding region; NS5-M3392V, M3392V mutation in the non-structural protein 5 (NS5) coding region; DENV, Dengue virus; ZIKV, Zika virus.

Review

EMBO reports

Molecular adaptations during viral epidemics

Nash D Rochman*, Yuri I Wolf & Eugene V Koonin**

nature communications

Article

https://doi.org/10.1038/s41467-023-42676-7

A single amino acid substitution in the capsid protein of Zika virus contributes to a neurovirulent phenotype

Received: 20 February 2023

Accepted: 17 October 2023

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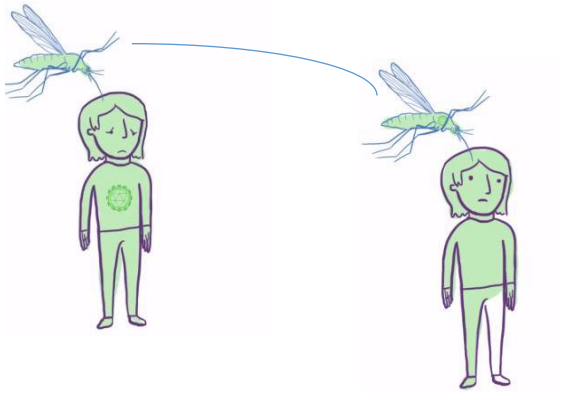
Check for updates

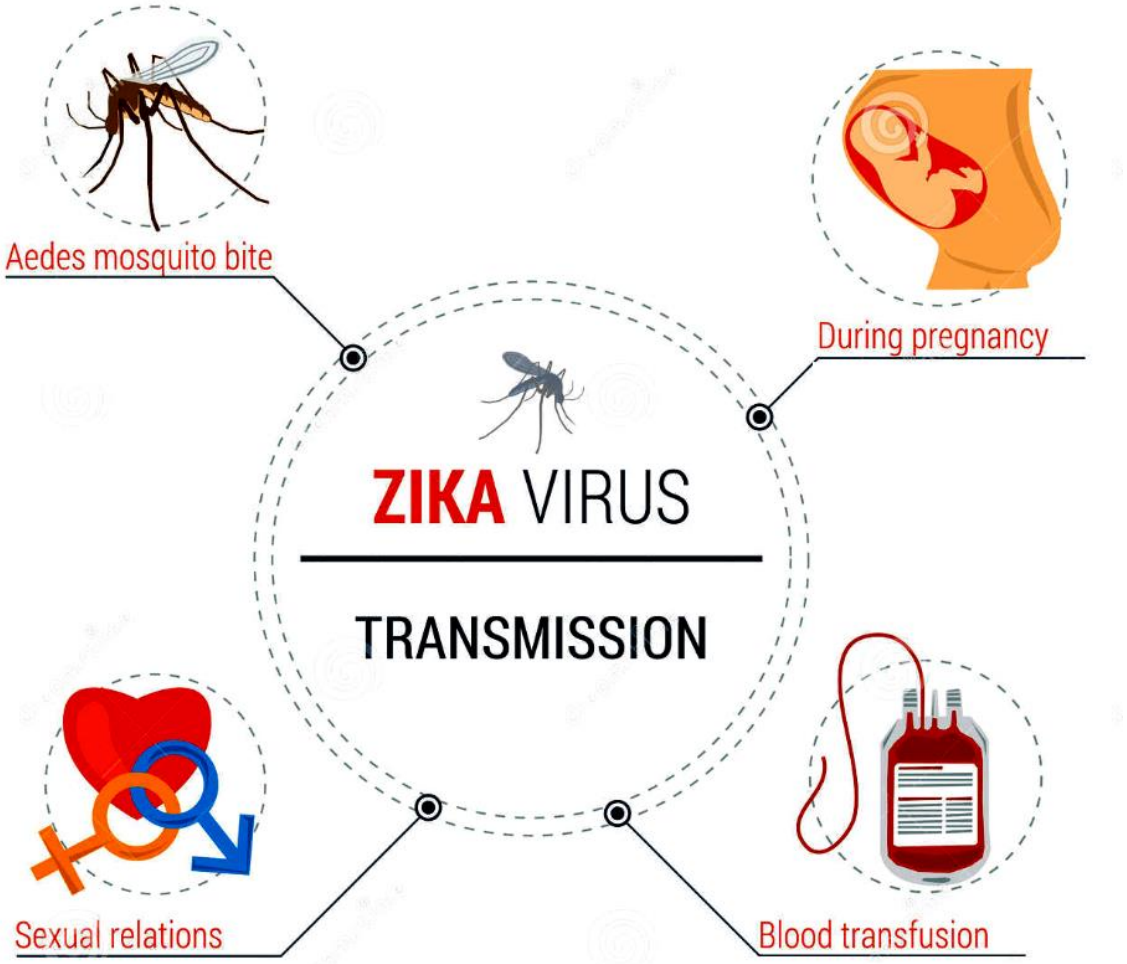
Guang-Yuan Song[1,2,6], Xing-Yao Huang[2,6], Meng-Jiao He[2,6], Hang-Yu Zhou[3,6], Rui-Ting Li[2], Ying Tian[1,2], Yan Wang[4], Meng-Li Cheng[2], Xiang Chen[2], Rong-Rong Zhang[2], Chao Zhou[2], Jia Zhou[2], Xian-Yang Fang[5], Xiao-Feng Li[2] ✉ & Cheng-Feng Qin[1,2] ✉

Increasing evidence shows the African lineage Zika virus (ZIKV) displays a more severe neurovirulence compared to the Asian ZIKV. However, viral determinants and the underlying mechanisms of enhanced virulence phenotype remain largely unknown. Herein, we identify a panel of amino acid substitutions that are unique to the African lineage of ZIKVs compared to the Asian lineage by phylogenetic analysis and sequence alignment. We then utilize

Zika Virus Transmission

Mosquito-borne





Materno-foetal

Zika RNA detected in amniotic fluid, antigen and RNA detected in placentas and brain tissues of newborns and from miscarriages

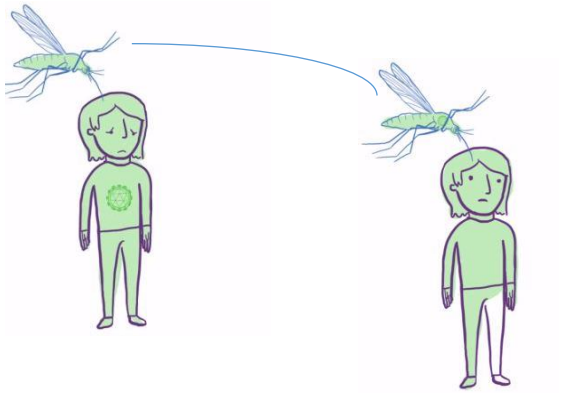
Sexual

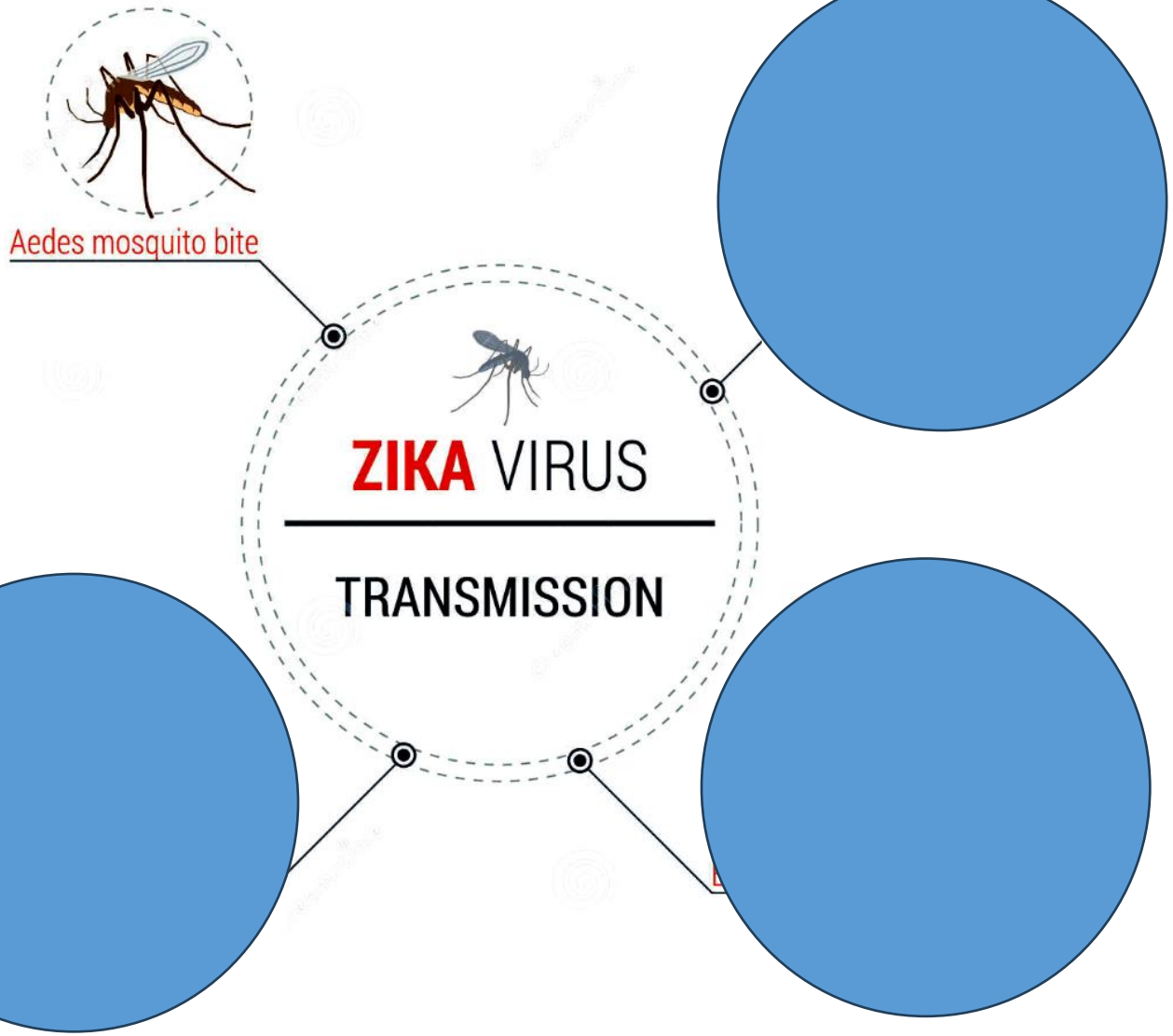
Male to male
Male to female
(Zika virus is present in Semen and vaginal fluids)

Transfusion Transmitted infection

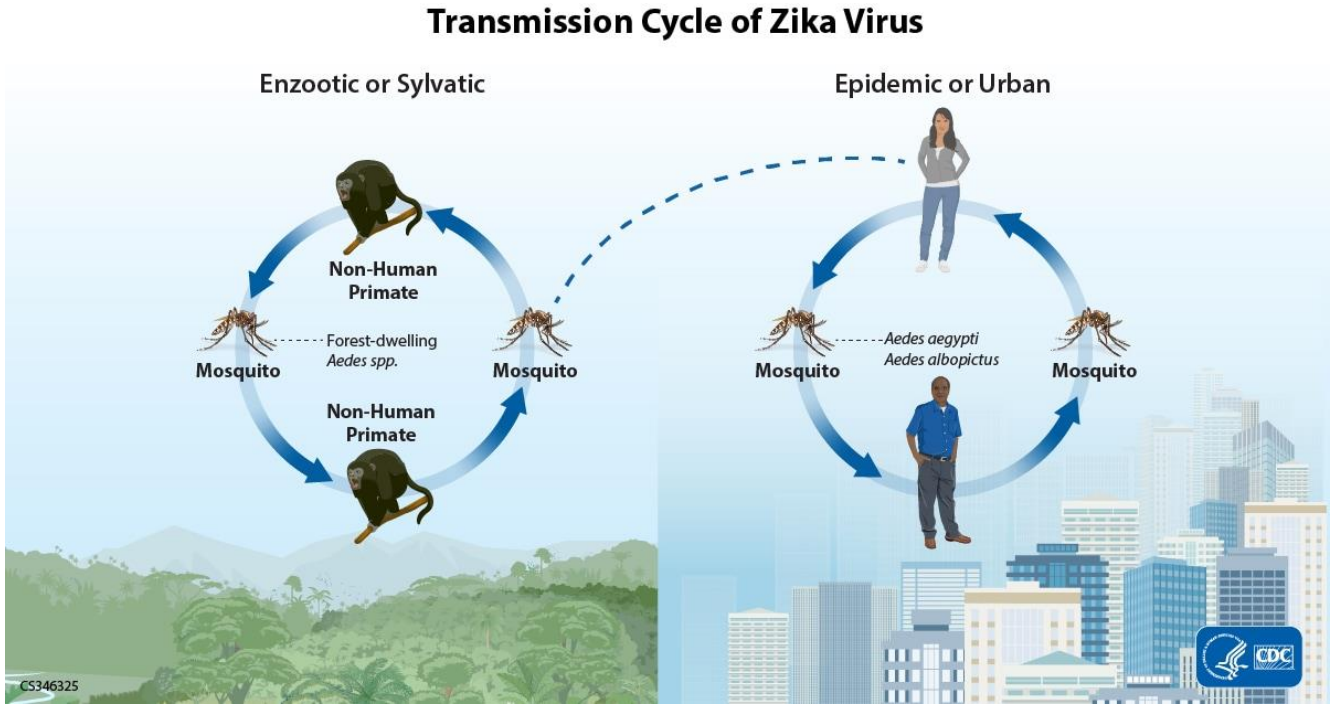
Zika Virus Transmission

Mosquito-borne

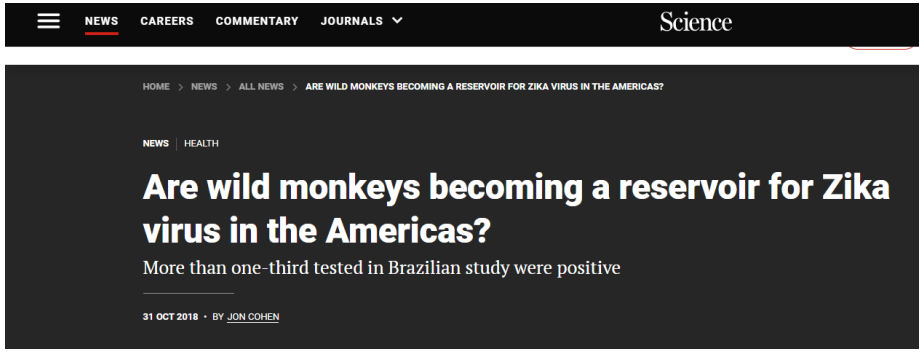




Zika virus cycles



First detection of zika virus in a sentinel monkey in the Zika forest in Uganda.



A Brazilian study found Zika virus in many wild marmoset monkeys, which could become part of a threatening transmission cycle for humans.

The virus has been isolated in monkeys, and antibodies have been detected in domestic sheep, goats, horses, cows, ducks, rodents, bats, orangutans, and carabaos.

Animal reservoir

Year of sampling	Location	Mosquito genus and species	Study/assay
Africa			
1948	Zika Forest, Uganda	*Aedes africanus*	Mosquito catches in Zika Forest and first isolation of ZIKV from *Aedes africanus* pooled specimens
1958	Zika Forest, Uganda	*Aedes africanus*	Virus isolation
1964	Zika Forest, Uganda	*Aedes africanus*	Virus isolation
1969	Uganda, Bwamba county, Zika Forest	*Aedes africanus, Aedes apicorgenteus*	Virus isolation from pc mosquitoes trapped in
1976–1980	Central African Republic	*Aedes africanus, Aedes opok*	Retrospective entomol and sequencing
1968–2002	West Africa: Côte d'Ivoire, Senegal, Burkina Faso, Central Africa Republic	*Aedes dalzieli, Aedes africanus, Aedes aegypti, Aedes furcifer, Aedes grahamii, Aedes luteocephalus, Aedes vittatus, Aedes opock*	Retrospective study; pl reverse transcription P(numerous recombinati
1962–2008	Senegal	*Aedes aegypti, Aedes dalzieli, Aedes fowleri, Aedes furcifer* (known as *Aedes taylori*), *Aedes luteocephalus, Aedes vittatus, Aedes neoafricanus, Aedes metallicus, Aedes minutus, Anopheles africanus, Anopheles coustani, coustani, Anopheles gambiae* s.l., *Mansonia uniformis* (the higher number of ZIKV isolation events was detected in *Aedes furcifer* (known as *Aedes taylori*), *Aedes luteocephalus*, and *Aedes dalzieli*)	Virus isolation in the n (*Aedes pseudoscutellaris* Identification of isolate with virus-specific imn confirmed by complem neutralization tests
2011	Southeastern Senegal	*Aedes africanus, Aedes hirsutus, Aedes metallicus, Aedes unilineatus*, and *Culex perfuscus* had the highest infection rates compared to *Aedes (Diceronyia) furcifer, Aedes (Fredwardsius) vittatus, Aedes taylori, Aedes luteocephalus, Aedes dalzieli, Aedes aegypti, Mansonia uniformis*, and *Anopheles coustani*, with the lower infection rates	Virus isolation, RT-PCR
2007	Gabon	*Aedes albopictus*	Retrospective sero-epi entomological study in sequencing of pooled s
Asia			
1969	Malaysia	*Aedes aegypti*	Virus isolation
NA; experiment in 2012	Singapore	(Local in Singapore) *Aedes aegypti*	Inoculation of Ugandar Singapore) *Aedes aegyp* mosquito-borne transn
NA; experiment in 2014	Yap Island, Federated States of Micronesia; human outbreak in 2007	Field collected *Aedes henselli* and *Culex quinquefasciatus* tested negative for ZIKV; *Aedes henselli* laboratory infection	Experiment; laboratory

ZIKV, Zika virus; NA, not applicable.

Year of sampling	Location	Animal	Assay virus/antibody	Number of animals tested	Positive animals (%)
1947	Zika Forest, Uganda	Rhesus monkeys	Intracerebral inoculation into mice and subsequent virus isolation	One rhesus diagnosed with the virus; two others underwent successful experimental inoculation	
1967–1968 1962 and 1964	Senegal 1967–1968 Ethiopia 1962 and 1964	Wild mammals, monkeys	Antibody detection with HI	Not reported	Wild mammals 2.4%, monkeys 25%
1969	Uganda, Kisubi, Bwamba county, Zika Forest	Bwamba county monkeys[a]	Antibody detection with HI test	Kisubi red-tail 21 Bwamba red-tail 52 Bwamba others 16	4/21 23/52 7/16
1978	Lombok, Indonesia; human outbreak in 1977	Ducks, goats, cows, horses, bats, rats, carabaos (water buffalo)	HI antibodies (HIA) and detection of antibodies (DA)	HIA: 15 horses 41 cows 13 carabaos 35 goats 78 chickens 52 ducks 71 bats 25 rats 17 wild birds DA: 6 horses, 8 cows, 1 carabao, 9 goats, 1 chicken, 2 ducks, 1 bat	HIA: 3/15 (20%) 4/41 (10%) 1/13 (8%) 7/35 (20%) 0/78 (0) 2/52 (4%) 6/71 (8%) 0/25 (0) 0/17 (0) DA: All tested negative
1982	Gabon	Monkeys	Antibody detection with HI/complement fixation test		
1983	Pakistan	Rodents, domestic sheep and goats (at slaughter), humans	Antibody detection	157 rodents 45 cows 33 buffaloes 46 sheep 48 goats	6/157(3.8%) 0 0 1(2.57%) 1(2.083%)
1996–1997	Malaysia, Borneo	Wild orangutans, semi-captive orangutans	Antibodies	40 wild orangutans, 31 semi-captive orangutans	5/40 (13%) wild, 1/31 (3%) semi-captive
1968–2002	West Africa: Côte d'Ivoire and Senegal, Burkina Faso, Central Africa Republic; retrospective study of viral isolates	Monkeys	RT-PCR, nucleotide sequencing, recombination detection	NA	
1962–2008	Senegal; retrospective study of viral isolates	Monkey (*Erythrocebus patas*), *Chlorocebus aethiops* (also named grivet and African green monkey)	Virus isolation in the mosquito cell line AP61 (*Aedes pseudoscutellaris*); identification of isolates by immunofluorescence with virus-specific immune ascitic fluid and confirmed by complement fixation or neutralization tests	NA	

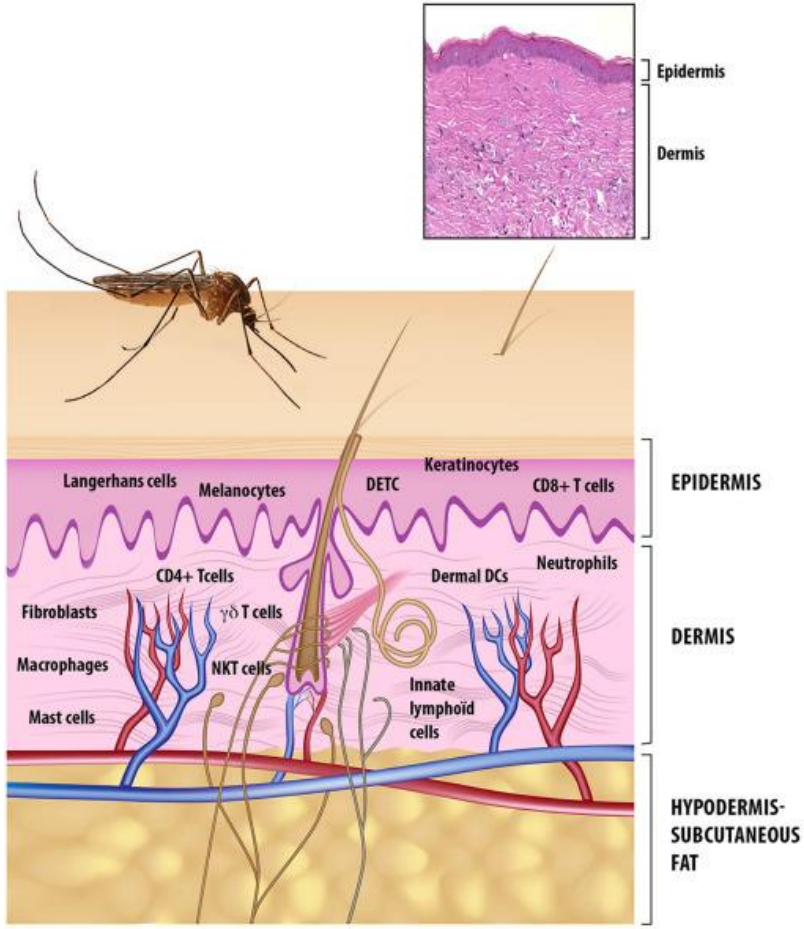
HI, hemagglutination inhibition; NA, not applicable.
[a] Red-tail monkey, black mangabey, and lowland colobus were positive for Zika virus.

Mosquito-borne transmission

The transmission cycle of mosquito-borne viruses is initiated when pathogen-containing fluids are ingested by the vector from an infected vertebrate during a blood meal.

Once the virus crosses the midgut barrier and has replicated in the mosquito body, it reaches the salivary glands, leading to the presence of high infectious titers in the saliva of infected arthropods.

During a subsequent blood meal, the proboscis of the infected mosquito probes the vertebrate host's skin, resulting in the extravascular delivery of most of salivary glands content in both the epidermis and dermis where resident and migratory cells will encounter the pathogen.

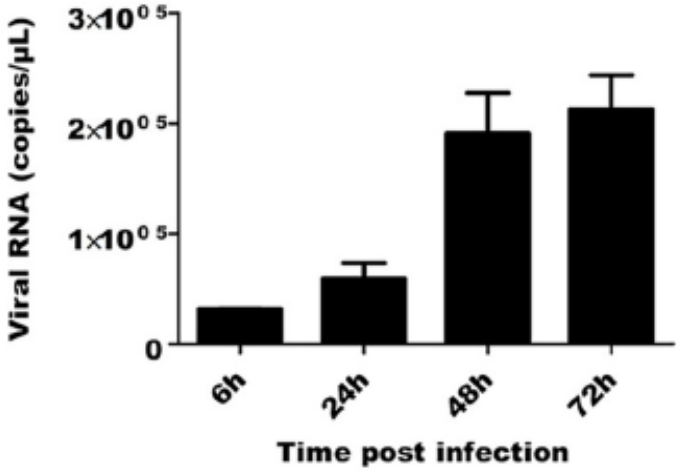


Biology of Zika Virus Infection in Human Skin Cells

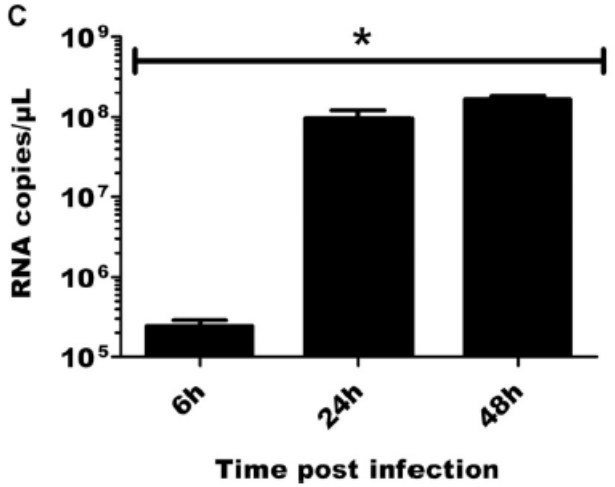
J. Virol September 2015 Volume 89 Number 17

Rodolphe Hamel,[a] Ophélie Dejarnac,[b] Sineewanlaya Wichit,[a] Peeraya Ekchariyawat,[a] Aymeric Neyret,[c] Natthanej Luplertlop,[d] Manuel Perera-Lecoin,[a] Pornapat Surasombatpattana,[e] Loïc Talignani,[a] Frédéric Thomas,[a] Van-Mai Cao-Lormeau,[f] Valérie Choumet,[g] Laurence Briant,[c] Philippe Desprès,[h] Ali Amara,[b] Hans Yssel,[i] Dorothée Missé[a]

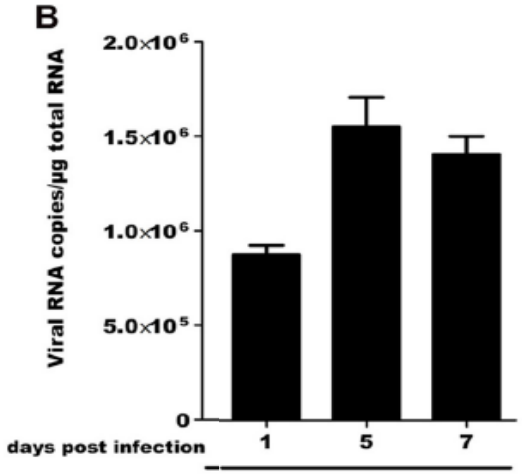
Primary keratinocyte



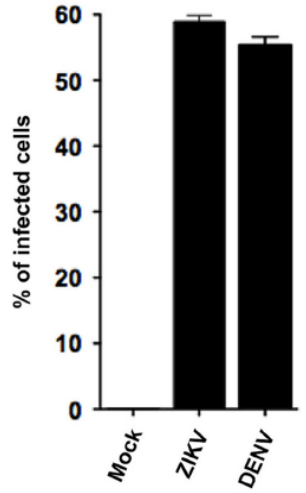
Primary dermal fibroblasts



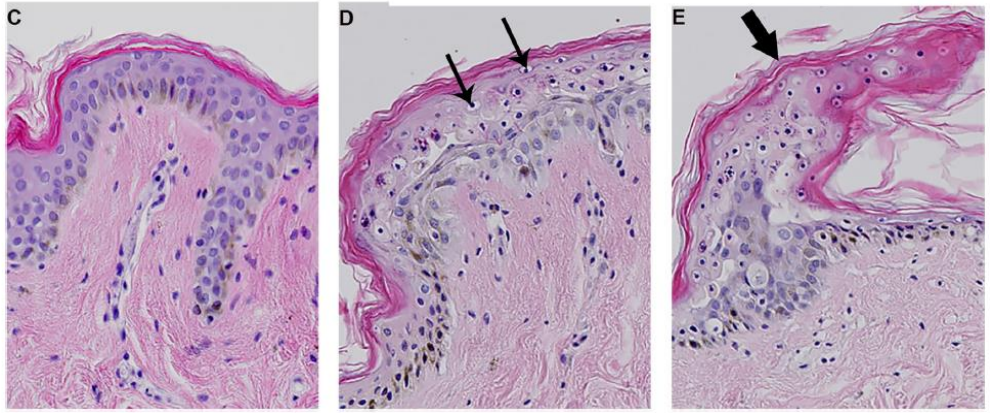
Skin biopsies



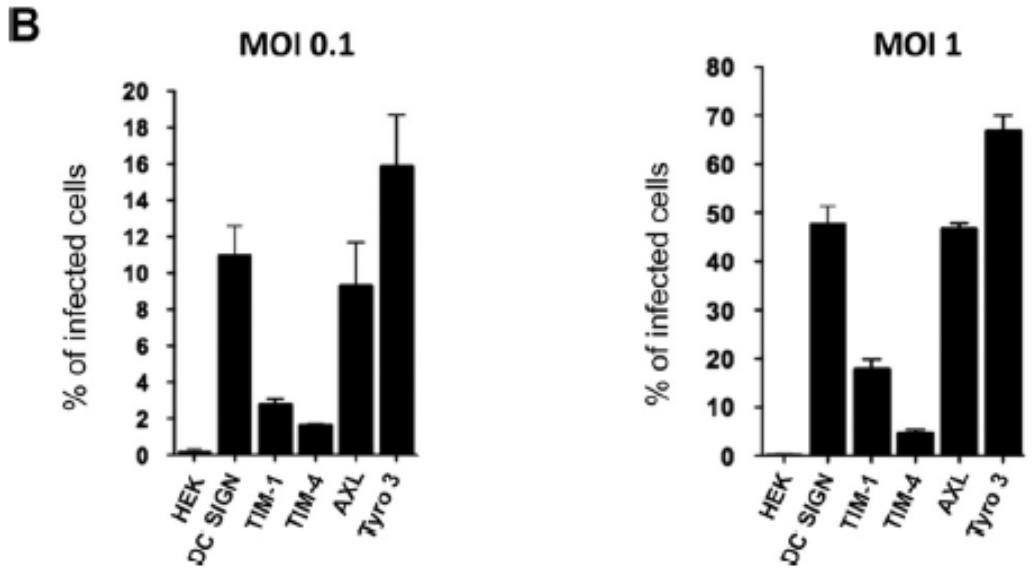
Immature DCs



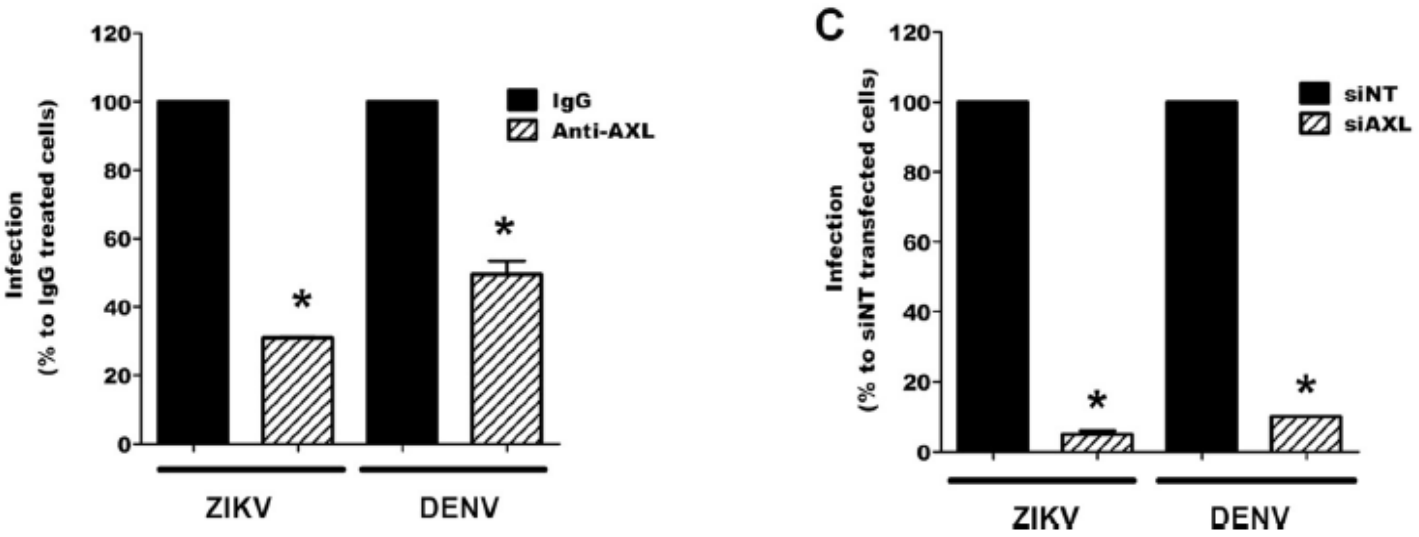
Microscopic observation of mock (C)- or ZIKV (D and E)-infected human skin biopsy specimens. Small arrows indicate keratinocyte cytoplasmic vacuolation. The large arrow indicates a superficial subcorneal edema, and also cytoplasmic vacuolation.



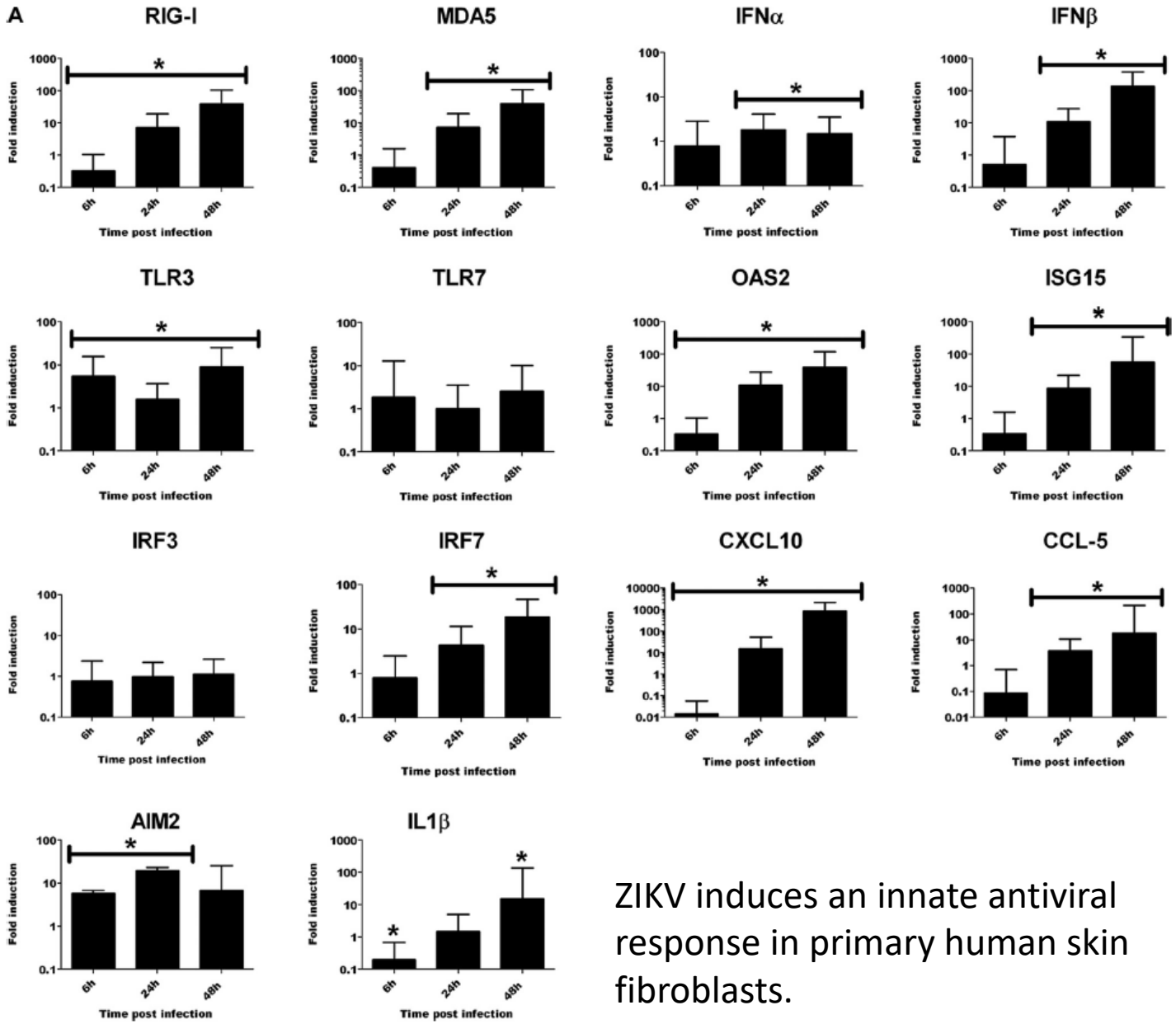
DC-SIGN, AXL, TYRO3, TIM-1 as ZIKV receptors



HEK293T cells expressing the indicated receptors were incubated with ZIKV (MOI0.1 and 1), and the percentage of infected cells was determined by measuring the expression of the viral envelope protein by flow cytometry at 24 hpi.

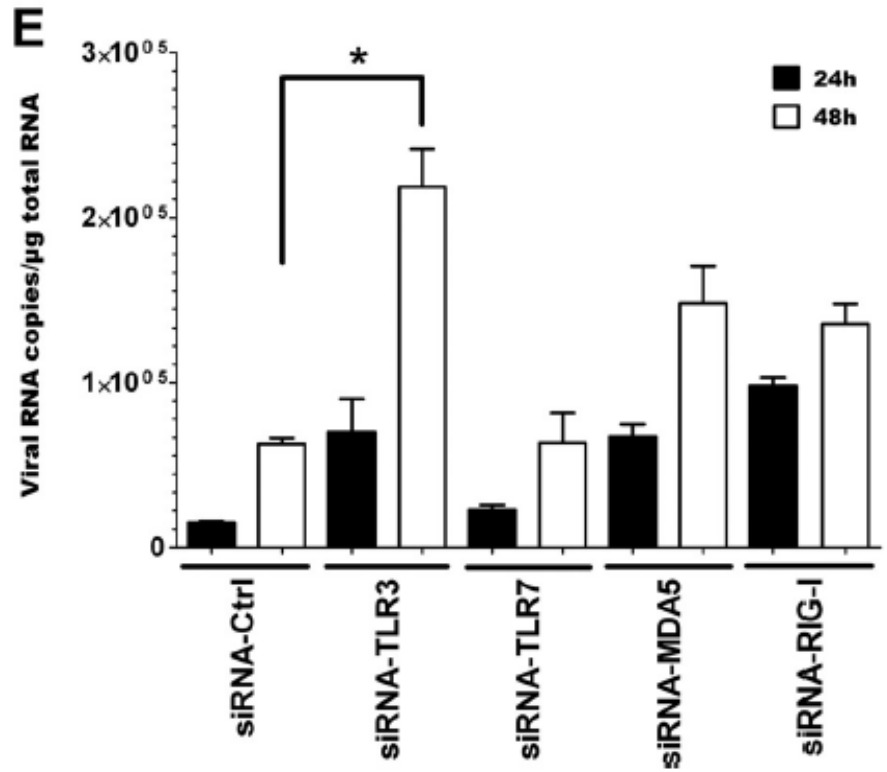


Human skin fibroblasts express AXL but not TIM-1.
Neutralizing anti-AXL Ab reduced and AXL siRNA abrogated the infection with ZIKV or DENV.

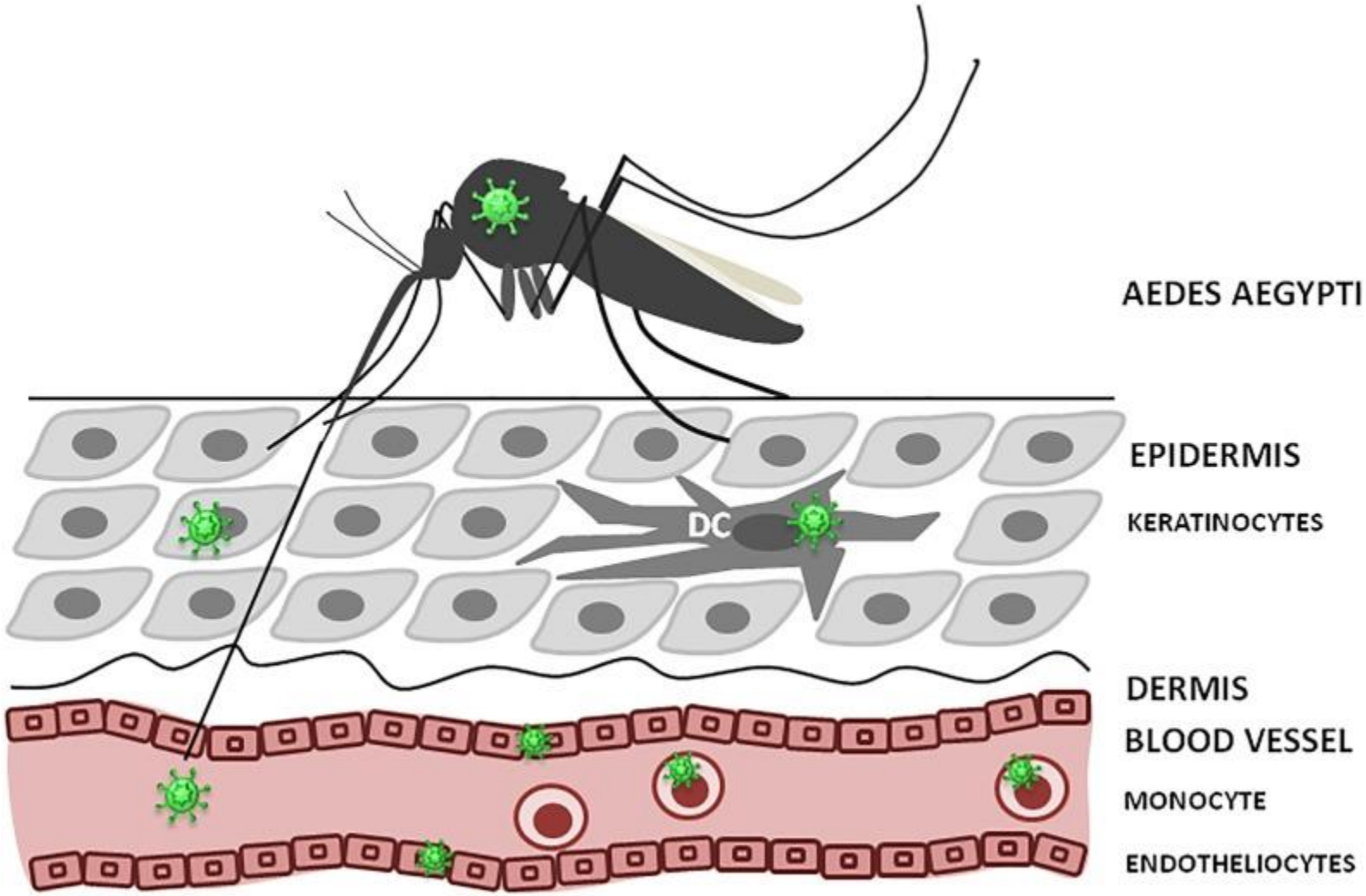


ZIKV induces an innate antiviral response in primary human skin fibroblasts.

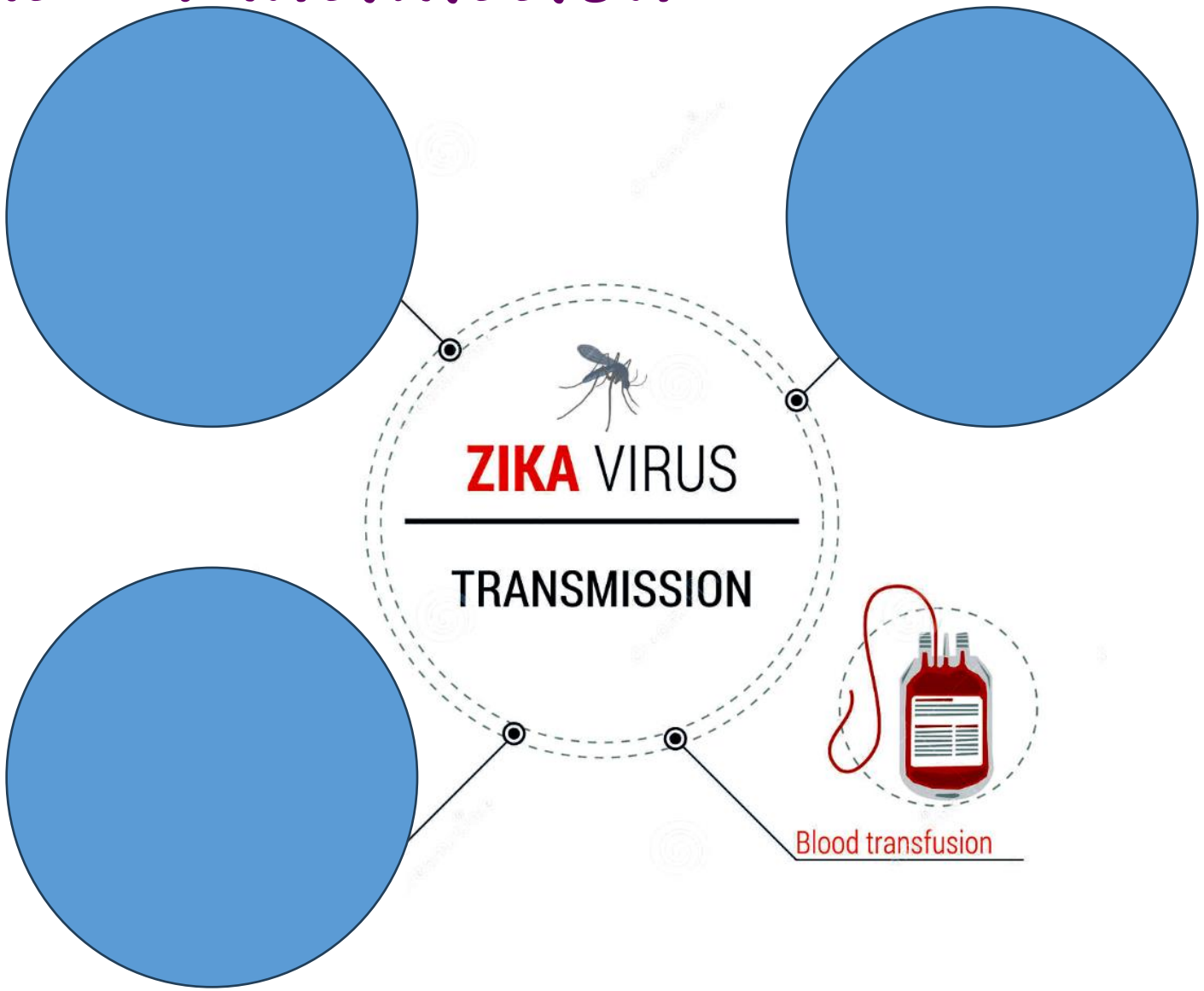
Inhibition of TLR3 expression, unlike that of the other PRRs, resulted in a strong increase in the viral RNA copy number 48 h following viral infection of the cells



Mosquito-borne transmission



Zika Virus Transmission



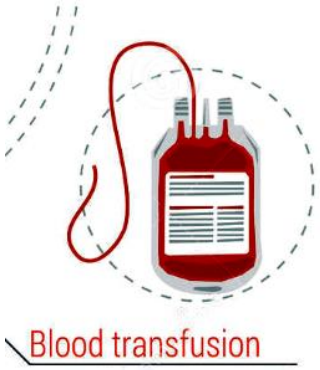
Transfusion transmission of Zika virus

- two probable cases of transfusion transmission

Evidence for Transmission of Zika Virus by Platelet Transfusion

N ENGL J MED 375;11 NEJM.ORG SEPTEMBER 15, 2016

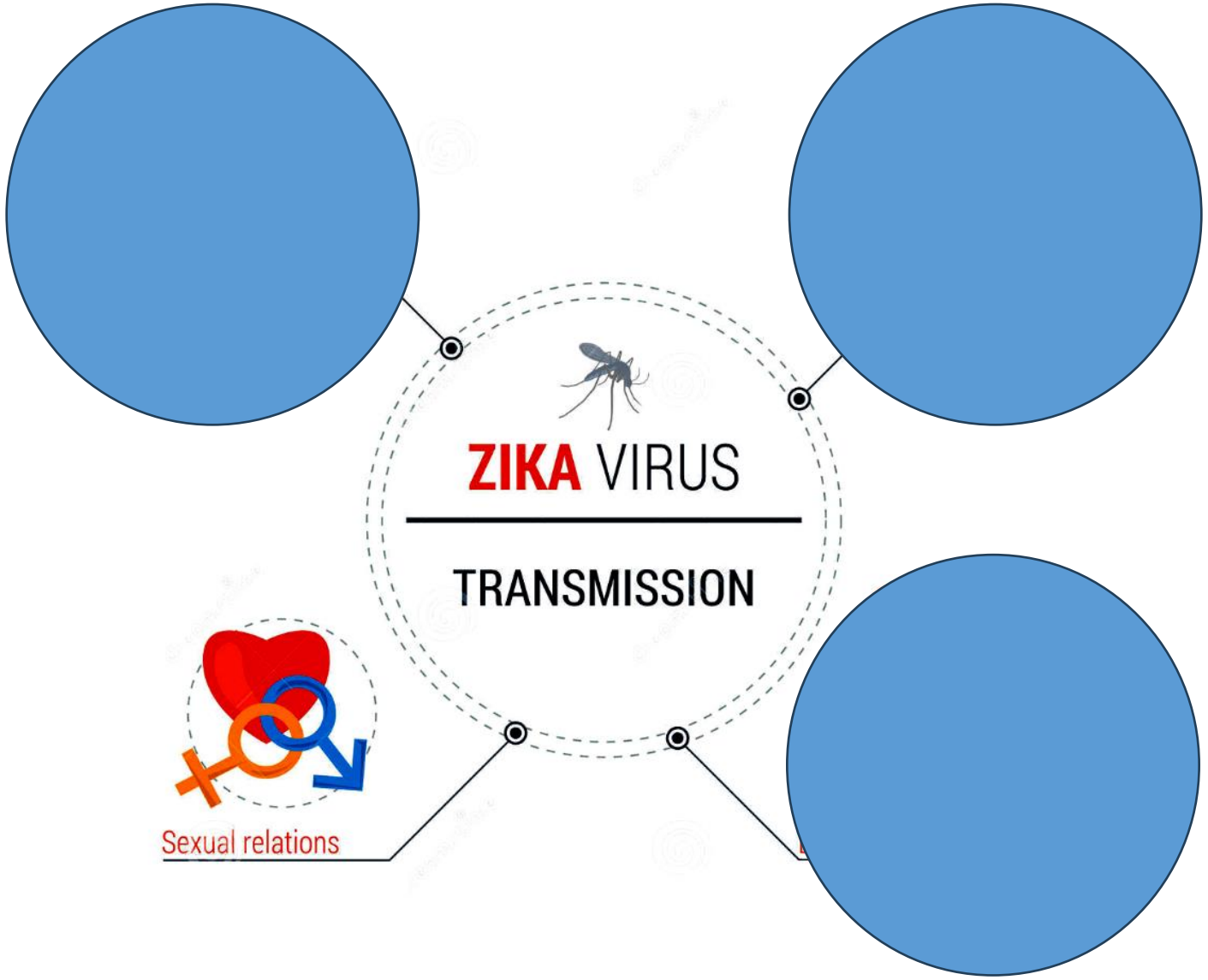
- donations occurred in March and April 2015
  - donors asymptomatic at the time of donation
  - donors developed symptoms 1 and 3 days post donation
  - one archived donor sample tested; positive for ZIKV RNA
- both recipients were positive for ZIKV RNA



2,8% of samples from blood donors resulted ZIKV RNA positive in French Polynesia during the epidemics

ZIKV may associate to blood cells and platelets

Zika Virus Transmission



Evidence of Sexual transmission

Probable Non–Vector-borne Transmission of Zika Virus, Colorado, USA

Brian D. Foy, Kevin C. Kobylinski, Joy L. Chilson Foy, Bradley J. Blitvich, Amelia Travassos da Rosa, Andrew D. Haddow, Robert S. Lanciotti, and Robert B. Tesh

Male (returning from Senegal 2008) to Female

An autochthonous case of Zika due to possible sexual transmission, Florence, Italy, 2014

G Venturi [1], L Zammarchi [2 3], C Fortuna [1], ME Remoli [1], E Benedetti [1], C Fiorentini [1], M Trotta [3], C Rizzo [4], A Mantella [2], G Rezza [1], A Bartoloni [2 3]

Male (returning from Thailand) to Female

Evidence of Sexual Transmission of Zika Virus

Eric D'Ortenzio, M.D. Sophie Matheron, M.D. Yazdan Yazdanpanah, M.D., Ph.D.

N ENGL J MED 374;22 NEJM.ORG JUNE 2, 2016

Male (returning from Brazil) to Female

Male-to-Male Sexual Transmission of Zika Virus – Texas, January 2016

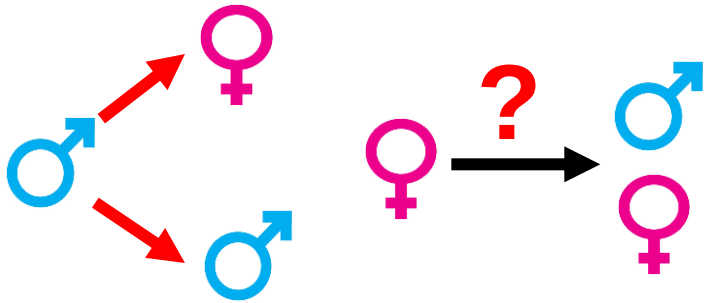
D. Trew Deckard, PA-C[1]; Wendy M. Chung, MD[2]; John T. Brooks, MD[3]; Jessica C. Smith, MPH[2]; Senait Woldai, MPH[2]; Morgan Hennessey, DVM[4,5]; Natalie Kwit, DVM[4,5]; Paul Mead, MD[4]

Morb Mortal Wkly Rep. 2016 Apr

returning from Venezuela

- Zika virus can be spread by a man to his sex partners.
- In known cases of sexual transmission, the men developed Zika virus symptoms. From these cases, we know the virus can be spread when the man has symptoms, before symptoms start and after symptoms resolve.

Sexual transmission



Zika virus can be sexually transmitted from a man to his sex partners.

The first documented case of sexual transmission of Zika virus was in 2008 from a man returning to USA from Senegal

Other recent reports described: in Italy in 2014 (imported from Thailand), in France in 2016 imported from Martinique and Brazil, 6 confirmed cases in USA (imported from Latin America)

The viral load in semen is often higher than in blood and long lasting.

Reports of replication-competent Zika virus isolated from semen till 62 days after onset of symptoms.

In known cases of sexual transmission, the man developed Zika virus symptoms.

It is not known whether infected men who never develop symptoms can transmit Zika virus to their sex partners.

WHO reccomendations: Travellers returning from areas with ongoing Zika virus transmission should be advised to use a condom for at least **8 weeks** after returning. If symptoms occur, men should use condoms for at least **6 months**. Use a condom with a pregnant partner **until the end of pregnancy.**

Zika in Semen

•The virus is present in semen longer than in blood.

Potential Sexual Transmission of Zika Virus

Didier Musso, Claudine Roche, Emilie Robin, Tuxuan Nhan, Anita Teissier, Van-Mai Cao-Lormeau

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 21, No. 2, February 2015

French Polynesia (Zika detected in semen)

Zika virus: high infectious viral load in semen, a new sexually transmitted pathogen?

*Jean Michel Mansuy, Marine Dutertre, Catherine Mengelle, Camille Fourcade, Bruno Marchou, Pierre Delobel, Jacques Izopet, Guillaume Martin-Blondel

Lancet Infect Dis 2016

Published Online March 3, 2016

Brazil and French Guyana Viral load in semen of 8,6 Log

Detection of Zika Virus in Semen

Barry Atkinson, Pasco Hearn, Babak Afrough, Sarah Lumley, Daniel Carter, Emma J. Aarons, Andrew J. Simpson, Timothy J. Brooks, Roger Hewson

Emerg Infect Dis. 2016 May

Cook Islands 2014

Sexual transmission up to 41 dps	Infectious particles in semen up to 69 dps	Viral RNA in semen up to 414 dps	Lower sperm count

Zika virus in the semen of infected men

Mansuy et al, Lancet Inf Dis 2016

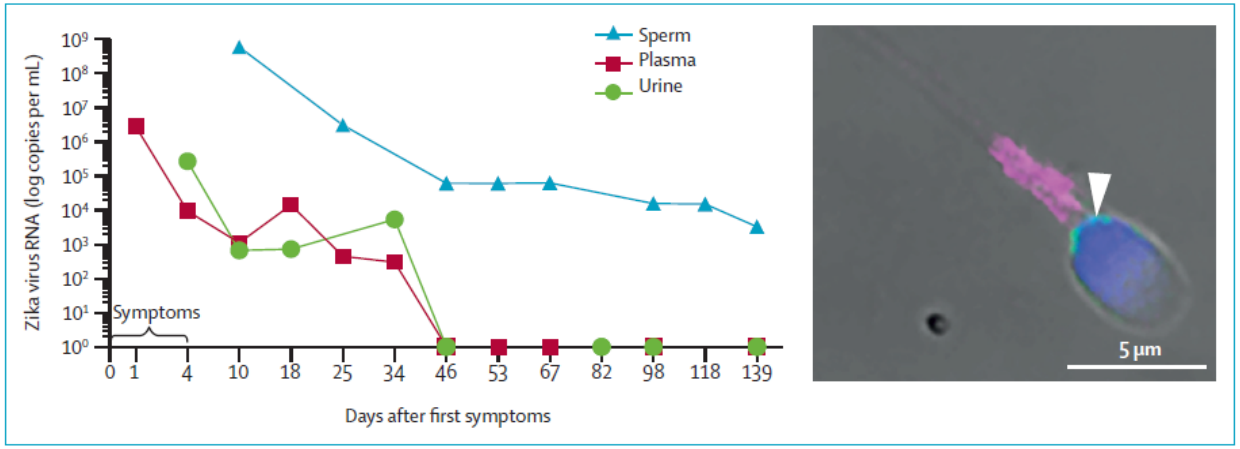
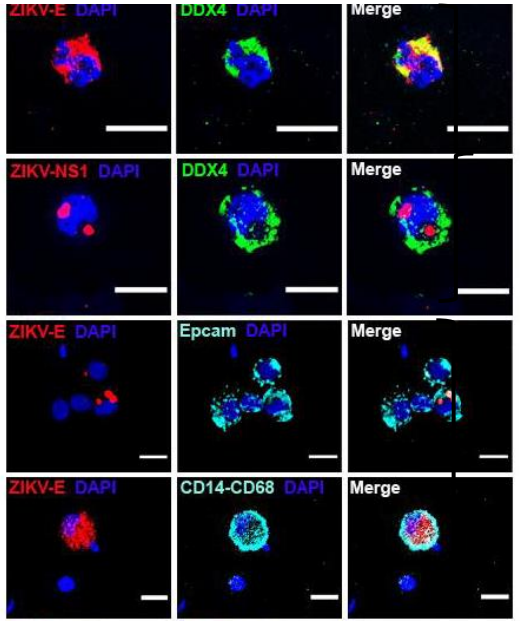


Figure: **Zika virus infects spermatozoa**
On the left are the kinetics of Zika virus RNA detection in plasma, urine, and semen quantified by real-time RT-PCR (RealStar Zika Virus RT-PCR Kit 1.0; Altona Diagnostics GmbH, Hamburg, Germany). On the right is immunohistochemical detection of Zika virus (green; arrowhead) on brightfield microscopy in the head of spermatozoa obtained from the patient; Tom20 is pink; Zika virus is green; and Hoechst stain is blue.

Mahé et al, Lancet Inf Dis 2020



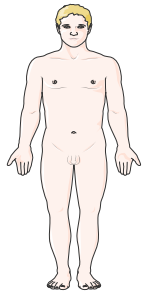
Testicular germ cells in semen are infected up to 5 months

Epithelial cells and **macrophages** in semen are infected up to 3 months
Epididymal origin?

Semen smears: IHC viral proteins + cell markers

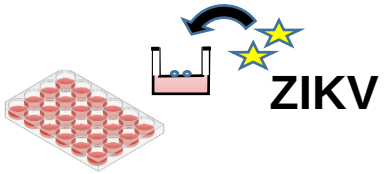
- ✓ **ZIKV associates to spermatozoa**
- ✓ **Germ cells macrophages and epithelial seminal cells are infected by ZIKV**
- ✓ **Germ cells are persistently infected for the longest duration (5 months)**

Zika virus infection of human testis

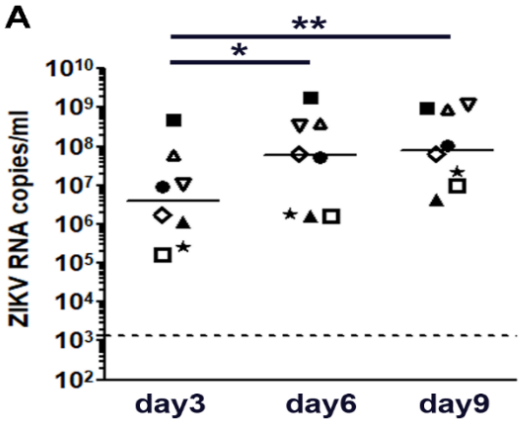


Organ donors

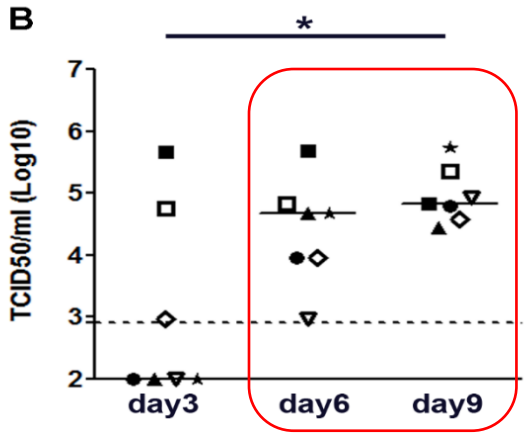
Ex vivo infection



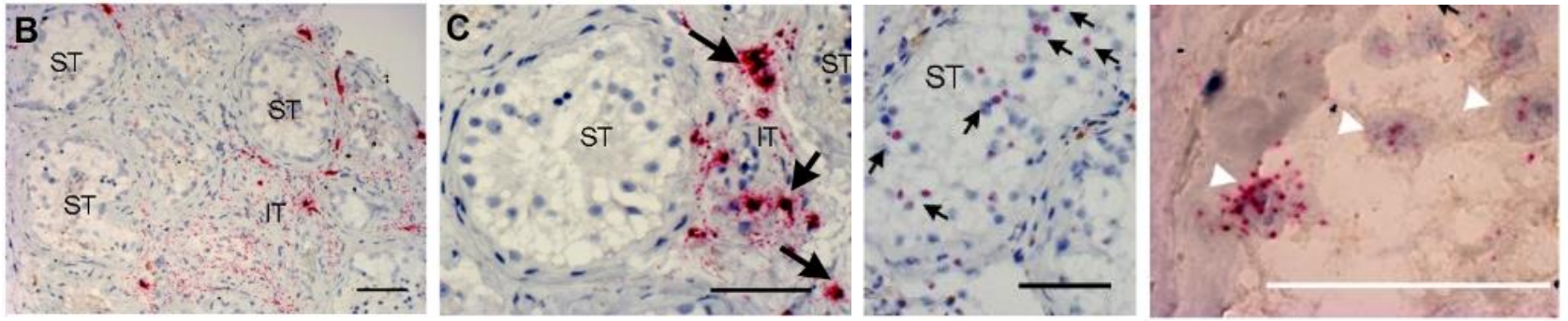
Viral RNA



Infectious viral particles



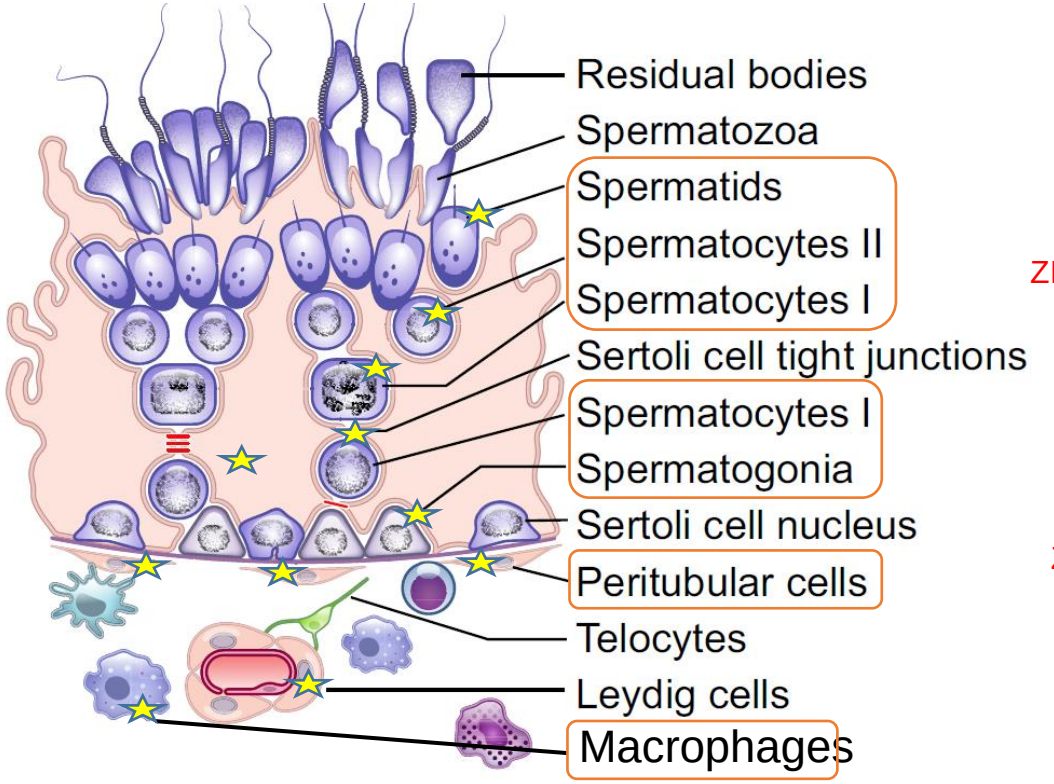
ISH RNAscope- ZIKV RNA



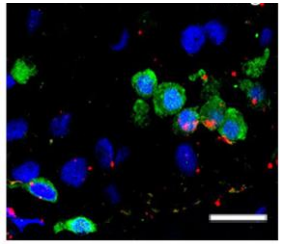
✓ **ZIKV replicates *ex vivo* in the human testis interstitial tissue and seminiferous tubules**

Matusali et al, Lancet Inf Dis 2016

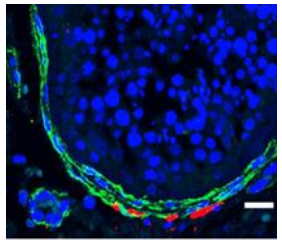
Target cells of ZIKV in the human testis ex vivo



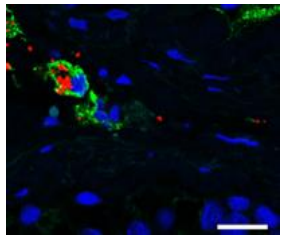
ZIKV RNA ISH/ DDX4 IHC

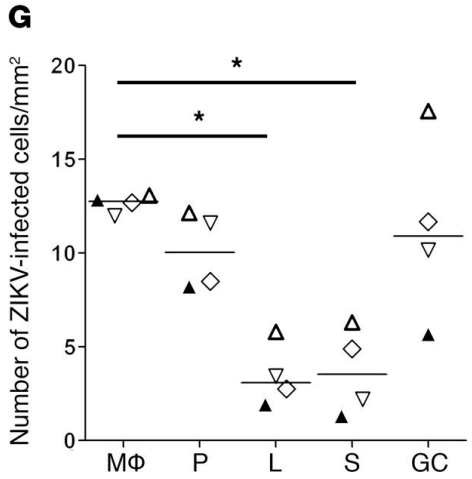


ZIKV RNA ISH/ SMA IHC



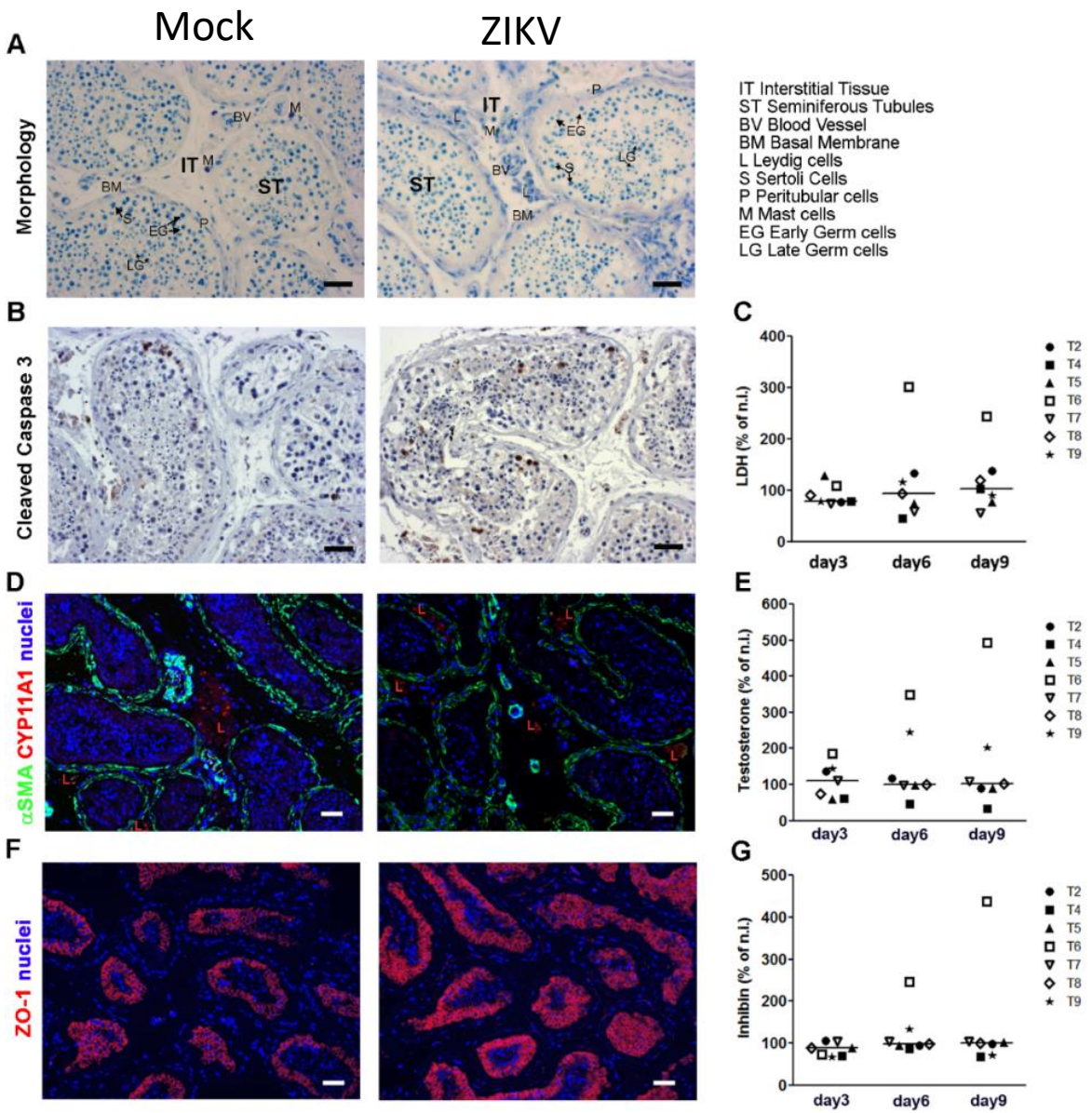
ZIKV RNA ISH/ CD68 IHC





✓ **ZIKV primarily infects resident macrophages, peritubular cells and germ cells ex vivo**

ZIKV has no effect on human testis explants histology, apoptosis, BTB or hormones production ex vivo



Cell apoptosis and tissue viability

Testosterone

Blood testis barrier

Inhibin B

Zika in female genital samples

Prolonged Detection of Zika Virus in Vaginal Secretions and Whole Blood

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 23, No. 1, January 2017

Up to 14 dso ZIKV RNA in vaginal swabs

Case Report: Prolonged Detection of Zika Virus RNA in Vaginal and Endocervical Samples from a Brazilian Woman, 2018

Taís E. da Cruz,[1]† Raquel P. Souza,[1]† Sandra M. Pelloso,[2] Fabrício Morelli,[1] Tamy T. Suehiro,[1] Edilson Damke,[1] Patrícia de S. Bonfim-Mendonça,[1] Vânia R. S. da Silva,[1] and Marcia E. L. Consolaro[1]*

Samples	Day 6	Day 31	Day 54
Whole blood	–	Negative	Negative
Plasma	–	Negative	Negative
Serum	2×10^3 copies/mL	Negative	Negative
Urine	–	Negative	Negative
Vaginal	–	2×10^2 copies/mL	Negative
Endocervical	–	2×10^2 copies/mL	Negative

– = not performed.

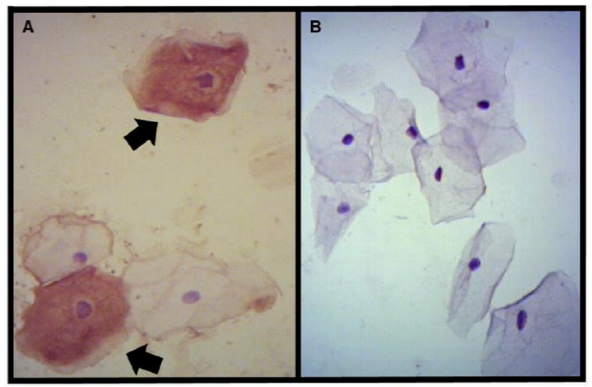


FIGURE 1. Immunocytochemistry staining on the vaginal–cervical cytological smear. (**A**) Positive immunostaining of Zika virus (ZIKV) antigens in squamous cells (arrow). (**B**) Negative immunostaining of ZIKV antigens in cells (×400 magnification). This figure appears in color at www.ajtmh.org.

Zika in female genital samples

Detection and persistence of Zika virus in body fluids and associated factors: a prospective cohort study

Guilherme Amaral Calvet[1✉], Edna Oliveira Kara[2], Camila Helena Aguiar Bôtto-Menezes[3,4], Marcia da Costa Castilho[3], Rafael Freitas de Oliveira Franca[5], Ndema Habib[2], Armando Menezes Neto[5], Gerson Fernando Mendes Pereira[6], Silvana Pereira Giozza[6], Ximena Pamela Díaz Bermúdez[7], Tatiana Jorge Fernandes[1], Kayvon Modjarrad[8], Patrícia Brasil[1], Nathalie Jeanne Nicole Broutet[2], Ana Maria Bispo de Filippis[9] & ZIKABRA Study Team*

ZIKV RNA was present in the vaginal fluids of 60.7% of the 184, a higher frequency when compared to a study conducted in Puerto Rico, where detection was observed in only 1.7% of 119 women Detection up to 67 days after symptom onset.

Prolonged Shedding of Zika Virus RNA in Vaginal Secretions, Nicaragua

Yaoska Reyes,[1] Natalie M. Bowman,[1] Sylvia Becker-Dreps, Edwing Centeno, Matthew H. Collins, Guei-Jiun Alice Liou, Filemón Bucardo

ZIKV RNA was present in the vaginal fluids up to 6 months fso

Most of our subjects were pregnant at enrollment (4 out of 5); pregnant women are known to shed Zika virus in blood for up to 3 times longer than nonpregnant women, possibly because of fetal infection.

Another study reported detection of Zika virus RNA in cervical cytology samples of 32 of 59 pregnant women compared with 18 of 109 nonpregnant women.

Fetal tissue may act as a reservoir for long-term infection, or pregnancy-related immunosuppression might delay viral clearance.

One patient shed Zika virus RNA in the reproductive tract after delivery, suggesting that there may be reservoirs of viral replication in the female reproductive tract.

Zika in female genital samples

Table 1 - Timeline of reports evaluating the kinetics of ZIKV in the lower genital tract of non-pregnant women.

Authors	ZIKV vaginal and/or cervical last detection (dpo)*	ZIKV RT-PCR**	Blood ZIKV load**	Urine ZIKV load**	ZIKV isolation from vaginal secretion
Prisant *et al.*[35]	~11	Positive	Negative	Negative	NA
Penot *et al.*[45]	~3-10	Sample 1: 5.3 log copies/mL Sample 2: 3.99 log copies/mL	3.8 log copies/mL	6.1 log copies/mL	Positive
Murray *et al.*[36]	14	Positive	Positive	Positive	NA
Nicastri *et al.*[44]	13	Positive	Negative	Positive	NA
Sánchez-Montalvá *et al.*[25]	~37-69	Positive	NI	NI	NA
Paz-Bailey *et al.*[21]	~3	Positive	NI	NI	NA
Tozetto-Mendoza *et al.*[48]	~18	Positive	Positive	NI	NA
Tobar *et al.*[46]	NI	Positive	NA	NA	NA
Reyes *et al.*[47]	180	Positive	NI	NI	NA
Cruz *et al.*[37]	~31-54	2×10^2 copies/mL	Negative	Negative	NA

NI: not informed; NA: not analyzed; ZIKV: Zika virus; RT-PCR: real-time reverse transcription–polymerase chain reaction. *dpo: days post onset of symptoms; **On the day of the last detection of ZIKV RNA in lower genital tract.

ZIKV RNA has also been detected in the ovaries of female mice and non-human primates. A human case of *in vitro* fertilization (IVF) described ZIKV RNA-positive oocytes. However, in comparison with the male genital tract, the consequences of ZIKV infection on the female genital tract (FGT) have not been extensively evaluated, so that little is known about the target cells of ZIKV in this site.

ZIKV replication in the FGT: in vitro and in vivo models

- **In vivo**

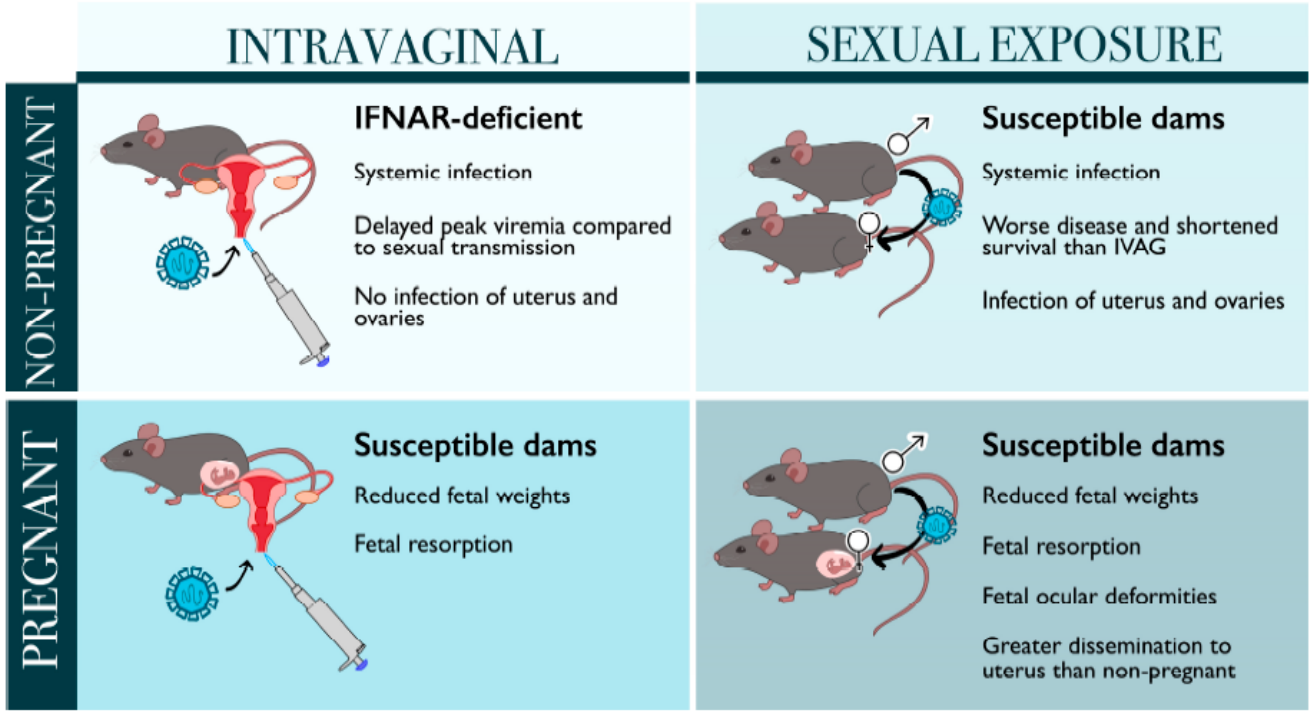
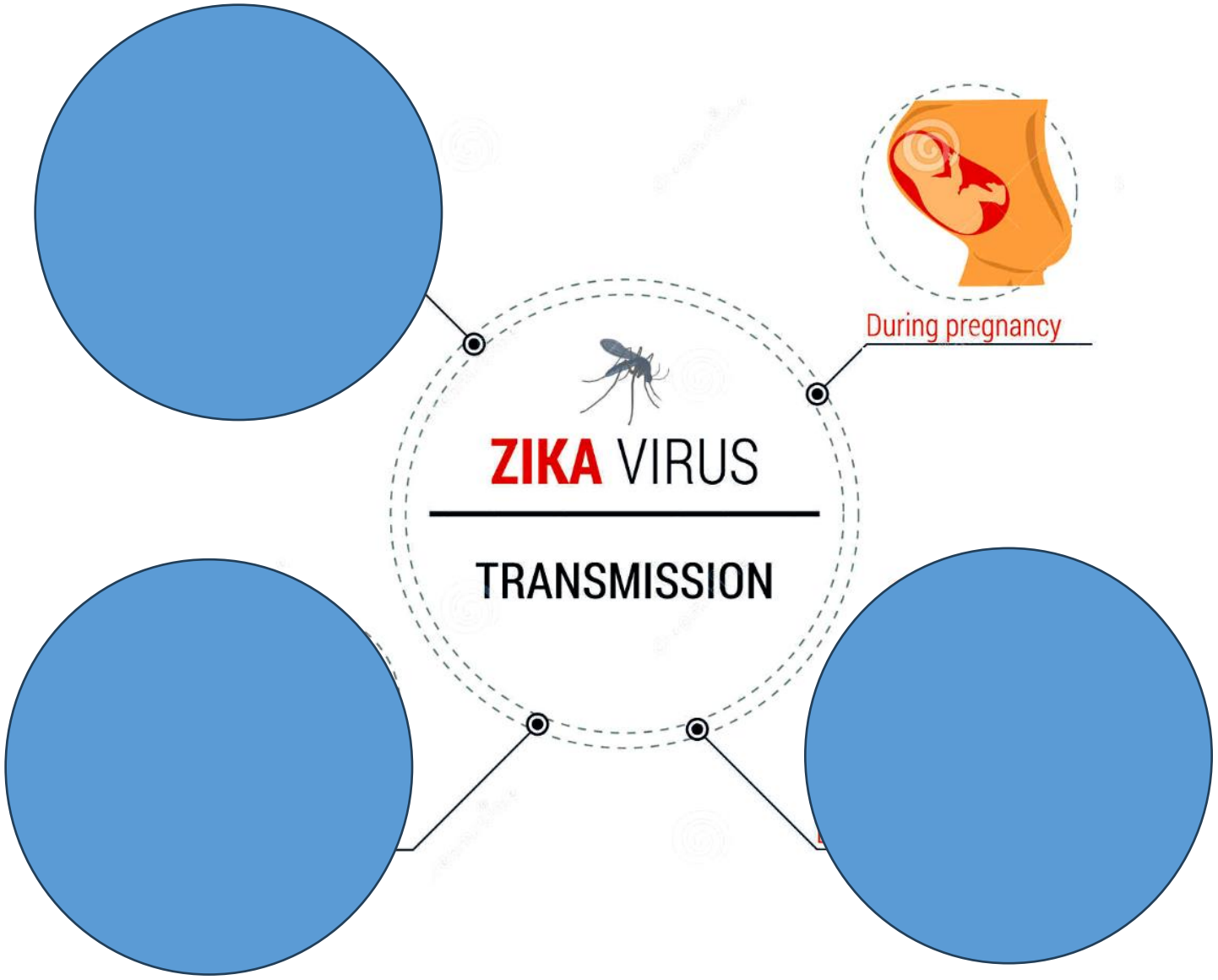


Figure 2. Models of sexually transmitted ZIKV infections in both pregnant and non-pregnant mice. Sexual transmission results in worse survival and greater dissemination to the uterus and ovaries in non-pregnant females compared to intravaginally infected (IVAG) mice. Both intravaginal infection and sexual exposure can impair fetal development, but these clinical outcomes have not been directly compared.

- **In vitro infections:** investigation on the effect of IFNs and sex hormones on viral replication.
  Protective role of IFNλ, IFNε and estradiol

Gli ormoni sessuali modulano l'infezione da virus zika

Zika Virus Transmission

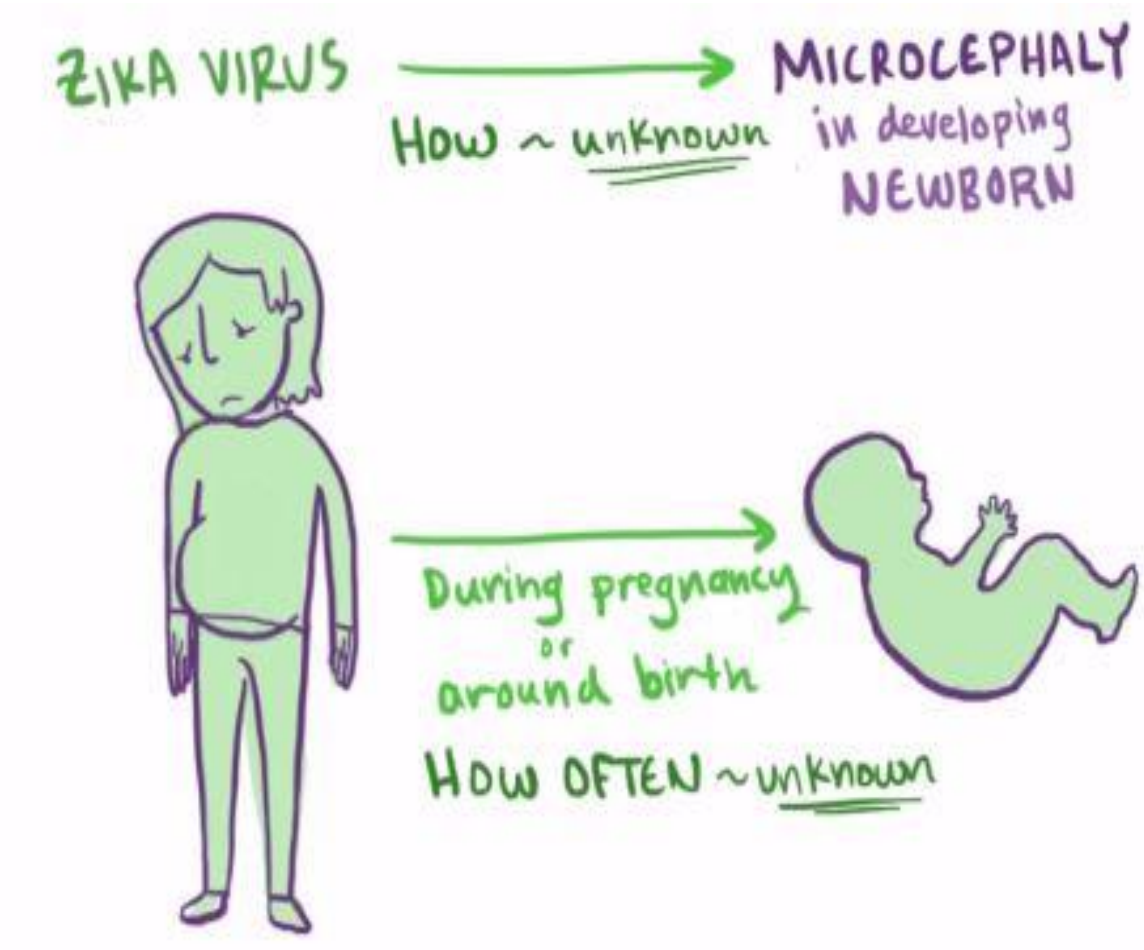


Materno-foetal transmission

- A pregnant woman can pass Zika virus to her fetus during pregnancy. **Zika is a cause of microcephaly and other severe fetal brain defects.**

- A pregnant woman already infected with Zika virus can pass the virus to her fetus **during the pregnancy or around the time of birth.**

- To date, **there are no reports of infants getting Zika virus through breastfeeding.**



Mechanisms of materno-fetal transmission of viruses:

The mechanisms by which viruses can be transmitted vertically are multifaceted and can involve entry into the gestational sac via **direct hematogenous spread**, **trophoblastic transcellular or paracellular pathways**, **transport within immune cells or infected sperm**, **pre-pregnancy uterine colonization**, introduction during **invasive procedures** during pregnancy, and/or **transvaginal ascending infection.**

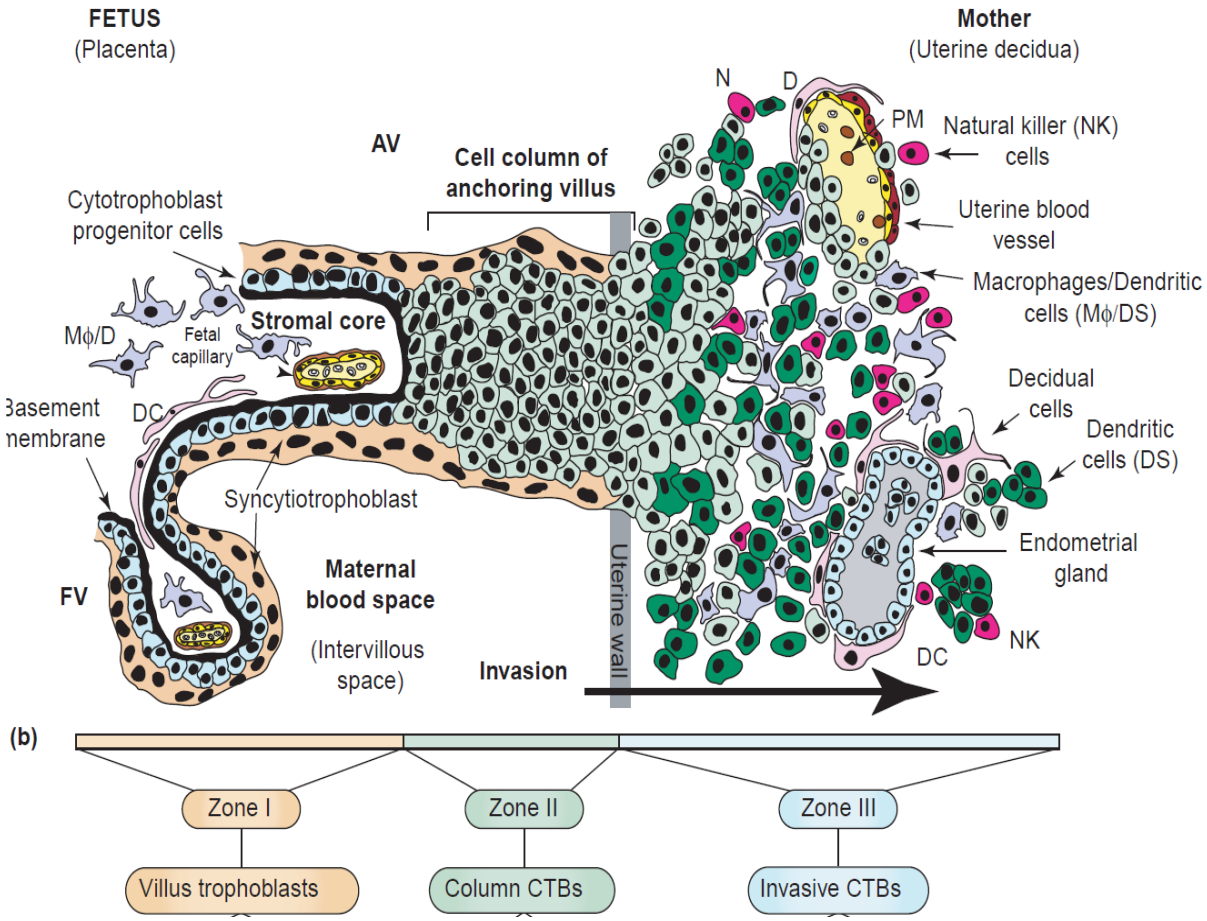


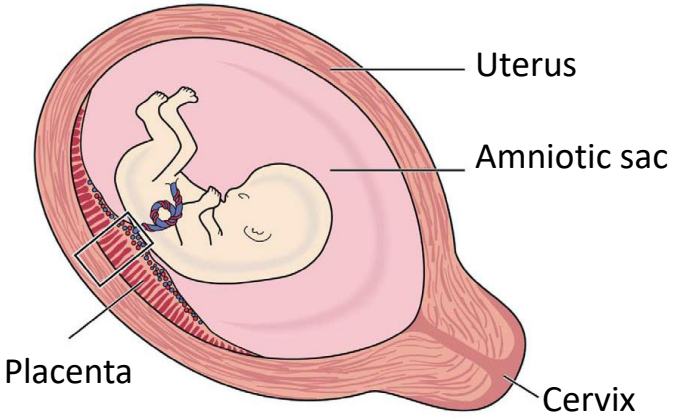
Table 1. Intrauterine viral infections

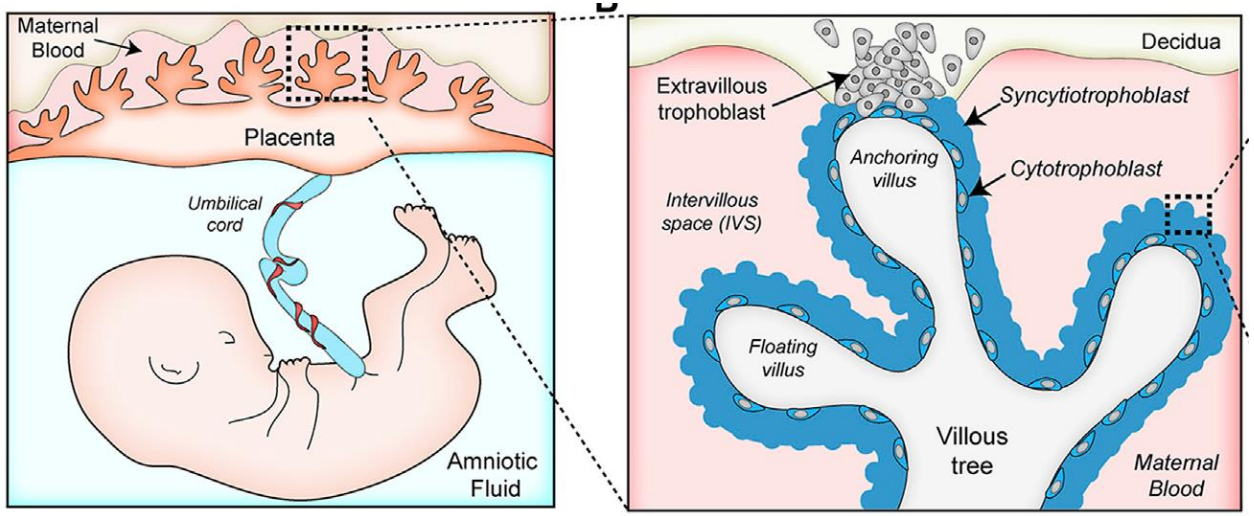
Virus	Infection	Refs
Cytomegalovirus	Prenatal infection affects 1–2% of live births. Virus replicates in the uterus, infects the placenta, then is transmitted to the fetus. Transmission rate is high in women with primary infection (50%).	[2,17,18]
Herpes simplex virus 2	Infrequent prenatal infection. Transmission primarily at delivery (80%). Possible ascending infection after membrane rupture.	[20,21,53,54]
Human immunodeficiency virus	Transmission primarily at delivery. Isolated cytotrophoblasts infected *in vitro*.	[23,24,55]
Hepatitis B virus	Transmission primarily perinatal. Some intrauterine infection from maternal blood (5%).	[56]
Hepatitis C virus	Intrauterine infection and at delivery (2–12%).	[57]
Parvovirus B19	Placental infection associated with inflammatory cytokines. Complications in early gestation.	[58]
Rubella virus	Placental infection during primary maternal infection. Transmission in first trimester (80%) and second trimester (25%).	[54]
Human papilloma virus	Infection at birth.	[25,53]
Varicella zoster virus	Congenital infection low (2%). Transmission during primary infection in late gestation (25–50%).	[54]

on the thickness of the separating layers and is affected by the number and type of layers. In the hemochorial placenta, the maternal blood vessels are completely destroyed by the fetal trophoblasts that directly contact maternal blood. Maximal exchange surface is provided by a tree-like branching pattern of the chorion, resulting in floating villi. Rats and mice have a hemotrichorial placenta, whereas humans, rhesus monkeys and guinea pigs have a hemodichorial and, at term, hemomonochorial placenta. In species with invasive implantation, the trophoblast forms a syncytium that can be passed only transcellularly by transport mechanisms, such as diffusion, facilitated transfer, active transport and vesicular transfer. In humans, the syncytiotrophoblast is the decisive barrier that limits or supports transplacental transfer processes. The architecture of the guinea pig and rhesus placentas, similar to the human, could allow studies of prenatal infection in these animal models.

Hemochorial: Trophoblast surface membrane covered by maternal blood.
Hemodichorial: Syncytium covered on one side by cytotrophoblasts.
Hemomonochorial: Syncytium without underlying cytotrophoblasts.
Hemotrichorial: Syncytium covered on two sides by cytotrophoblasts.
Intervillous space: Maternal blood space between placental villi.
Invasive cytotrophoblasts: Differentiating cells that leave the basement membrane, switch to an endothelial phenotype and invade the maternal–fetal junctional zone.
Syncytiotrophoblast: Formed by trophoblast cell fusion, covers the villus surface.
Trophoblast: Epithelial cells that compose the developing placenta.
Villus stroma: Connective tissue, fibroblasts, macrophages and fetal blood vessels that are surrounded by trophoblasts.

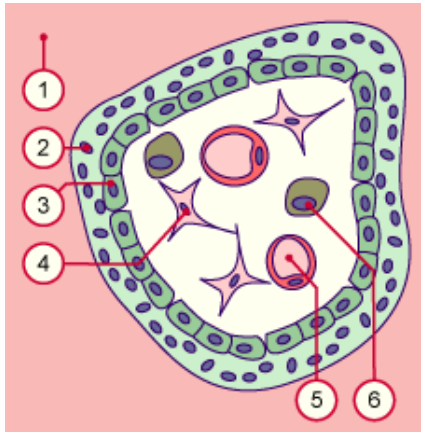
Placenta



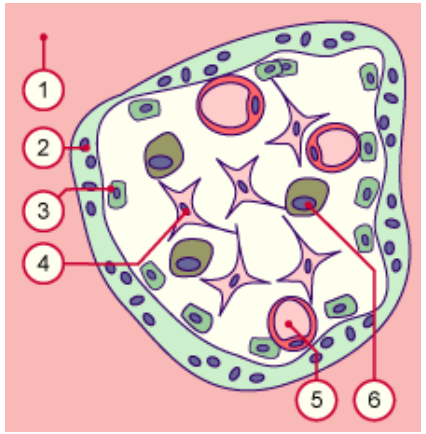


1 Intervillous space
2 Syncytiotrophoblast
3 Cytotrophoblast
4 Villus mesenchyma
5 Fetal capillaries
6 Hofbauer (MΦ)

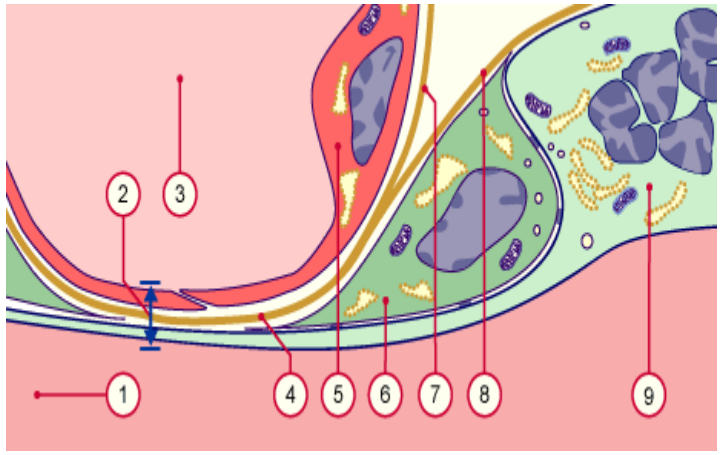
1st trimester villi



2nd trimester villi



3rd trimester villi



1 Intervillous space (with maternal blood)
2 Placental barrier of a terminal villus
3 Fetal capillaries
4 Merged basal membranes of fetal capillary and syncythiothrophoblast
5 Endothelial cells
6 Rare cytotrophoblast cells
7 Basal membrane of the capillaries
8 Basal membrane of the trophoblast
9 Syncytiotrophoblast (nuclei rich region)

Placenta and Zika in the literature

Martines RB CDC report of **1 IHC positive placenta from a miscarriage (week 11 or 13, not specified)**

de Noronha L et al. Mem Inst Oswaldo Cruz: 5 cases report: **Placenta positive for Zika in case 1** (cutterage for loss of fetal heartbeats **12 GW IHC+ in Hofbauer cells**, symptoms in the mother during the 7th week) and **case 5 (healthy newborn Zika detected by RT-QPCR**, symptoms in the mother during the eigth month)

Miner JJ Cell: Zika Virus infection during pregnancy in **Mice** causes placental damage and fetal demise **ZIKV identified within trophoblasts of the maternal and fetal placenta** (female mice lacking interferon signaling)

Bayer A. et al Cell Host and microbes: **Human primary Trophoblast from full term placentas are resistant to Zika Virus infection. Suggested role of protection for IFN-III**

Quicke KM et al. Cell Host and microbes: **Infection of placental macrophages from term placenta** (>37weeks)

ZIKA virus reveals broad tissue and cell tropism during the first trimester of pregnancy

Hicham El Costa[1,2,*], Jordi Gouilly[1,*], Jean-Michel Mansuy[2], Qian Chen[1], Claude Levy[3], Géraldine Cartron[4], Francisco Veas[5], Reem Al-Daccak[6], Jacques Izopet[1,2] & Nabila Jabrane-Ferrat[1]

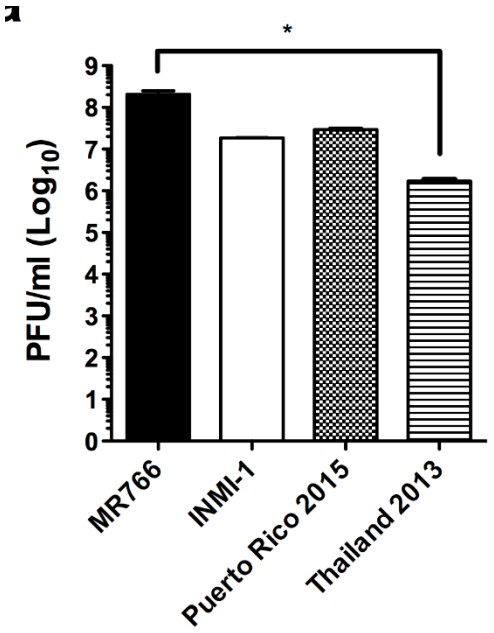
The outbreak of the Zika Virus (ZIKV) and its association with fetal abnormalities have raised worldwide concern. However, the cellular tropism and the mechanisms of ZIKV transmission to the fetus during early pregnancy are still largely unknown. Therefore, we *ex vivo* modeled the ZIKV transmission at the maternal-fetal interface using organ culture from first trimester pregnancy samples. Here, we provide evidence that ZIKV strain circulating in Brazil infects and damages tissue architecture of the maternal *decidua basalis*, the fetal placenta and umbilical cord. We also show that ZIKV replicates differentially in a wide range of maternal and fetal cells, including decidual fibroblasts and macrophages, trophoblasts, Hofbauer cells as well as umbilical cord mesenchymal stem cells. The striking cellular tropism of ZIKV and its cytopathic-induced tissue injury during the first trimester of pregnancy could provide an explanation for the irreversible congenital damages.

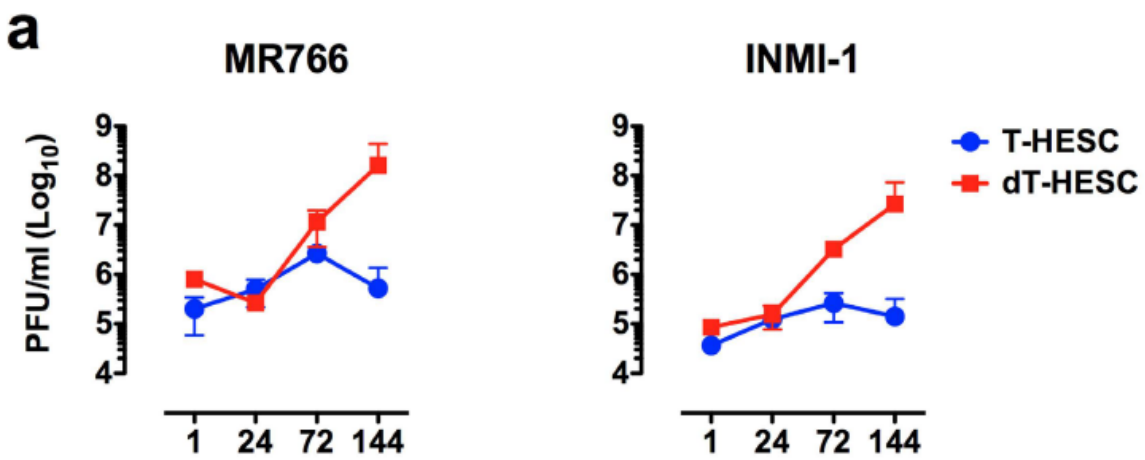
Human Endometrial Stromal Cells Are Highly Permissive To Productive Infection by Zika Virus

Isabel Pagani[1,*], Silvia Ghezzi[1,*], Adele Ulisse[2], Alicia Rubio[3], Filippo Turrini[1], Elisabetta Garavaglia[4], Massimo Candiani[4,5], Concetta Castilletti[6], Giuseppe Ippolito[6], Guido Poli[5,7], Vania Broccoli[8], Paola Panina-Bordignon[2,†] & Elisa Vicenzi[1,†]

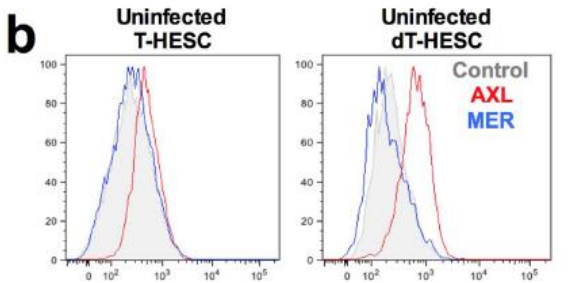
Zika virus (ZIKV) is a recently re-emerged flavivirus transmitted to humans by mosquito bites but also from mother to fetus and by sexual intercourse. We here show that primary human endometrial stromal cells (HESC) are highly permissive to ZIKV infection and support its *in vitro* replication. ZIKV envelope expression was detected in the endoplasmic reticulum whereas double-stranded viral RNA colocalized with vimentin filaments to the perinuclear region. ZIKV productive infection also occurred in the human T-HESC cell line together with the induction of interferon-β (IFN-β) and of IFN-stimulated genes. Notably, *in vitro* decidualization of T-HESC with cyclic AMP and progesterone upregulated the cell surface expression of the ZIKV entry co-receptor AXL and boosted ZIKV replication by *ca.* 100-fold. Thus, endometrial stromal cells, particularly if decidualized, likely represent a crucial cell target of ZIKV reaching them, either via the uterine vasculature in the viremic phase of the infection or by sexual viral transmission, and a potential source of virus spreading to placental trophoblasts during pregnancy.

Primary endometrial stromal cells

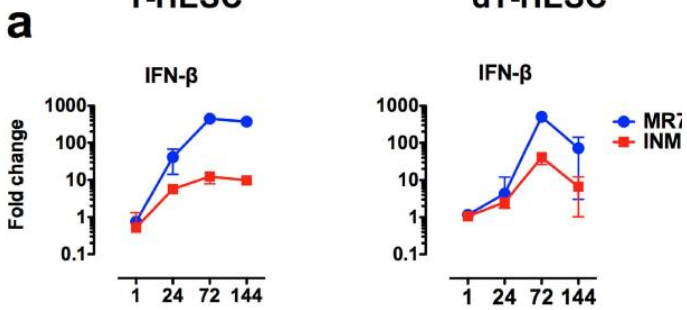




(African lineage) (Asian lineage)



Higher expression of AXL in d-T-HESC



A more rapid insurgence of IFN-β expression was observed in T-HESC *vs.* dT-HESC

dT-HESC=decidualized immortalized Human endometrial stromal cells

Zika Virus Infects Early- and Midgestation Human Maternal Decidual Tissues, Inducing Distinct Innate Tissue Responses in the Maternal-Fetal Interface

Yiska Weisblum,[a,b] Esther Oiknine-Djian,[a,b] Olesya M. Vorontsov,[a,b] Ronit Haimov-Kochman,[c] Zichria Zakay-Rones,[b] Karen Meir,[d] David Shveiky,[c] Sharona Elgavish,[e] Yuval Nevo,[e] Moshe Roseman,[e] Michal Bronstein,[f] David Stockheim,[g] Ido From,[a,b] Iris Eisenberg,[c] Aya A. Lewkowicz,[c] Simcha Yagel,[c] Amos Panet,[b] Dana G. Wolf[a]

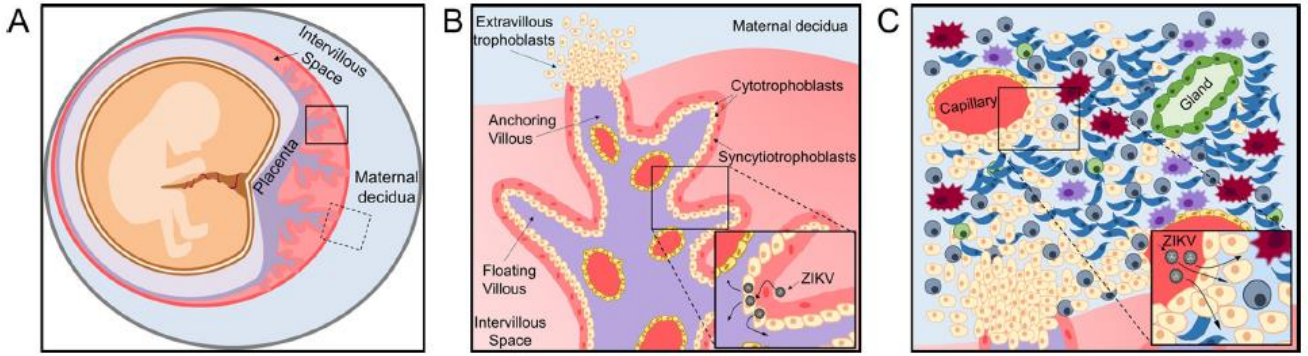


FIG 1 Schematic presentation of the maternal-fetal interface, depicting potential ZIKV transmission routes. (A) Chimeric maternal-fetal interface: the fetus-derived placental villi, composed of floating villi and anchoring villi, invading the maternal decidua. The solid and dashed squares mark a placental villus and the maternal decidua, shown in detail in panels B and C, respectively. (B) Placental anchoring and floating villi, bathed in maternal blood within the intervillous space. The surface of the villous tree is composed of a multinucleated syncytiotrophoblast cell layer, covering a subjacent layer of cytotrophoblasts. The villous core contains stromal cells, Hofbauer cells, and fetal blood capillaries. Extravillous trophoblasts invade and anchor the placenta to the maternal decidua. (C) Overview of the maternal decidua. Invasive extravillous trophoblasts, originating from anchoring villi (see also panel B), partially replace the resident maternal endothelium and commingle with multiple types of maternal cells, including epithelial, decidual, endothelial, and immune cells: decidual NK cells, macrophages, dendritic cells, and T cells. The enlarged insets in panels B and C depict potential routes of maternal-fetal viral transmission: from the maternal [illegible] via the placental villi (B) or through the maternal decidua (C) aspects.

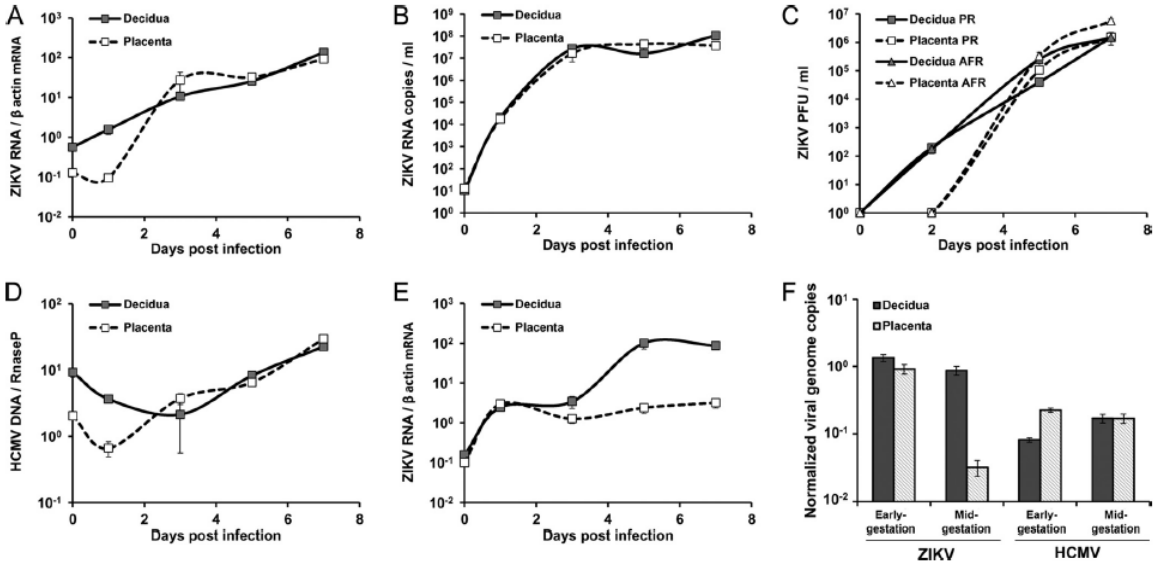


FIG 2 ZIKV infection kinetics in maternal-decidual and fetus-derived chorionic villus organ cultures. Decidual and chorionic villus cultures were infected in parallel with ZIKV or HCMV (5×10^4 PFU/well). (A, B, and E) Levels of ZIKV RNA, determined by quantitative RT-PCR, at the indicated times postinfection. The levels of tissue-associated ZIKV RNA in infected tissues, normalized to β-actin, are shown in panels A and E. The copy number of extracellular ZIKV RNA measured in the supernatants of the early-gestation-infected tissues is shown in panel B. (C) Infectious ZIKV progeny titers in the supernatants of the same infected tissues at the indicated times postinfection, determined by a standard plaque assay. (D) Levels of HCMV DNA, normalized to RNase P, in tissues infected in parallel to those in panel A. (E) Levels of tissue-associated ZIKV RNA following infection of tissues obtained at midgestation. (F) Comparison of normalized tissue-associated ZIKV and HCMV genome copies following infection of tissues obtained at early gestation versus midgestation. The data shown are representative of 3 independent experiments. Each point represents the mean ± SEM from 5 biological replicates. PR, ZIKV strain PRVABC59, isolated in Puerto Rico in 2015; AFR, ZIKV strain MP1751, isolated in Uganda, Africa, in 1962.

Interestingly, we found that midgestation decidual tissues remained susceptible to ZIKV replication, whereas midgestation chorionic villi (obtained from the same donors and infected in parallel) demonstrated significantly reduced viral replication (2-log reduction) compared to the decidual tissues (P 0.001) (Fig. 2E and F)

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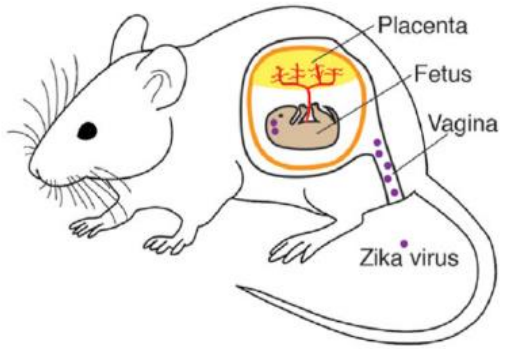
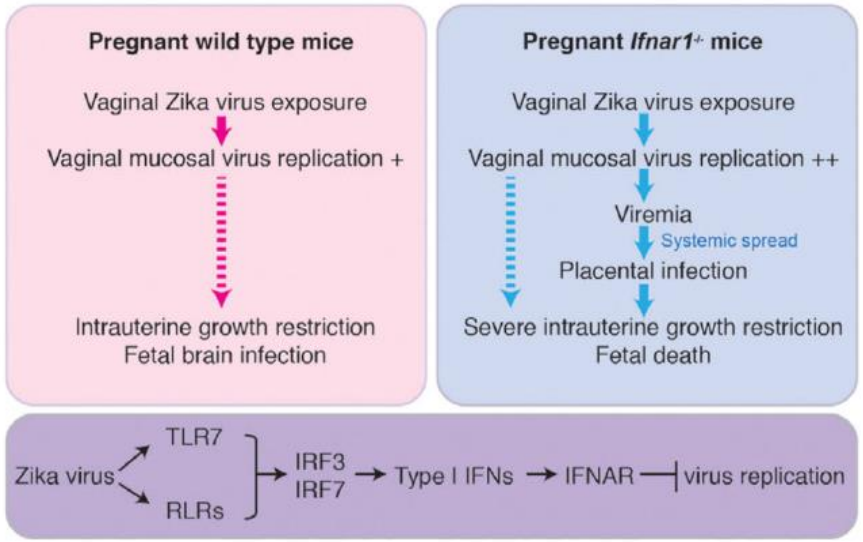
Vaginal Exposure to Zika Virus during Pregnancy Leads to Fetal Brain Infection

Laura J. Yockey[1], **Luis Varela**[2], **Tasfia Rakib**[1], **William Khoury-Hanold**[1], **Susan L. Fink**[1,3], **Bernardo Stutz**[2], **Klara Szigeti-Buck**[2], **Anthony Van den Pol**[4], **Brett D. Lindenbach**[5], **Tamas L. Horvath**[2], and **Akiko Iwasaki**[1,6,7,*]



SUMMARY

Zika virus (ZIKV) can be transmitted sexually between humans. However, it is unknown whether ZIKV replicates in the vagina and impacts the unborn fetus. Here, we establish a mouse model of vaginal ZIKV infection and demonstrate that, unlike other routes, ZIKV replicates within the genital mucosa even in wild-type (WT) mice. Mice lacking RNA sensors or transcription factors IRF3 and IRF7 resulted in higher levels of local viral replication. Furthermore, mice lacking the type I interferon (IFN) receptor became viremic and died of infection after a high-dose vaginal ZIKV challenge. Notably, vaginal infection of pregnant dams during early pregnancy led to fetal growth restriction and infection of the fetal brain in WT mice. This was exacerbated in mice deficient in IFN pathways, leading to abortion. Our study highlights the vaginal tract as a highly susceptible site of ZIKV replication and illustrates the dire disease consequences during pregnancy.



Maternal Zika Virus (ZIKV) Infection following Vaginal Inoculation with ZIKV-Infected Semen in Timed-Pregnant Olive Baboons

Sunam Gurung,[a] Hugh Nadeau,[a] Marta Maxted,[a] Jamie Peregrine,[a] Darlene Reuter,[c] Abby Kimberly Hyatt,[a] Krista Singleton,[a] James F. Papin,[b,c] Dean A. Myers[a]

It is critical to establish NHP models of the vertical transfer of ZIKV that recapitulate human pathogenesis. We hypothesized that vaginal deposition of ZIKV-infected baboon semen would lead to maternal infection and vertical transfer in the olive baboon (*Papio anubis*). Epidemiological studies suggest an increased rate of CZS in the Americas compared to the original link to CZS in French Polynesia; therefore, we also compared the French Polynesian (FP) ZIKV isolate to the Puerto Rican (PR) isolate. Timed-pregnant baboons ($n = 6$) were inoculated via vaginal deposition of baboon semen containing 10^6 focus-forming units (FFU) of ZIKV ($n = 3$ for FP isolate H/PF/2013; $n = 3$ for PR isolate PRVABC59) at midgestation (86 to 95 days of gestation [dG]; term, 183 dG) on day 0 (all dams) and then at 7-day intervals through 3 weeks. Maternal blood, saliva, and cervicovaginal wash (CVW) samples were obtained. Animals were euthanized at 28 days ($n = 5$) or 39 days ($n = 1$) after the initial inoculation, and maternal/fetal tissues were collected. Viremia was achieved in 3/3 FP ZIKV-infected dams and 2/3 PR ZIKV-infected dams. ZIKV RNA was detected in CVW samples of 5/6 dams. ZIKV RNA was detected in lymph nodes but not the ovaries, uterus, cervix, or vagina in FP isolate-infected dams. ZIKV RNA was detected in lymph nodes (3/3), uterus (2/3), and vagina (2/3) in PR isolate-infected dams. Placenta, amniotic fluid, and fetal tissues were ZIKV RNA negative in the FP isolate-infected dams, whereas 2/3 PR isolate-infected dam placentas were ZIKV RNA positive. We conclude that ZIKV-infected semen is a means of ZIKV transmission during pregnancy in primates. The PR isolate appeared more capable of widespread dissemination to tissues, including reproductive tissues and placenta, than the FP isolate.

TABLE 1 Inoculation and sampling procedures[a]

Dam	Procedure(s) on day after initial inoculation							
	−4	0	4	7	11	14	21	28
FP1	Δ	X, O	Δ	X, O	Δ	Δ	Δ	Ω
FP2	Δ	X, O	Δ	X, O	Δ	Δ	Δ	Ω
FP3	Δ	X, O	Δ	X, O	Δ	X, Δ	Δ	Ω
PR1	Δ	X, O	Δ	X, O	Δ	Δ	Δ	Ω
PR2	Δ	X, O	Δ	X, O	Δ	Δ	Δ	Ω
PR3	Δ	X, O	Δ	X, O	Δ	X, Δ	Δ	Ω

l with IACUC approval. X, ng the French Polynesian lations were repeated at samples; Δ, inal wash, blood, and

TABLE 2 ZIKV RNA in maternal reproductive tissues and maternal lymph nodes

Dam	ZIKV RNA level (no. of copies/g)[a]						
	Maternal reproductive tissue				Maternal lymph nodes		
	Uterus	Cervix	Vagina	Ovary	Inguinal	Mesenteric	Axial
FP isolate							
FP1	–	–	–	–	2.5E05	3.3E06	1.0E05
FP2	–	–	–	–	7.6E05	2.0E05	5.4E05
FP3	–	–	–	–	7.7E03	–	5.0E03
PR isolate							
PR1	3.2E03	–	6.9E03	–	2.7E03	–	4.1E03
PR2	4.7E04	–	–	–	2.7E05	5.8E05	3.2E05
PR3	–	–	2.8E03	–	1.2E04	7.1E04	–

TABLE 3 ZIKV RNA in placental tissues

Dam	ZIKV RNA level (no. of copies/g) in placental tissue at site[a]:					
	1	2	3	4	5	6
FP isolate						
FP1	–	–	–	–	–	–
FP2	–	–	–	–	–	–
FP3	–	–	–	–	–	–
PR isolate						
PR1	2.0E05	1.7E05	–	1.7E05	1.6E05	7.9E04
PR2	–	7.5E04	3.6E05	–	3.6E03	6.8E03
PR3	–	–	–	–	–	–

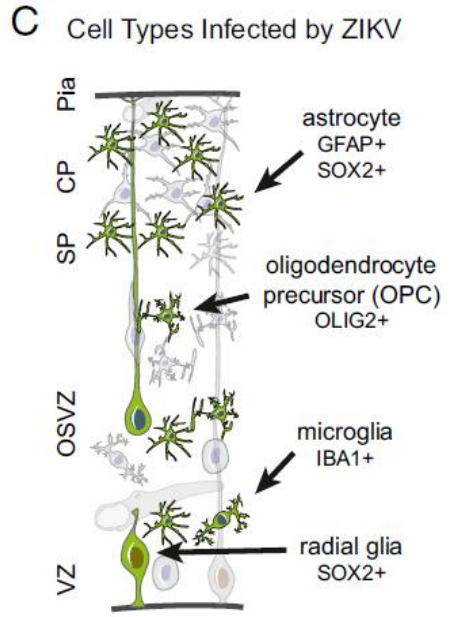
[a]–, below the level of detection.

Zika in the fetal brain

Zika virus cell tropism in the developing human brain and inhibition by azithromycin

Hanna Retallack[a,1], Elizabeth Di Lullo[b,c,1], Carolina Arias[a,d], Kristeene A. Knopp[a], Matthew T. Laurie[a], Carmen Sandoval-Espinosa[b,c], Walter R. Mancia Leon[b,c], Robert Krencik[e,f], Erik M. Ullian[e], Julien Spatazza[b,g], Alex A. Pollen[b,c], Caleigh Mandel-Brehm[a], Tomasz J. Nowakowski[b,c], Arnold R. Kriegstein[b,c,2], and Joseph L. DeRisi[a,2]

Ex vivo brain biopsies: ZIKV preferentially infected neural stem cells, astrocytes, oligodendrocyte precursor cells, and microglia, whereas neurons were less susceptible to infection.



Zika Virus Infects Human Fetal Brain Microglia and Induces Inflammation

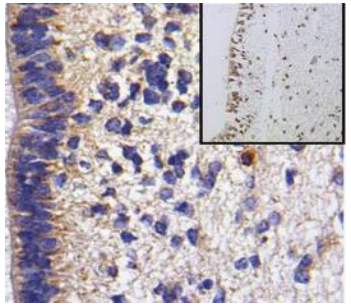
Fok-Moon Lum,[1] Donovan K. S. Low,[1] Yiping Fan,[2] Jeslin J. L. Tan,[1] Bernett Lee,[1] Jerry K. Y. Chan,[1,2,3,4] Laurent Rénia,[1] Florent Ginhoux,[1] and Lisa F. P. Ng[1,5]

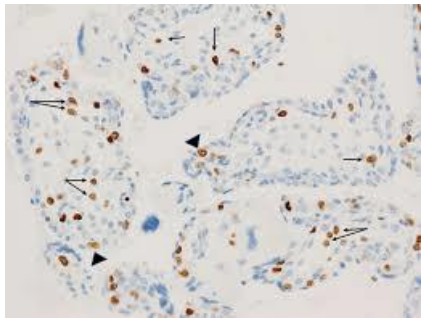
[1]Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR), [2]Department of Reproductive Medicine and [3]KK Research Centre, KK Women's and Children's Hospital, and [4]Cancer and Stem Cell Program, Duke–NUS Medical School, Singapore; and [5]Institute of Infection and Global Health, University of Liverpool, United Kingdom

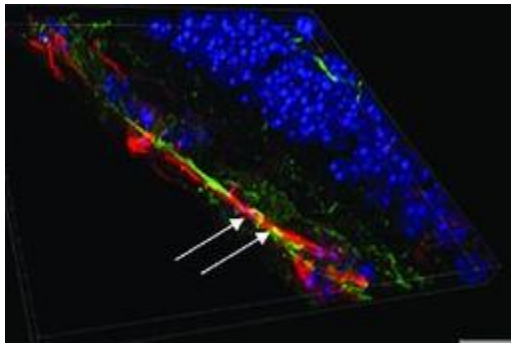
Methods. Investigations were performed with brain cell preparations obtained from 9 donors. Virus infectivity was assessed by detection of virus antigen by flow cytometry together with various hematopoietic cell surface markers. Virus replication was determined by viral RNA quantification. Cytokine levels in supernatant obtained from virus-infected fetal brain cells were measured simultaneously in microbead-based immunoassays.

Results. We also show that ZIKV infection was particularly evident in hematopoietic cells with microglia, the brain-resident macrophage population being one of the main targets. Infection induces high levels of proinflammatory immune mediators such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), and monocyte chemotactic protein 1 (MCP-1).

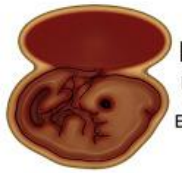
Conclusions. Our results highlight an important role for microglia and neuroinflammation during congenital ZIKV pathogenesis.







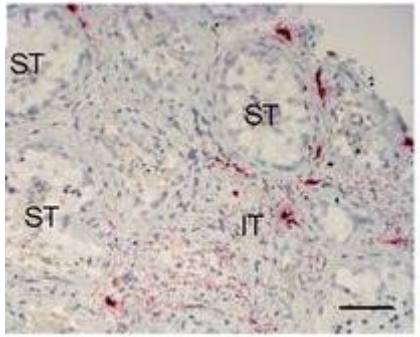
Brain
Neural progenitors
Mature neurons
Astrocytes

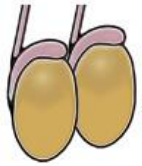


Placenta
Hofbauer cells
Trophoblasts
Endothelial cells

Eye
Ganglion cells
Bipolar neurons
Optic nerve
Cornea
Anterior chamber aqueous humor

ZIKV Tropism

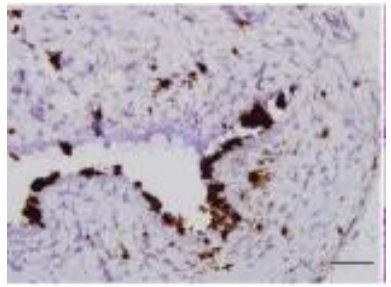


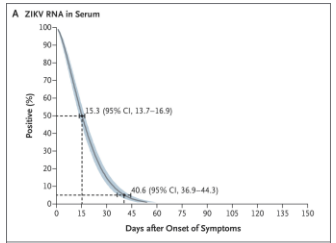


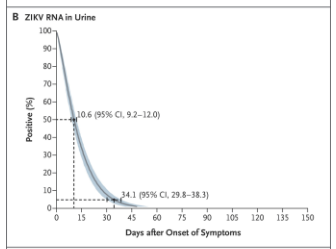
Testis

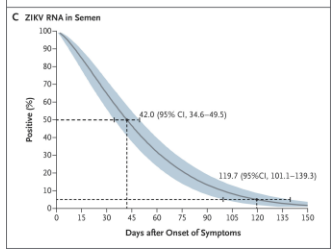
Uterus and Vagina
Vaginal epithelium
Uterine fibroblasts

Body fluids
Tears
Saliva
Semen
Cervical mucus
Urine



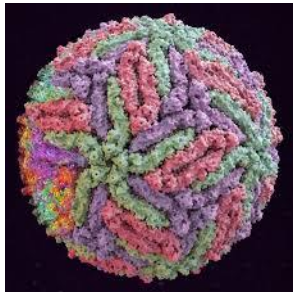






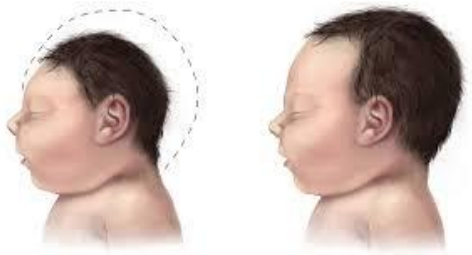
Zika natural history

Where and when: from Africa to America travelling East



Who and why: african and asian lineages have epidemic potential

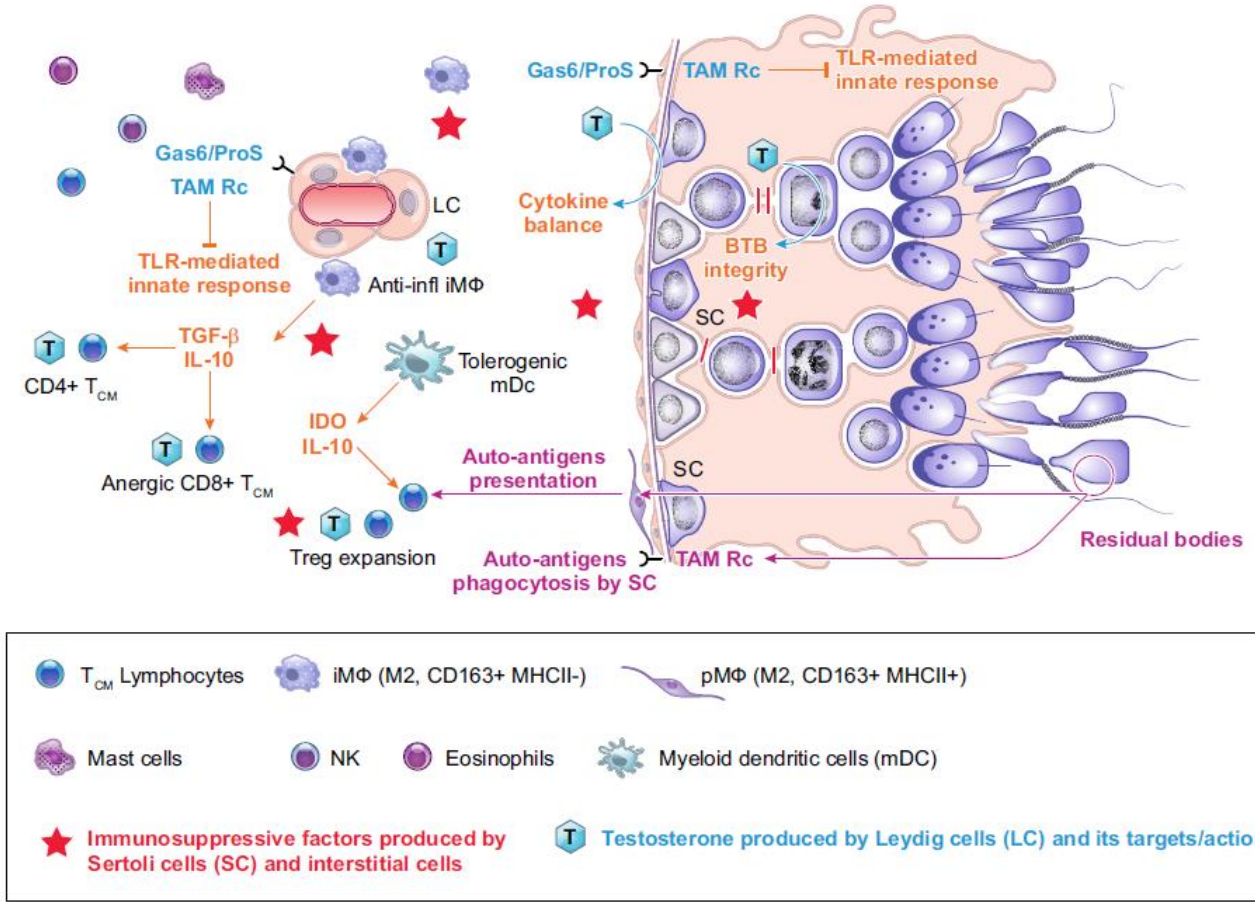
How: arthtopode and alternative route of transmission



Which target: wide range of target organs

The testis is an immune-privilege organ

- **Restrict acquired immunity** to protect germ cells from non-self recognition: **systemic immune response deleterious for fertility**



Actors :

- **Blood-testis barrier :** Sertoli cell tight junctions
- **Range of immuno-suppressive mechanisms**:

  - Secretion of anti-inflammatory molecules: IL-10, TGFβ, IDO, testosterone…
  - Resident immune cells: anti-inflammatory macrophages, tolerogenic DC, Treg, anergic T cells…
  - TAM inhibition of TLR-mediated responses in Sertoli, macro, DC
  - Immunosuppressive testosterone

Ideal shelter for viruses, unless breakage of immune privilege by inflammation (eg Mumps orchitis) or strong antiviral immunity

Le Tortorec...Dejucq-Rainsford, Physiol Rev

Open Forum Infectious Diseases

BRIEF REPORT

Rescue of Replication-Competent ZIKV Hidden in Placenta-Derived Mesenchymal Cells Long After the Resolution of the Infection

THE *AEDES* MOSQUITO

The *Aedes aegypti* mosquito, which transmits dengue, chikungunya and yellow fever, is the main vector for Zika. The mosquito has distinctive white markings on its legs and thorax, and breeds in clean, standing water.

BLOOD SUCKERS

Mosquitoes become infected with Zika when they bite an infected person.

BREEDING

The female's eggs develop in water but can survive for up to one year without water.

HABITAT

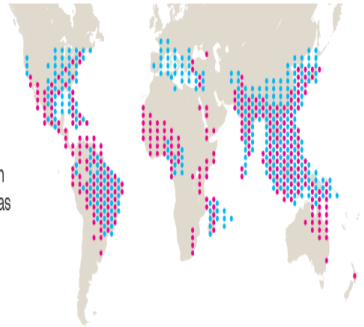
Aedes mosquitoes often live in urban areas.

PEAK TIME

Peak biting activity occurs in early mornings and late afternoons.

GLOBAL DISTRIBUTION

A. aegypti *A. albopictus*

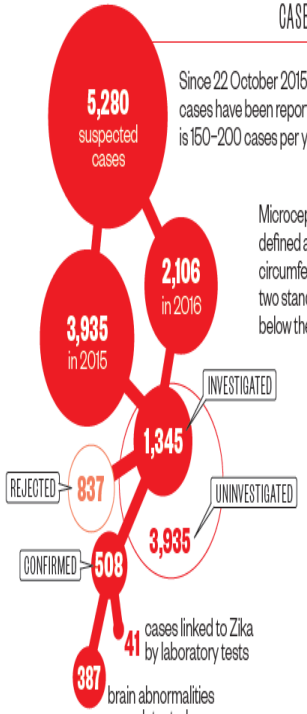


Aedes aegypti lives in tropical and subtropical climates, but *Aedes albopictus*, which is also capable of transmitting Zika, can survive in cooler climates, such as Southern Europe. *A. albopictus* has not yet been shown to be a significant vector in the field.

MICROCEPHALY

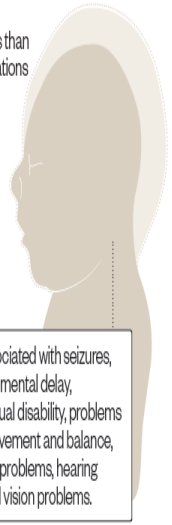
A causal link between Zika virus and microcephaly has not yet been established but evidence is mounting.

CASES IN BRAZIL



Since 22 October 2015, 5,280 suspected microcephaly cases have been reported in Brazil; the usual figure is 150–200 cases per year.

Microcephaly is usually defined as a head circumference less than two standard deviations below the average.

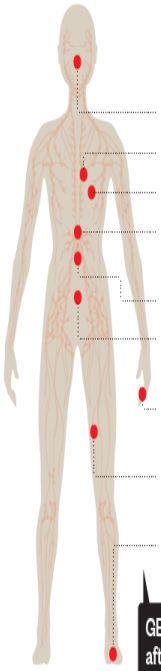


It is associated with seizures, developmental delay, intellectual disability, problems with movement and balance, feeding problems, hearing loss and vision problems.

GUILLAIN-BARRÉ SYNDROME

An increase in rare neurological condition Guillain-Barré syndrome (GBS) has been reported in Zika hit areas, but a causal link is yet to be established.

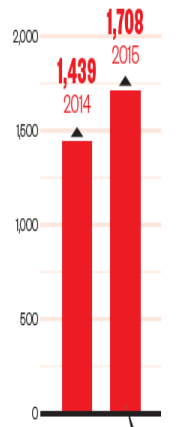
SYMPTOMS INCLUDE



- Difficulty moving eyes, talking, chewing or swallowing
- Fast heart rate
- Difficulty breathing
- Paralysis
- Severe lower back pain
- Loss of bladder control
- Tingling in fingers and toes
- Muscle weakness in legs and upper body
- Difficulty walking steadily

GBS usually occurs 2–4 weeks after an acute infection.

CASES IN BRAZIL



Brazil first reported an association between Zika and GBS in July 2015.

VACCINE DEVELOPMENT

Several companies are working to develop a vaccine against Zika but a commercially available product is likely to be several years away.

Strategy
- Live, attenuated
- DNA-based
- Inactivated
- Recombinant

COMPANY	EXPERIENCE	
Bharat Biotech, Hyderabad, India	Japanese encephalitis, chikungunya	
National Institute of Allergy and Infectious Diseases (NIAID), Maryland, with Butantan Institute, Brazil	Dengue	
National Institute of Allergy and Infectious Diseases (NIAID), Maryland, United States	West Nile Virus	
Inovio Pharmaceuticals, Plymouth Meeting, Pennsylvania, and GeneOne Life Science, Seoul, South Korea	West Nile Virus, dengue, chikungunya, MERS, Ebola	
NewLink Genetics, Massachusetts, United States	Ebola	
The Jenner Institute, Oxford, UK	Hepatitis C, Flu, Ebola, malaria, HIV	
Protein Sciences, Meriden, Connecticut, United States	Influenza, SARS, Ebola	
Hawaii Biotech, Honolulu, United States	Dengue, West Nile virus, chikungunya	
Sementis, Melbourne, Australia	Chikungunya	
Sanofi Pasteur, Paris, France	Dengue, yellow fever	TBC

Merck, GlaxoSmithKline, Johnson & Johnson, Pfizer and Takeda have all said that they evaluating their vaccine technologies for the potential of developing a Zika vaccine.

Infographics: Alisdair Macdonald (alisdairmacdonald.co.uk). Editorial adviser: Peter Horby, professor of emerging infectious diseases and global health, University of Oxford.

Table 1. Intrauterine viral infections

Virus	Infection	Refs
Cytomegalovirus	Prenatal infection affects 1–2% of live births. Virus replicates in the uterus, infects the placenta, then is transmitted to the fetus. Transmission rate is high in women with primary infection (50%).	[2,17,18]
Herpes simplex virus 2	Infrequent prenatal infection. Transmission primarily at delivery (80%). Possible ascending infection after membrane rupture.	[20,21,53,54]
Human immunodeficiency virus	Transmission primarily at delivery. Isolated cytotrophoblasts infected *in vitro*.	[23,24,55]
Hepatitis B virus	Transmission primarily perinatal. Some intrauterine infection from maternal blood (5%).	[56]
Hepatitis C virus	Intrauterine infection and at delivery (2–12%).	[57]
Parvovirus B19	Placental infection associated with inflammatory cytokines. Complications in early gestation.	[58]
Rubella virus	Placental infection during primary maternal infection. Transmission in first trimester (80%) and second trimester (25%).	[54]
Human papilloma virus	Infection at birth.	[25,53]
Varicella zoster virus	Congenital infection low (2%). Transmission during primary infection in late gestation (25–50%).	[54]

on the thickness of the separating layers and is affected by the number and type of layers. In the hemochorial placenta, the maternal blood vessels are completely destroyed by the fetal trophoblasts that directly contact maternal blood. Maximal exchange surface is provided by a tree-like branching pattern of the chorion, resulting in floating villi. Rats and mice have a hemotrichorial placenta, whereas humans, rhesus monkeys and guinea pigs have a hemodichorial and, at term, hemomonochorial placenta. In species with invasive implantation, the trophoblast forms a syncytium that can be passed only transcellularly by transport mechanisms, such as diffusion, facilitated transfer, active transport and vesicular transfer. In humans, the syncytiotrophoblast is the decisive barrier that limits or supports transplacental transfer processes. The architecture of the guinea pig and rhesus placentas, similar to the human, could allow studies of prenatal infection in these animal models.

Hemochorial: Trophoblast surface membrane covered by maternal blood.
Hemodichorial: Syncytium covered on one side by cytotrophoblasts.
Hemomonochorial: Syncytium without underlying cytotrophoblasts.
Hemotrichorial: Syncytium covered on two sides by cytotrophoblasts.
Intervillous space: Maternal blood space between placental villi.
Invasive cytotrophoblasts: Differentiating cells that leave the basement membrane, switch to an endothelial phenotype and invade the maternal–fetal junctional zone.
Syncytiotrophoblast: Formed by trophoblast cell fusion, covers the villus surface.
Trophoblast: Epithelial cells that compose the developing placenta.
Villus stroma: Connective tissue, fibroblasts, macrophages and fetal blood vessels that are surrounded by trophoblasts.

Viral receptors and tropism

It has been reported that the entry of the virus is through the interaction of E protein with TAM (Tyro3, Axl, and Mer), TIM (T cell/transmembrane, immunoglobin, and mucin), AXL (AXL receptor tyrosine kinase) or DC-SIGN (Dendritic cell-specific intracellular adhesion molecule-3-G rabbing N on- integrin) receptors

the attachment depends on the interaction between viral E protein and receptor. AXL, Tyro3, TIM1 and DC-sign have been identified to be the receptors for ZIKV to infect its permissive cells (52, 53). But the conclusive statement still needs further investigation because recent results have been disputed (54, 55). Then, viral entry is completed by endocytosis via endosomes. As the capsid breaks apart in the cytoplasm, there is a release of the viral RNA into endoplasmic reticulum (51). The positive-sense RNA genome will be translated by the host ribosomes attached to the endoplasmic reticulum, which results in a polyprotein processed by proteolytic activity for the structural and non-structural proteins (51). When all parts are assembled correctly, the virion will be transported out of the cell by endosomal sorting complexes through ERGIC (endoplasmic reticulum-Golgi intermediate compartment) to the Golgi apparatus (51). As exocytosis is used as a mature virion exits the cell in which a single ZIKV can rapidly increase the amount of the virus as the virus will take over the host immune defense system (51).