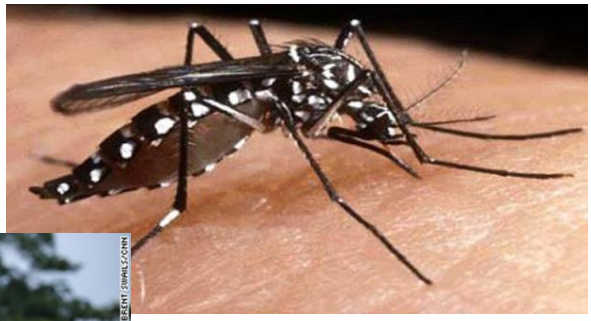
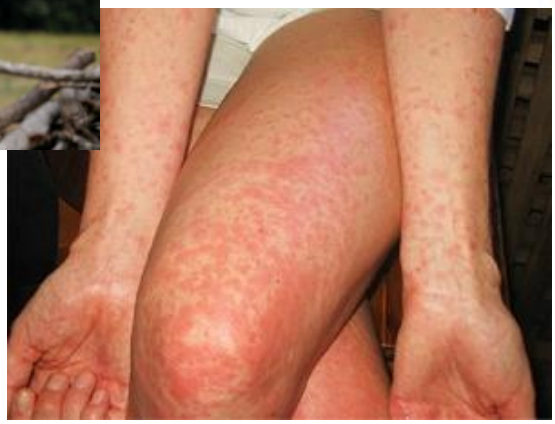
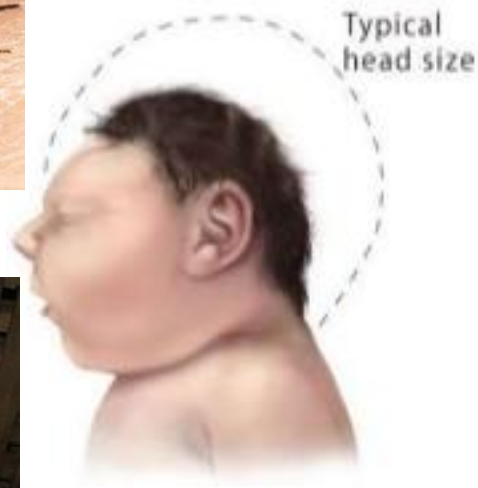
Internal Medicine Ground Rounds
September 16, 2016

Zika
The Arbovirus du Jour







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This is to acknowledge that Robert W. Haley, MD has disclosed that he does not have any financial interests or other relationships with commercial concerns related directly or indirectly to the program. Dr. Haley will not be discussing off-label uses in his presentation.

Biographical Information:

Robert W. Haley, M.D., is Professor of Internal Medicine, Distinguished Teaching Professor, and holder of the U.S. Armed Forces Veterans Distinguished Chair in Medical Research Honoring America's Gulf War Veterans endowed by Ross Perot and the Perot Foundation. After serving 10 years at the U.S. Centers for Disease Control and Prevention (CDC), he joined the UT Southwestern faculty, founding the Division of Epidemiology which he heads. In addition to attending on Parkland Medicine and teaching a course in epidemiology for the clinical investigator and SAS computing for research in the Department of Clinical Science, his research currently focuses on the neurological and genetic basis for sarin-related Gulf War illness and the possible role of paraoxonases in coronary atherosclerosis and congestive heart failure, and he leads the Texas Medical Association's clean air policy development. While in medical school, Dr. Haley worked in Dr. James Luby's virology laboratory on an epidemiologic study that demonstrated continuing transmission of arboviruses in Dallas County. Recently he rekindled that interest with a collaborative analysis of Dallas' 2012 West Nile virus epidemic that appeared in *JAMA*.

Purpose and Overview:

The purpose is to review the literature on the ongoing Zika epidemic, much of which has appeared in the past 4 months. Although ZIKV was identified in Africa in 1947 and caused epidemics of a mild illness on South Pacific islands in the 2000s, it was recognized as a significant public health threat only in 2015 with the discovery of an epidemic of microcephaly in offspring of Brazilian women who recalled a mild rash illness months earlier. In the succeeding months it spread to other Latin American countries and to south Florida. Zika is spread almost entirely by the *Aedes aegypti* mosquito which breeds close to human activity and persists largely through a mosquito-human-mosquito cycle. Zika is spread rarely by sexual transmission, mostly from symptomatic men to women, but asymptomatic and female-to-male spread have been described. ZIKV appears to damage fetal brains by persistently attacking human neural progenitor cells and avoiding immunologic response. Diagnosis of Zika infection is through PCR testing of serum and urine during the acute illness, positive PCR persisting longer in urine, and by serologic testing for anti-Zika IgM with confirmation by PRNT. In the U.S. autochthonous spread is contained by detecting cases and mounting intensive mosquito control in a Zika alert zone around the case. Aerial insecticide spraying is less effective for Zika than for West Nile epidemics. Genetically modified male mosquitoes appear to be a safe, effective strategy for *Aedes aegypti* eradication in circumscribed epidemic areas, but "perifocal" insecticide treatment and a Zika or polyvalent arboviral vaccine will be needed for continental eradication.

Educational Objectives:

1. Review the epidemiologic evidence for epidemics of Zika illness, microcephaly and Guillain-Barré syndrome.
2. Learn how characteristics of the *Aedes aegypti* mosquito and its breeding habits close to human activity determine the epidemiology and control of Zika infection.
3. Explore the recent evidence on the pathophysiology of how ZIKV damages fetal brains.
4. Review the evidence for unusual modes of transmission of Zika infection such as sexual transmission, female to male sexual transmission, and asymptomatic transmission.
5. Understand the strategies for diagnosing and managing Zika infection in patients, controlling foci of autochthonous spread, personal protection, and Zika eradication.

Introduction

In the past 12 months epidemic Zika virus (ZIKV) infection has swept through South and Central America and now into south Florida, spawning secondary epidemics of Guillain-Barré syndrome and infant microcephaly.[1] With tens of thousands of acute Zika infections, Brazil has reported over 4,300 cases of microcephaly, 1,845 of which meet the strict case definition, in contrast to 62 in the rest of Latin America, 21 in the U.S. and 1 in Canada. So far the U.S. mainland has confirmed 2,686 travel-associated Zika infections, 56 autochthonous, mosquito-borne infections (all in South Florida), 23 sexually transmitted cases, and 7 cases of Zika-associated Guillain-Barré syndrome.

ZIKV is but the latest of a parade of arthropod-borne viruses (arboviruses) to have swept through the Americas, including yellow fever, St. Louis encephalitis, dengue fever, West Nile, and chikungunya. The Zika story is a cautionary tale of how a little known exotic, seemingly innocuous pathogen can suddenly, with little warning, erupt in modern Western cities with serious health effects and limited means of combatting it.

A Sudden Epidemic of Microcephaly in Brazil

IN AUGUST 2015, something strange began happening in the maternity wards of Recife, a seaside city perched on the northeastern tip of Brazil where it juts out into the Atlantic.

"Doctors, pediatricians, neurologists, they started finding this thing we had never seen," said Dr. Celina M. Turchi, an infectious diseases specialist at the Oswaldo Cruz Foundation, Brazil's most famous scientific research institute.

"'Children with normal faces up to the eyebrows, and then you have no foreheads," she continued. "The doctors were saying, 'Well I saw four today,' and 'Oh, that's strange because I saw two.'

Some of the children seemed to breastfeed well and did not seem to be ill, she said.

Others cried and cried, in a weird, high-pitched wail, as if they were in constant pain and could not be comforted.

Some had seizures, one after the other, their tiny bodies wracked by spasms. If the seizures lasted long enough, they could disrupt the babies' breathing and heartbeat. Those babies often died after a few days.

Other seemed unable to flex their arms and legs or their eyes jumped around erratically, not seeming to focus, perhaps not seeing anything.

Others did not react to noises and appeared to be deaf.

Others could not swallow. If they did not get intensive care and feeding through nose tubes, they, too, soon died."[2]

Over the last half of 2015 the background rate of microcephaly (6 per 10,000 live births) increased 40-70 fold in the 4 hardest hit Brazilian states.[3]

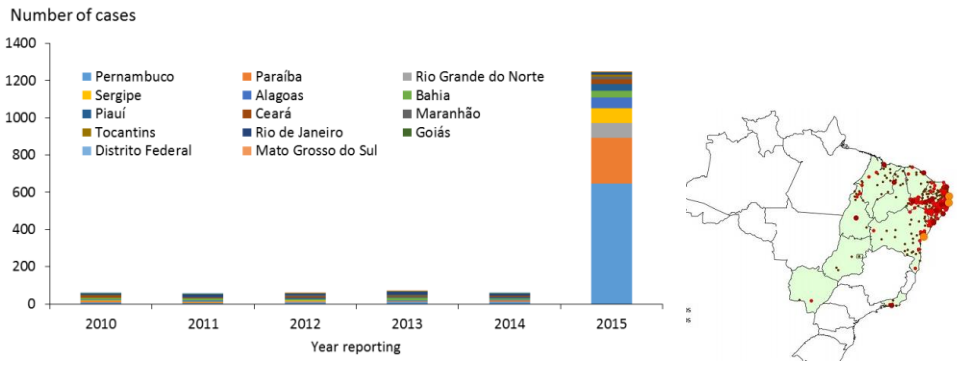


Fig. 1. Cases of microcephaly in Brazil by state and year.

Microcephaly

Microcephaly ("small head") is defined as head circumference significantly (>2 or 3 SD) below the norm for gestational age and gender (Fig. 2). It may be caused by failure of brain growth to expand the pliable skull or massive brain shrinkage with skull collapse. Its many known causes include TORCH syndrome (Toxoplasma, Other including Rubella, CMV, and Herpes), rarely chikungunya and dengue virus infections (probably not West Nile infection), and non-infectious causes such as drug or alcohol abuse, fetal alcohol syndrome, untreated PKU, toxic chemicals (e.g., mercury), radiation, severe malnutrition and fetal chromosome abnormalities (e.g., Downs, craniosynostosis).

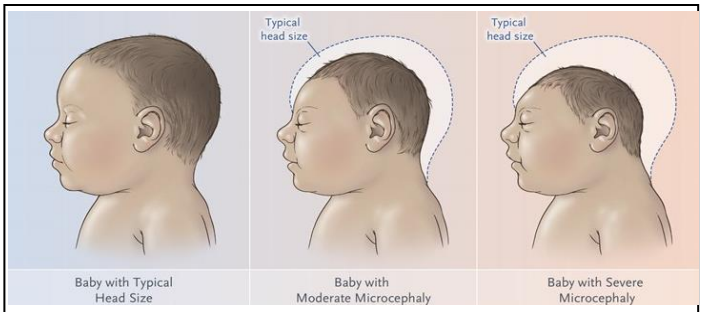


Fig. 2. Grades of microcephaly (www.cdc.gov)

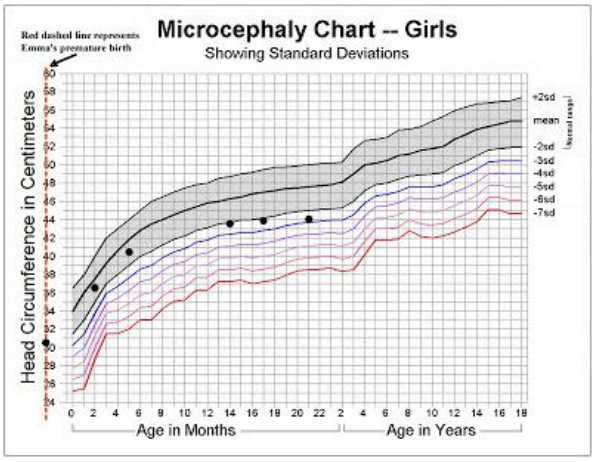


Fig. 3. Identification of microcephaly

The condition is rarely diagnosable before early 3rd trimester (average 28 weeks) when low head circumference begins lagging behind the normal wide variation of normal (Fig. 3)

Zika-related microcephaly is usually associated with MRI findings including cerebral calcifications (at the cortex-white matter junction), dilated ventricles, flattened gyri (smooth brain surface), cerebellar or brainstem hypoplasia, or absent corpus callosum.[4]

Doença Misteriosa

> As the horrified doctors compared notes, one thing stood out: many of the mothers mentioned that, months earlier, they had had the *doença misteriosa*—Portuguese for the 'mystery disease'—that had first appeared nine months earlier in Recife, Salvador, Natal, and Fortaleza, the cities of Brazil's arid northeast.
>
> Back then, the disease had not seemed a big deal. Everyone appeared to have the symptoms: an itchy pink rash; fever and chills; bloodshot eyes; headaches and joint pains.
>
> Nonetheless, many people had gone to local clinics or emergency rooms because they were worried. The symptoms looked like early malaria or yellow fever. They looked most like the first symptoms of dengue, a disease Brazilians feared because of its unique power to deliver the double tap: The first time you get dengue, you were miserable. It was called "break-bone fever" because it felt as if someone had take a sledgehammer to your arms, legs, and neck. Still, you usually recovered. It was the second bout that could kill you. A second infection—with a different one of dengue's four strains—was worse, and had a chance of turning into dengue hemorrhagic fever.[2]

Acute Zika is usually a mild illness, with incubation period mostly 4-7 days (up to 14), lasting around 6-12 days, featuring the following symptoms: itchy maculopapular rash (90%); fever, low grade and brief (65%); arthralgias or arthritis (65%); non-purulent conjunctivitis, keratitis and uveitis (55%); headache (45%); peripheral edema (19%); vomiting (10%); upper and lower extremity edema; aphthous ulcers; testicular pain and hematospermia (rare).[5] Most importantly, 80% of infections are entirely asymptomatic. Virus becomes undetectable in blood, and seroconversion occurs as the symptoms end, between 6 and 12 days from onset.[6]

Extensive interviews with patients and doctors placed the start of the acute Zika epidemic in December 2014 (Fig. 4).[2] Clinical and laboratory evidence found activity beginning at least by January 2015 and peaking in May.[7]

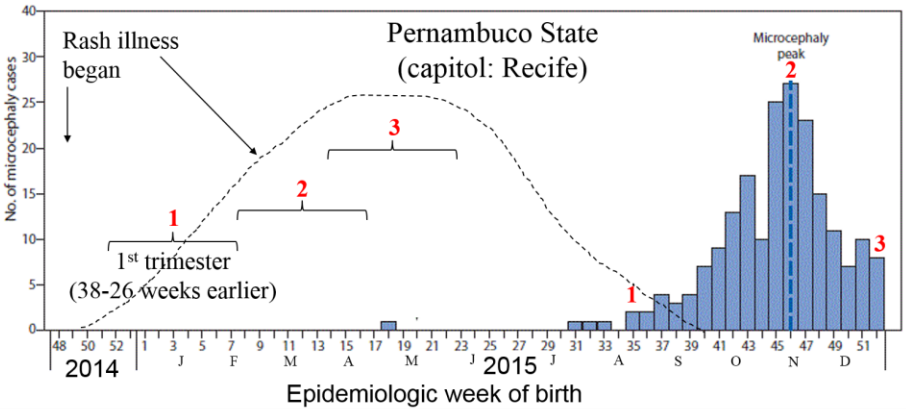


Fig. 4. Findings of the initial epidemic investigation of the microcephaly epidemic in Brazil.

The first cases of microcephaly began in August 2015; cases peaked in mid-November and declined into early January 2016 (Fig 4). Counting back 38-26 weeks from the first microcephaly cases ("1" in Fig. 4) places their 1st trimester in late December to mid-February, coincident with the start of Zika activity.[8] Likewise, the 1st trimester of cases at the peak of the microcephaly epidemic ("2" in Fig. 4) was from mid-February to early April, approaching the peak of the epidemic of acute Zika illness.

An epidemic investigation of 19 states in northeastern Brazil where records were available found the rate of microcephaly of 2.80 (1.86-4.05) cases per 10,000 births in the 15 states with laboratory-confirmed Zika activity, compared with 0.60 (0.22-1.31) in the 4 states with no Zika activity.

The Zika Virus

A member of the *Flavividae* family along with dengue, yellow fever, Japanese encephalitis & West Nile (Table 1). It was named for the Ziika Forest in Uganda, where in 1947 it was identified by researchers in a febrile monkey. It is transmitted primarily in urban settings in a **mosquito → human → mosquito cycle** involving primarily ***Aedes aegypti*** (the yellow fever mosquito), but to a far lesser extent by ***Aedes albopictus*** (the Asian Tiger). It is also transmitted vertically, sexually and most likely by transfusion. A single-stranded RNA virus, prone to mutating, ZIKV has mutated significantly in the 70 years since it was first discovered.[9] It has been sustained in the jungles of Africa through a **mosquito → primate → mosquito cycle**.

Table 1. Major arboviruses in 4 families that cause human disease

	Flaviviridae	*Togaviridae*	*Bunyaviridae*	*Reoviridae*
Active in U.S.	Yellow fever* Dengue* Zika* St. Louis† West Nile† Powassan¶	Chikungunya* Western equine* Eastern equine¶	La Crosse* California* Jamestown Canyon*† Heartland virus¶	Colorado tick fever¶ Bluetongue¶
Not in U.S.	Japanese† Murray Valley† Spondweni* Tick-borne encephalitis¶	Venezuelan equine*† Ross River*† O'nyong'nyong¶	Crimean-Congo hemorrhagic fever¶ Bunyamwera* Rift Valley fever† Toscana¶	Equine Enceph-alosis virus¶ Banna† African Horse sickness¶

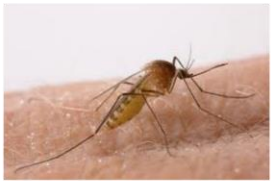
Main vector: **Aedes* †*Culex* ¶Other (mosquitoes, ticks, sand flies, midges)

Aedes vs *Culex*

At present the two most serious arboviral threats to the U.S. are West Nile virus (WNV) encephalitis and Zika. To understand the stark difference in public health approaches to control them, it is important to contrast their key characteristics (Table 2, next page).

Aedes, the main vector for yellow fever, dengue, chikungunya and Zika, is a large mosquito that has only humans (primates) as host; whereas, *Culex*, a small mosquito and the main WNV vector, primarly feeds on birds, with humans as incidental victims only in large bird epizootics. Consequently, in human epidemics from *Aedes*, only rare mosquitoes are infected, while in human epidemics from *Culex*, mosquitoes are infected at high rates—determining different surveillance strategies.

Table 2. Contrasting characteristics of *Aedes* and *Culex* mosqitoes

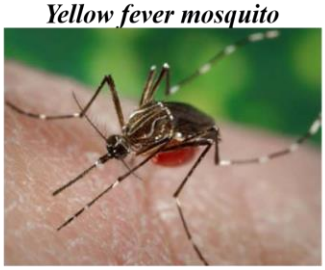
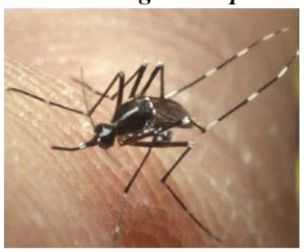
Aedes	*Culex*
	
• Spreads yellow fever, dengue, chikungunya, Zika	• Spreads West Nile
• Mosquito→**human**→mosquito	• Mosquito→**bird**→mosquito ↘human
• Sipper – must bite 3 or 4 times	• Gulper – needs to bite only once
• Human loving – lives near homes	• Canopy dweller – lives in tree tops
• Day biter	• Dawn/dusk biter

Since *Aedes* bite multiple times, live in and around human houses, and are day biters, the best control measures are mosquito traps, insecticides with residual activity, and elimination of breeding conditions. Since *Culex* bite only once, largely dwell in the tops of trees, and are dusk/dawn biters, aerial insectide spraying at night is highly effective.

Aedes aegypti vs *Aedes albopictus*

A. aegypti is a far more efficient vector for its viruses in the Americas than *Aedes albopictus* because of trait differences (Table 3). Because *A. albopictus* has a more extensive geographical distribution (Fig. 5), however, there is concern that it could play a bigger role in spreading Zika than is presently expected, extending the range of Zika further into the northeast and upper mid-west. *A. albopictus* has been the main vector for recent dengue epidemics in Hawaii.[1]

Table 3. Contrasting characteristics of *Aedes aegypti* and *Aedes albopictus* mosqitoes

Yellow fever mosquito	*Asian tiger mosquito*
	
• Imported with slave trade in 1600s	• Imported from Asia in used tires in 1980s
• Lyre-shaped silvery pattern	• Single silvery stripe
• Urban: breeds/lives near humans	• Sylvan: breeds/lives in vegetation
• Loves man-made containers	• Prefers tree holes and vegetation
• Slips indoors, hides in closets	• Mostly outdoor, garden mosquito
• Sneaky biter (primarily humans)	• Aggressive biter (animals & humans)
• Highly efficient transmitter	• Inefficient transmitter (important??)

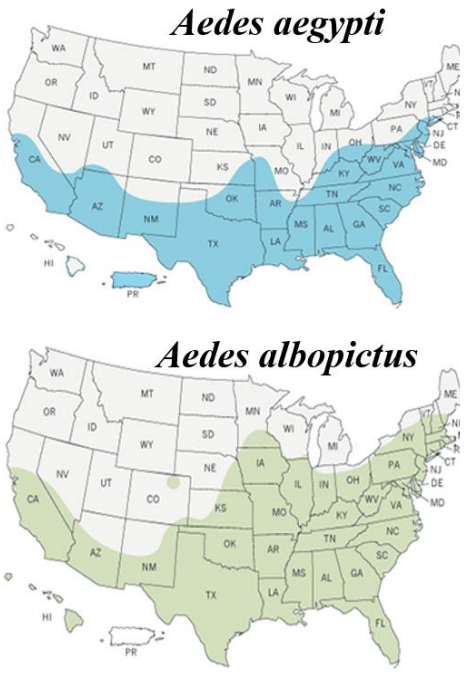


Fig. 5. New extended geographical distributions two *Aedes* species

The range of *Aedes aegypti* has expanded northward over the past several decades, orignally ending just south of Dallas over to South Carolina, but now extending up into Missouri to Massachusettes (Fig. 6). This has occurred at the same time as the northern and southern jet streams have moved poleward by atmospheric warming, particularly in the northern hemisphere, consequently widening the tropical belt.[10] The range of *Aedes* is highly related to temperature and rainfall.

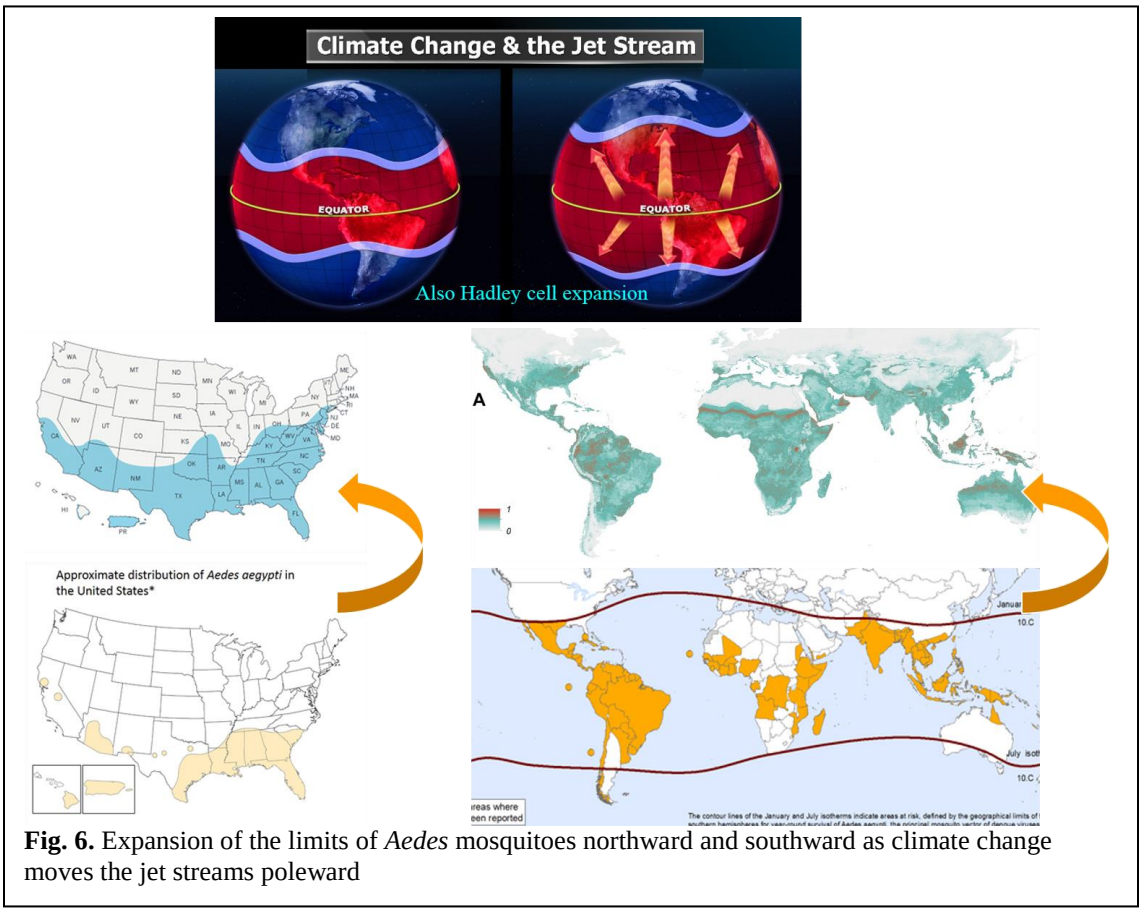


Fig. 6. Expansion of the limits of *Aedes* mosquitoes northward and southward as climate change moves the jet streams poleward

Aedes aegypti evolved in close relationship with man. It breads most readily in water sources close to human habitats. Beginning in the 1980s the accumulation of plastic trash that resists biodegradation has proved especially advantageous for the vector. Consquently, dengue, chikungunya and now Zika viruses spread explosively in Latin American favelas in which trash accumulates and window screens are scarce.

Fig. 7. *Aedes aegypti* spreads infection explosively in favelas with plastic trash and old tires plus windows without screens.

Near Eradication of *Aedes aegypti* from Latin America, 1945-1970

From 1945 to 1970 the Pan American Health Organization (PAHO) nearly eradicated the *Aedes aegypti* mosquito from Latin America by a military-style campaign to eliminate standing water and "perifocal" spraying with DDT (Fig. 8). **With the lack of U.S. participation**, PAHO abandoned it in the 1970s. Plastic trash, pesticide resistance, and increased travel allowed *Aedes* to return, leading to resurgence of dengue in the 1980s and the first appearance of chikungunya in 2013 and Zika in 2015.

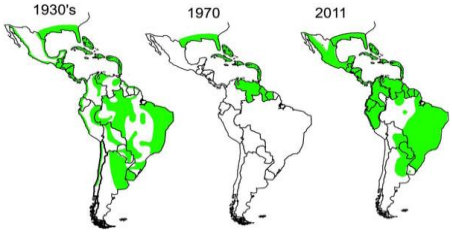


Fig. 8. Near eradication of Aedes aegypti, 1945-1970

International Spread of Zika

After its discovery in Uganda, East Africa, in 1947, Zika was next identified in 3 ill humans in Nigeria, West Africa, in 1953.[1] Serosurveys in the 1950s found high rates of past Zika infection in Africa, Southeast Asia, the Phillipines, and Malasia. From there an evolved Asian strain began spreading across the Pacific (Fig. 9).

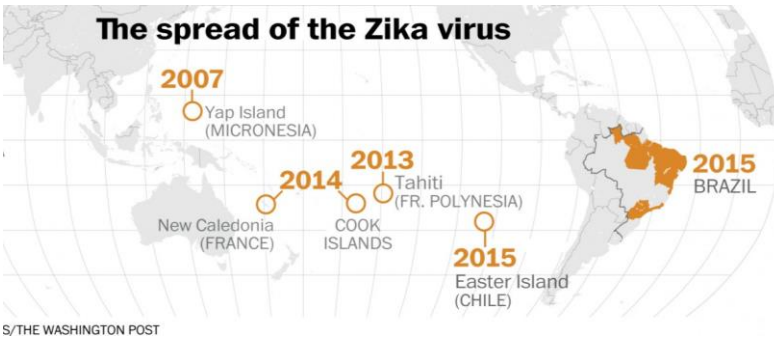


Fig. 9. The spread of Zika virus across the Pacific

The Yap Epidemic, 2007

In 2007 the first Zika epidemic to be recognized occurred on **Zap Island**, Federated States of Micronesia, a chain of islands located east of the Phillipines and north of Indonesia with a population of 7,391. Physicians on the island reported an outbreak of an unusual illness involving a rash, fever, conjunctivitis and arthralgias, too mild to be dengue.

Under a relationship dating back to World War II, Micronesia received epidemic assistance from CDC's Arbovirus Reference Laboratory in Ft. Collins, CO. A group of CDC Epidemic Intelligence Service officers and a Ft. Collins team sent serum samples from cases back to the Ft. Collins Lab for PCR and serological testing for IgM and neutralizing antibody. Of 185 patients tested, 26% had confirmed Zika, 32% probable Zika, 39% suspected, and 3% with no evidence of Zika.[5]

In a serosurvey of 200 randomly selected households on Zap Island, they found that **73%** of 557 individuals had Zika IgM antibodies, suggesting recent Zika infection, and **18%** of the seropositive residents had had a compatible clinical illness.[5] While the rate of seropositivity affected all age groups about equally, the attack rate of clinical Zika illness increased with age.

A later look-back study of medical records found no cases of microcephaly related to the Zika epidemic, but because of the small size of the population, only 3 additional cases would have been predicted.

In an environmental survey they found that 87% of the 170 randomly selected houses had water containers infested with mosquito larvae. Of the 1366 water-holding containers found, 43% contained mosquito larvae. Large rain barrels contained thousands of larvae, while coconut shells contained only a few. Although 9 mosquito species were found, 83% of the infested containes had *Aedes hensilli*, and no ther species inhabited more than 3%.[5,11]

The Zika Epidemic in French Polynesia (Tahiti), 2013-2014

The next Zika epidemic occurred from October 2013 to April 2014 in the islands of French Polynesia with its main population center on Tahiti.[12] A serosurvey estimated that **66%** of its 270,000

population had been infected.

Look-back for microcephaly. Although an increase in microcephaly births was not suspected during the Zika epidemic in French Polynesia, when it became an issue in Brazil 18 months later, a look-back study documented an apparent increase in microcephaly coincident with the Zika epidemic.[12]

The review found 8 cases of microcephaly during the Zika epidemic (Fig. 10). With a baseline rate of 2 cases of microcephaly per 10,000 births, the observed rate of 95 per 10,000 gave a relative increase of 53 (95% CI 7-1061).

This was comparable to the relative increase between 42 and 77 fold in 4 Brazilian states.[3]

Statistical modeling of time intervals found the best fit of cases to exposure in the 1st trimester of pregnancy (P = 0.0007).[12]

Guillain-Barré syndrome. As the Zika epidemic was peaking, physicians began seeing excess cases of GBS, eventually 42 in all, and 37 (88%) of the cases had a prior illness typical of Zika (Fig. 11).[13] The presentation was typical of GBS, with ascending paralysis, abnormal nerve conduction studies, 16 in ICU, and 12 on ventilators. None died, and 24 improved by 3 months. Compared with the pre-Zika incidence of 5 cases per year, 42 cases in 7 months amounted to a 21-fold increase in incidence. While GBS was known to follow other flavivirus infections, including chikungunya, West Nile, dengue, Japanese encephalitis, and yellow fever vaccine, the high rate in the Zika outbreak was unique.

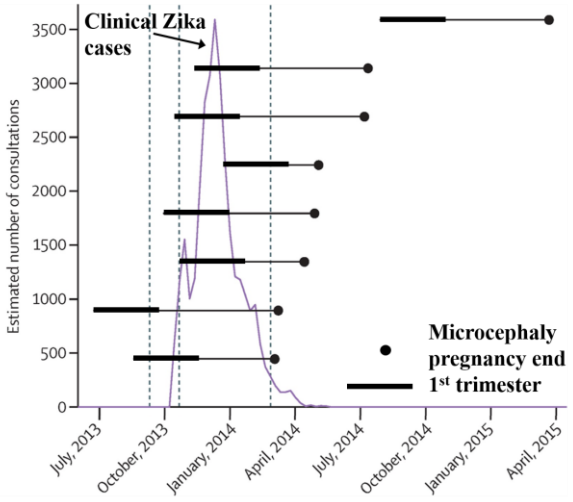


Fig. 10. Look-back study for microcephaly, French Polynesia, 2013-2014

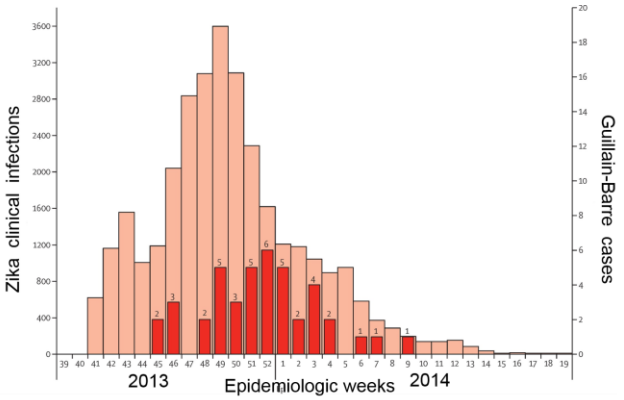


Fig. 11 The first Zika-associated epidemic of Guillain-Barré syndrome, French Polynesia, 2013-2014

	Cases	RI*
Bahia State, Brazil	155	2.7
Colombia	320	3.1
Dominican Republic	45	2.5
El Salvador	184	2.0
Honduras	71	2.6
Suriname	15	5.0
Venezuela	684	9.8
Puerto Rico	30	--
U.S.	7	--

RI = Relative increase over baseline

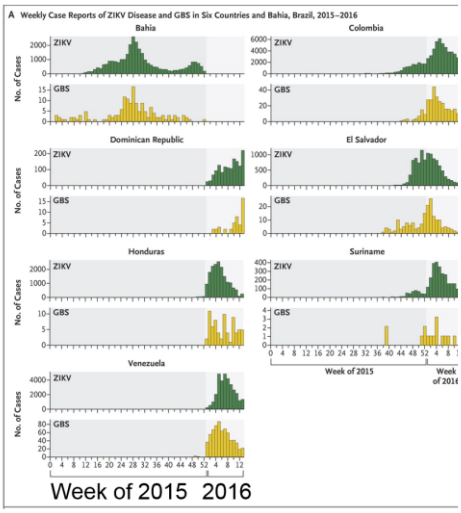


Fig. 12. Guillain-Barré syndrome in the Americas

Guillain-Barré syndrome in the Americas

The 2015 Brazilian Zika epidemic saw an increase in GBS, represented by that in Bahia state where the best records were kept (Fig. 12).{dos Santos, 2016 253 /id} The Zika epidemic spread virtually simulaneously to 6 nearby countries where Zika cases were also accompanied by GBS. The relative increase over baseline GBS rates ranged from 2.0 to 9.8 (Fig. 12).

Could the Increase in GBS Have Been Due to Dengue Instead of Zika?

Since dengue infection was occurring in the areas of the Zika outbreaks and GBS has been reported following dengue, the question has arisen whether the GBS might have been due really to dengue or to co-infection with dengue and Zika. The answer appears to be negative from several observations. For example, in the Brazilian data, the increase in GBS exactly coincided with the appearance of Zika and too long after dengue activity in the area (Fig. 13).{dos Santos, 2016 253 /id}, appendix The incubation for GBS following an infection is usually no longer than 1-4 weeks.

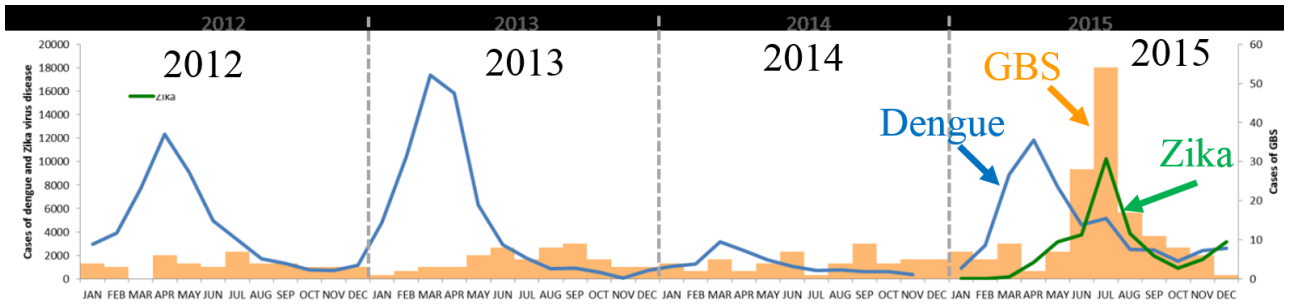


Fig. 13. Timing of GBS with the dengue and Zika epidemics, Bahia State, Brazil, 2015

How Long Have the Zika Epidemics Lasted?

To date the reported Zika epidemics have been self limited, usually spanning the warm months (Table 4).{dos Santos, 2016 253 /id} The longest running Zika epidemic occurred in northeastern Brazil (here represented by Bahia State where the best records existed) where it lasted for just over a year.

	Duration (wks)	Months	Year
Yap State, Micronesia	16	Apr – Jul	2007
French Polynesia	28	Oct – Apr	2013
Bahia State, Brazil	52	Dec – Jan	2014-2016
Colombia	22	Oct – Apr(?)	2015-2016
Dominican Republic	13	Jan – May(?)	2015
El Salvador	28	Oct – Apr(?)	2015-2016
Honduras	13	Dec – Mar	2015-2016
Suriname	28	Oct – Apr(?)	2015-2016
Venezuela	17	Jan – May(?)	2015

Table 4. Duration of Zika epidemics

What Is the Evidence that Zika Infection Causes Microcephaly?[15]

Epidemiology

- >20-fold increase in rates of microcephaly followed closely the first appearance of ZIKV infection in French Polynesia and Brazil.
- Consistent association with 1st trimester Zika exposure (like Rubella)

Viral detection by Brazilian Ministry of Health

- ZIKV isolated from CSF of miscarried microcephalic fetuses.
- ZIKV isolated from brain, CSF, heart lungs, liver, spleen, kidney of an infant dying after birth
- Zika IgM in CSF of 12 children born with microcephaly. Testing negative for TORCH (Toxoplasma, Other agents, Rubella, CMV, Herpes) and dengue and chikungunya.
- ZIKV isolated from amniotic fluid of 2 pregnant mothers with history of Zika infection and microcephalic fetus by ultrasound.
- ZIKV found by PCR in placental cells from an 8-week miscarriage.

Confirmation in the U.S.

- CDC confirmed ZIKV in brain of 4 microcephalic infants and placentas.
- Mlakar et al. found PCR+ for ZIKV in brain and placenta of 29 week miscarriage.

How Does Zika Virus Affect Fetal Brains?

In May 2016, Tang et al. reported inoculating cell cultures of various neuronal precursor cells from different points in the cell-cycle progression with the original African isolate of ZIKV (Uganda 1947).[16] They found that Zika infects **human neural progenitor cells (hNPC)** with high efficiency compared with **human embryonic stem cells**, human induced **pluripotent stem cells (hiPSC)** and immature **neurons** (Figs. 14 and 15). This resulted in cell death and dyregulation of the cell-cycle progression.

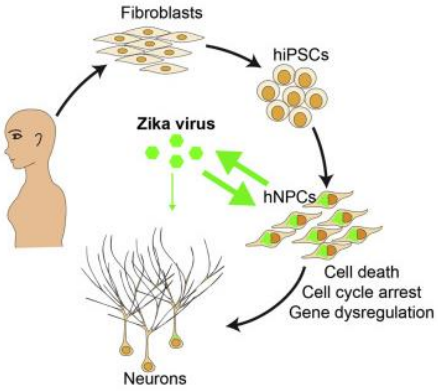


Fig. 14. Where ZIKV attacks the cell-cycle progression

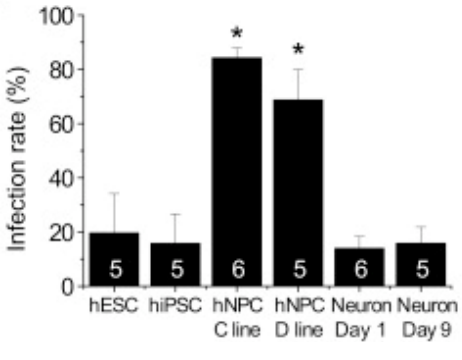


Fig. 15. Quantification of ZIKV's infection efficiency for different cell types in the cell-cycle progression. Numbers are replications.

Also in a May 2016 publication, Nowakowski et al. reported surveying expression of candidate receptors for ZIKV entry and identified increased expression of **Axl** (Tam) receptors in ZIKV infection as a possible determinant of cell tropism (Fig. 16).[17]

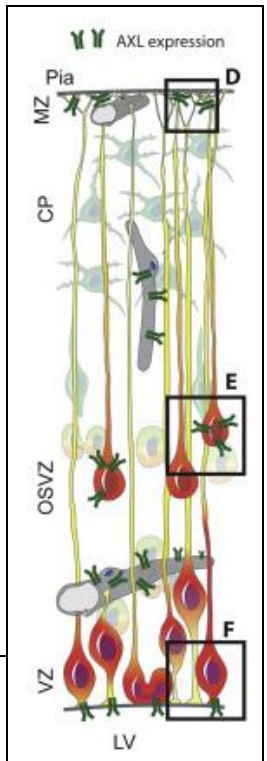


Fig. 16. Cell types that strongly express AXL receptor, including radial glia and brain vasculature

Then in June 2016, **Hanners et al.** in **John Schoggins' laboratory** in the UT Southwestern Microbiology, Pediatric ID, and Neuroscience departments reported a landmark advance in understanding the pathophysiology of Zika in the developing fetus.[18] While prior studies had examined the effects of the original African ZIKV strain, Hanners et al. obtained a **Western hemisphere strain** from Puerto Rico in 2015 and studied its effects on cell cultures of human neural progenitor cells.

They made three important observations (Fig. 17). First, they corroborated Tang's finding of an acute cytopathic effect on hNPCs. Second, they found that Zika-infected hNPCs are poorly immunogenic (Fig. 18). Third, ZIKV establishes a persisting infection that continues to exert a cytopathic effect over time, at least out to 28 days (Fig. 19).

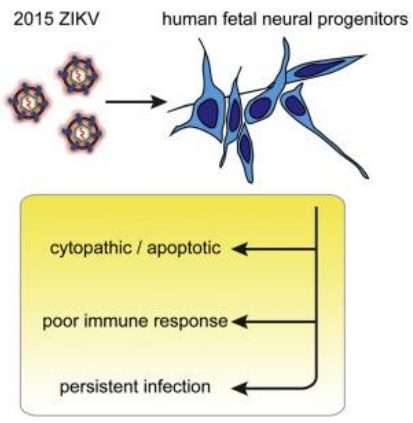


Fig. 17. Summary of findings of Hanners et al.

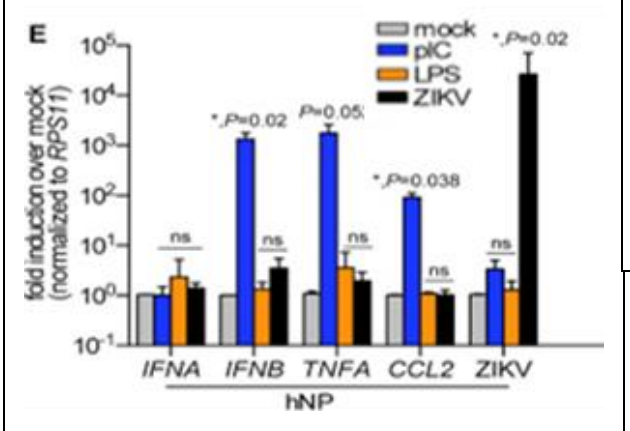


Fig. 18. ZIKV-infected hNPCs produce no cytokines. pIC is a viral RNA mimic.

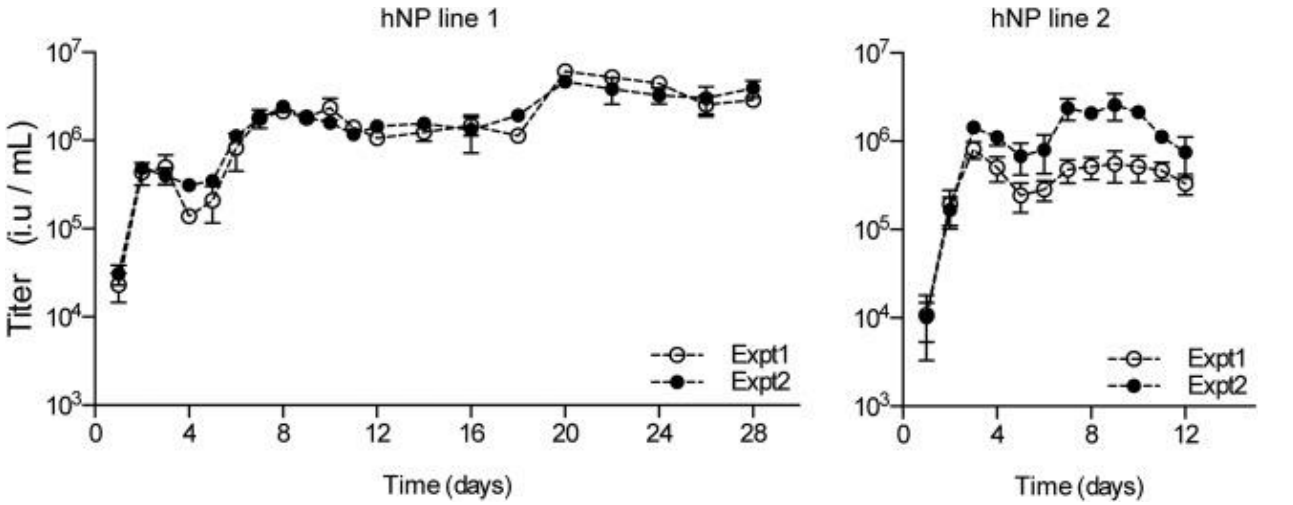


Fig. 19. ZIKV produces a persisting infection that continues to exert cytopathic effect for at least 28 days, replicable in two hNPC lines

Modes of Transmission of ZIKV

The overwhelming majority of cases of Zika infection are acquired through the bite of an infected female *Aedes aegypti* mosquito, but other modes of transmission have been documented. These include the following[1]:

- Bit by other mosquito species (e.g., *A. albopictus*)
- Vertical transmission from mother to fetus
- Laboratory acquisition (2 reports)
- Subcutaneous injection of infected monkey brain (1 report)
- Transfusion. Donated blood tests positive by RT-PCR, but no cases of transmission reported.
- Breast milk of Zika-infected mothers contains virus, but no cases of transmission reported.
- Sexual transmission

Sexual Transmission

Until recently no arboviral infection was known to be spread by sexual transmission. The initial report that raised this possibility for Zika appeared in 2011.

An American scientist returned from Senegal, Africa, to his home in northern Colorado. His home was above 6,500 feet altitude, where *Aedes aegypti* is not found. He had vaginal intercourse with his wife on the day of return. Nine days later, he developed typical Zika illness including perineal pain and bloody semen. Four days after his onset his wife became ill with a typical Zika illness. Zika infection was later confirmed in the man's blood by HAI serology, confirmed by plaque-reduction neutralization test (PRNT).

Since that report 11 papers have reported cases of sexual transmission. Their findings are as follows:

- Male to female except one case between gay men and one suspected female to male.
- In most, intercourse occurred during illness or shortly before.
- 5 studies confirmed Zika in seminal fluid of the sexual donor.
- Zika RNA load was higher in semen than serum.
- In 2 cases symptoms included testicular pain and hematospermia.
- 3 semen samples showed replicating virus, indicating infectivity.
- Sexual transmission has come mostly from symptomatic men; one exception reported.
- In 1 case Zika RNA was found in semen 62 days after illness onset.
- 2 men had ZIKV RNA in semen **6 months** after exposure, but virus was not grown.

Asymptomatic Sexual Transmission

In June 2016 the first case of asymptomatic sexual transmission of Zika infection was reported.[19] A French couple visited an in-vitro fertilization clinic and scheduled a procedure to be performed after their return from a 2 week vacation on the Caribbean island of Martinique. While there in early March 2016, ZIKV transmission was occurring (Fig. 20). Upon their return, they were informed that new French guidelines required testing for Zika infection at least 28 days after returning from an area of Zika transmission. So 39 days later they returned to the clinic and had urine and serum tested.

The man's urine was PCR positive for ZIKV, but his serum was PCR negative. His serum was serologically positive for anti-Zika IgM, negative for anti-dengue IgM, and positive for anti-flavivirus IgG. This indicated recent Zika infection long enough in the past to clear Zika viremia and generate both IgM and IgG.

His wife was PCR positive in both serum and urine, indicating very recent infection. Time to clearance of Zika virus from blood with 95% assurance is 2.4 to 18.9 days.[6]

The most likely conclusion is that the man was infected during their vacation in early March and infected his wife through sexual transmission between the 21st and 36th days after return to France. Neither partner ever developed symptoms or signs of Zika illness.

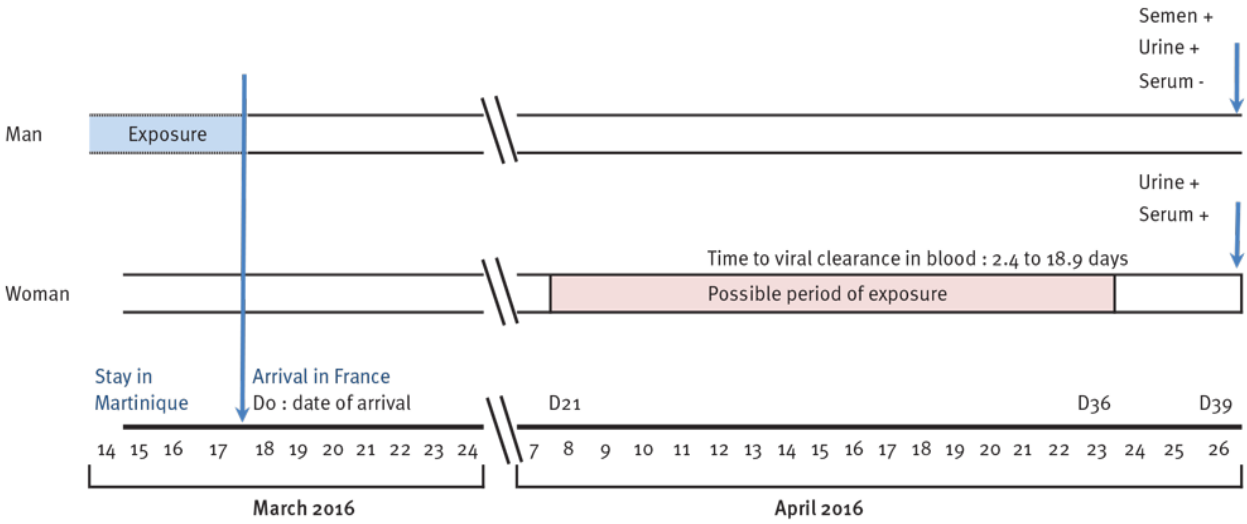


Fig. 20. Case report of suspected sexual transmission of ZIKV in an entirely asymptomatic couple, France, March-April 2016. Timelines show key dates of possible Zika exposure and results of PCR testing of semen, urine and serum.

Suspected Sexual Transmission from a Woman to a Man

The following case report, published in July 2016, is the first, and so far the only, report of sexual transmission from a woman to a man.[20]

- A nonpregnant woman in her 20s had unprotected vaginal sex on the day (day 0) she returned from travel to an area of ongoing Zika transmission.
- The next day (day 1) she developed symptoms of Zika illness. PCR was positive for ZIKV in blood and urine.
- 7 days after intercourse (day 6), the male partner in his 20s developed typical Zika illness
- On day 9 PCR was positive in his urine but negative in his blood.
- The man had not been outside the U.S., confirmed only vaginal sex, had no penile lesions, had noticed no bleeding during intercourse, and had had no other recent sexual partners or mosquito bites.

Two prior studies suggest a route of female-to-male transmission.

- ZIKV was found by PCR in a woman's cervical mucous, genital swab and endocervical swab 3 days after onset of Zika illness.[21]
- ZIKV was found in vaginal secretions of 3 nonpregnant female primates up to 7 days after inoculation.[22]

Complications of Zika Virus Illness

Summary

- In babies of mothers infected in 1st & early 2nd trimesters (est. risk 1%, 29% and 8%)
  - Fetal death, miscarriage, stillbirth
  - Microcephaly, seizures, mental retardation
  - Chorioretinal atrophy, optic nerve hypoplasia, and blindness
  - As yet unsupported concern for more subtle brain damage in infants (ADHD, bipolar, schizophrenia) infected later in pregnancy, but not after birth.
- In adults
  - Guillain-Barré syndrome
  - Immune thrombocytic purpura (ITP)
  - Possible acute disseminated encephalomyelitis in adults

Immune Thrombocytic Purpura (ITP)

Eleven cases of post-Zika ITP have been reported in 4 papers. These occurred in French Polynesia (4), Suriname (1), Columbia (3) and Puerto Rico (2).[23] Four of the 11 died of hemorrhagic complications. The first Zika-related death in Puerto Rico was due to brain hemorrhage from ITP.

Acute Disseminated Encephalomyelitis (ADEM)

ADEM is an acute multiple sclerosis-like illness, closely following typical Zika illness. Two cases were reported during the Zika epidemic in Pernambuco State, Brazil.[24]

Little Evidence of Severe Zika Infection in Immunosuppressed

There is little evidence of more severe Zika disease or prolonged viremia or shedding in Zika infection.[25] One report described 2 cases of fatalities possibly related to Zika infection. One was an adult with lymphoblastic leukemia and the other a child with myeloid leukemia.

How Did Zika Spread to Brazil?

How Zika spread to Brazil has been debated (Fig. 21). The first theory was that it was simply a continuation of Zika's eastward flow of island-hopping across the Pacific, with the final leap by travel from Easter Island. However, air travel to Easter Island is primarily to Chile, which, because of its high altitude has no *Aedes aegypti* and thus no Zika cases.

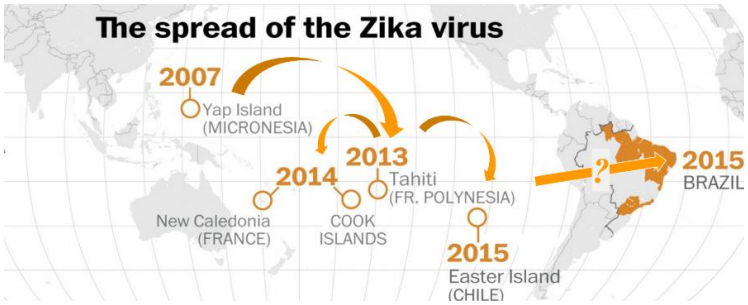


Fig. 21. The spread of Zika virus across the Pacific

The second theory arose from the temptation to blame soccer. The World Cup, held in June-July 2014, featured games in cities of northeastern Brazil—Recife, Natal and

Salvador—that were at the center of the Zika epidemic starting in December of that year. However, no teams from the South Pacific islands participated.

The third theory implicated the Va'a Sprints, a high profile outrigger canoe competition held in in Rio de Janeiro in August 2014, just 3 months before the outbreak began. Outrigger canoe teams from French Polynesia, Cook Island, and Easter Island, where Zika had been circulating, participated.

"Molecular Clock" Analysis

Based on the constant rate of mutation of Zika's single-stranded RNA, the analysis found that the Brazilian clade shared commonality with the French Polynesian virus—an advanced version of the Asian strain, not the original African strain (Fig. 22).[26]

The predominant Brazilian clade (B) arose around December 2013 from the common ancestor (clade A) of the Brazilian and French Polynesian clades, which had arisen around May-June 2013. This December 2013 genesis coincided with increasing travel from Zika-active countries related to the **FIFA Confederations Cup Soccer Tournament** in Brazil in June 2013, which included a soccer team from Tahiti. If true, the virus lay hidden for 18 months before becoming epidemic.

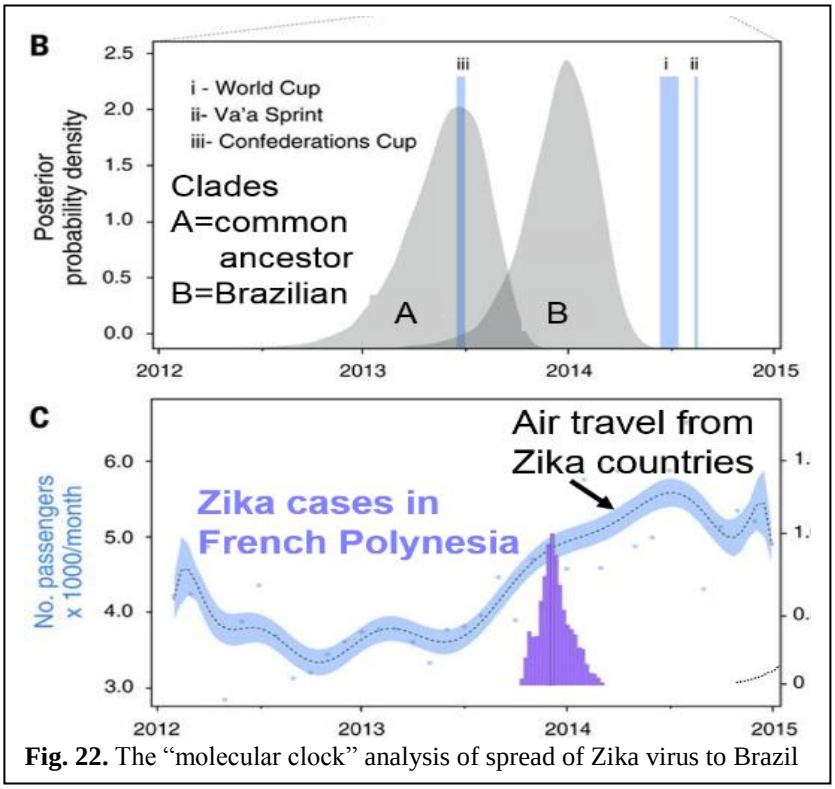


Fig. 22. The "molecular clock" analysis of spread of Zika virus to Brazil

Management of Zika Infection

Since there is no treatment for the disease or its complications except supportive care, emphasis should be on avoiding getting infected in the first place. Recommendations for personal protection include:

- Use EPA-registered insect repellant containing DEET.
- Wear protected clothing (long sleeves, long pants and socks).
- Stay in places with air conditioning or window and door screens.
- Or sleep under a mosquito net.

People who contract Zika infection should be counselled to try to prevent spreading it to others or to their developing fetus:

- Avoid being bitten by *Aedes* mosquitoes during the acute illness.
- Travelers to endemic regions wear DEET during trip and 3 weeks after return
- Women avoid pregnancy for 2 months after illness or return from trip to an endemic region
- Men and women abstain or practice protected sex for 6 months after acute illness
- Do not donate blood for 4 months after acute illness

Diagnosis of Zika Infection

Who Should Get Zika Testing?

Since most Zika testing is provided free by CDC and regional public health laboratories, it is provided only for those meeting the following criteria:

- Person with >1 Zika symptom within 4 weeks of:
  - Travel to an area with Zika transmission (see *cdc.gov/zika/geo*) OR
  - Unprotected sex with a partner who spent time in such an area.
- Pregnant woman*
  - Travel to Zika area during pregnancy or 8 weeks before conception
  - Unprotected sex with a partner who spent time in such an area
- Patient with Guillain-Barré syndrome with a Zika exposure history
- Infant born to a woman with a positive or inconclusive Zika test
- Infant with microcephaly etc. born to a woman with an exposure history
- Patient with a compatible illness and an alternate mode of acquisition (e.g., transfusion, transplant)

*Women with exposure wanting to get pregnant but not meeting the criteria are not being tested for now. (They are advised to avoid pregnancy for 2 months and have protected sex for 6 months).

Diagnosis of Zika Infection

Test for the viral RNA by PCR *during the acute illness*

- Blood is 51% sensitive (best in first 5 days of illness)
- Saliva is 81% sensitive
- Urine is 92% sensitive
  - Urine and saliva tests may remain positive longer than blood.

Blood test for IgM antibodies *after end of acute illness*

- Because of cross-reactivity with dengue infection and Japanese encephalitis vaccine (international travelers), positive Zika serology requires confirmation by the plaque reduction neutralization test (PRNT)
- The PRNT is a very labor intensive test to be done only when indicated.

Source of Zika Testing

Dallas County Health and Human Services (DCHHS) provides free testing of blood and urine for patients meeting the criteria.

- PCR on serum and urine
- IgM and IgG on serum

Special blood and urine specimen preparation provided by the outpatient labs of Baylor, Medical City and Methodist hospitals. To obtain testing for a patient, the physician completes the form from the DCHHS website*, makes out a prescription for Zika testing, and sends these with the patient to the laboratory. (*see http://www.dallascounty.org/department/hhs/zika.html)

Commercial testing of blood and urine for Zika is now available from 3 laboratories:

Viracor

- PCR ($165)
- IgM and IgG ($700)

LabCorps and Quest

- PCR only

How to Contain Autochthonous Spread from Zika Importations?

Travel Volume to the U.S. from Zika Transmission Zones

Over 200 million passenger journeys are made annally to the U.S. by air, land, and sea from areas with local Zika virus tranmission.[27] Texas (by land) and Florida (by air and sea) have the largest numbers. Women of child-bearing age make approximatel 50 million passenger journeys and pregnant women, approximately 2.3 million. Their points of departure and arrival are shown in Fig. 23. This large influx of travelers potentially arriving during the incubation period of an acute Zika illness ensures a steady load of acute Zika illness in the U.S.

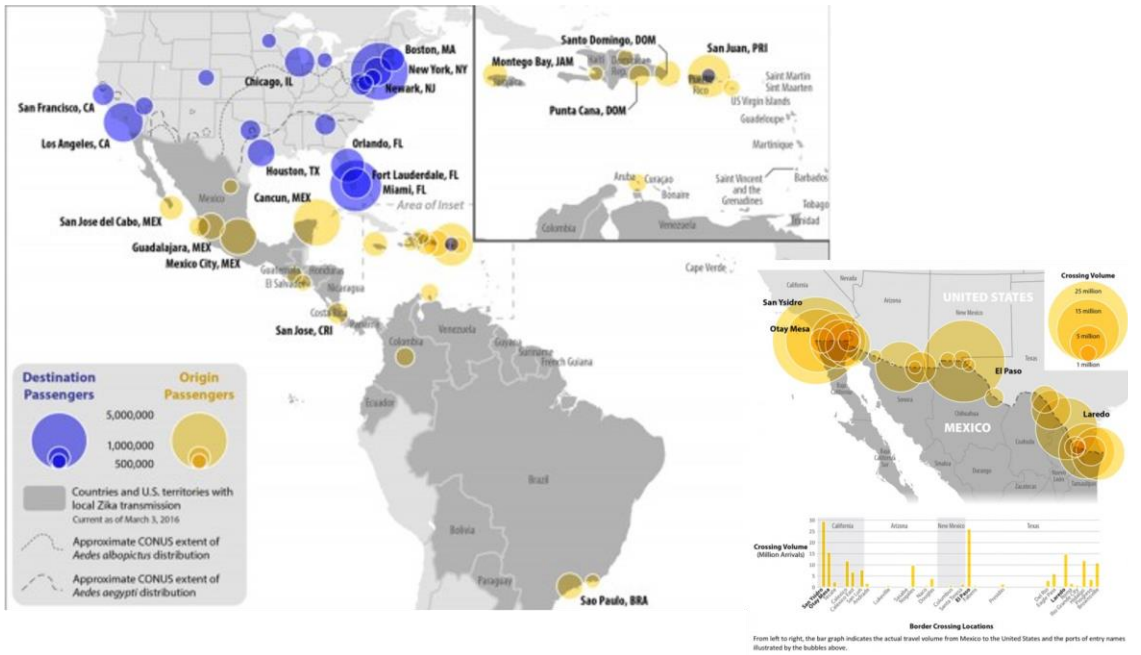


Fig. 23. Travel volume to the U.S. from Zika transmission zones.

The probability that a traveler with Zika viremia will be bitten by an *Aedes aegypti* mosquito and transmit the illness to other neaby residents is strongly retarded by the ubiquitous air conditioning, window screens and regular trash collection in the U.S. Our greatest vulnerability is in southern Florida and the Gulf Coast areas of Texas, where pockets of poverty afford less protective living conditions, mosquito and viral activity can persist nearly year round, and travel volumes are highest.

CDC Strategy for Eradicating a Zika Focus

The CDC strategy to contain and eradicate foci of autochthonous spread from an imported infection involve these elements:

- Surveillance to detect autochthonous cases
- Issue a **Zika Alert** for a square mile around the case (3-5 times further than an *Aedes* mosquito ranges) (Fig. 24).
- Travel warning and resident education (avoid bites)
- Intense mosquito control
  - Mosquito surveillance
  - Door-to-door inspection to eliminate water sources
  - Ground spraying (aerial spraying may not help)
  - Continue until no new cases for 2 incubation periods (2x14=28 days)

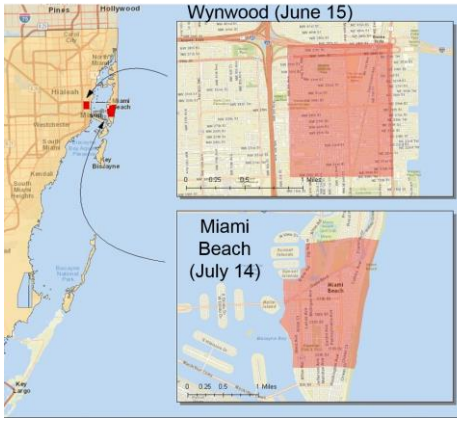


Fig. 24. Zika alert zones in Florida

What Can We Do To Protect Ourselves from Zika and Its Complications?

- Women living in areas of ongoing Zika transmission should avoid pregnancy until the epidemic is over, and time pregnancies for 1st trimester to avoid high-transmission months (warm season). CDC, PAHO and WHO are avoiding this recommendation for political reasons.
- Pregnant women living in areas of ongoing Zika transmission should wear DEET insect repellant, long sleeves and long pants.
- Eliminate water sources around the home, and use mosquito dunks where necessary.
- Use the U.S. Army's proven mosquito trap (*Trap-N-Kill*™), not insecticide spraying.{Zeichner, 2011 217 /id;Long, 2015 222 /id}
- It is safe to travel to areas with no Zika epidemic and anywhere above 6,500 ft. elevation.
- If exposed, avoid pregnancy for 2 months and practice protected sex for 6 months.

Trap-N-Kill – developed by U.S. Army (requires maintenance)

Fig. 25. The mosquito trap (*Trap-N-Kill*™) developed, validated and used by the U.S. Army to control mosquitoes throughout the world.

The Most Promising Public Health Approaches to Avoiding Zika Epidemics

Insectide Spraying

In contrast to West Nile epidemics where aerial spraying for *Culex* mosquitoes ("canopy dwellers") alone can end an epidemic overnight, aerial spraying is ineffective against epidemics spread by *Aedes aegypti* ("stealth mosquito"). Those fighting dengue and chikungunya epidemics in south Florida and south Texas say "you can't spray your way out of a dengue [or Zika] epidemic."[30] However, truck or backpack spraying may be a useful adjunct to a comprehensive conrol program.[31]

Genetically Modified Mosquitoes

Since the 1950s male mosquitoes were irradiated to render then sterile and were released to mate with females in a program that by the 1980s had eradicated screw worm from the southern US, Mexico and Belize.

A more efficient approach has been developed to alter the genome of male *Aedes aegypti* mosquitoes by gene editing. The genetically modified males are released into the environment to fertilize females with dud sperm; their offspring die soon after hatching. In 2009 experimental trials in the Cayman Islands, Panama and Brazil, this approach reduced *Aedes aegypti* populations by 99%.

Genetically modified male mosquitoes do not bite, and they die soon after fertilizing females, so they have no lasting genetic effects in their own species or on other species.

Field trials in Florida and Brazil have received government approval but are pending public comment. A Purdue University poll showed that 78% of Americans support genetically modifed mosquitoes. While likely effective in stopping focal, urban epidemics transmitted by *A. aegypti*, It is not practical for continental disease eradication.

"Perifocal" Insecticide Treatment

The central technique of the PAHO program that eradicated *Aedes aegypti* from South and Central America from 1945 to 1970 was **"perifocal" insecticide treatment**.[32] The technique was based on the fact that *A. aegypti* females lay a small number of eggs in many sites (are "skip ovipositors"). Field staff go house to house finding infested containers and spraying them *plus the*

surrounding meter with DDT that persistes for several months. As the female goes about depositing eggs, she has a high probability of contacting the residual insecticide. While DDT has been banned (from the effects of its massive agricultural use, not from disease control use), newer persistent insecticide (e.g., deltamethrin in a liquid polymer that dries to form a persisting residue) have been developed that kill female *Aedes* as effectively as DDT but are not detrimental to the environment.[32]

Although this technique is labor intensive and therefore expensive, it is probably the only environmental approach likely to eradicate *Aedes aegypti* on a large scale.

Vaccine

Probably the best way of protecting populations from Zika virus infection as well as other arbirival diseases is the development of a polyvalent arboviral disease vaccine to produce herd immunity to the arboviruses that are threatening now and those that will emerge in the future. We have had a very successful vaccine against Japanese Encephalitis virus for decades, and work on a dengue vaccine is advanced. Herd immunity would interrupt the mosquito-human-mosquito cycle and potentially eradicate the virus. A vaccine specific to Zika is at least 3 years away, assuming resolution of U.S. Congressional gridlock, while a polyvalent arboviral vaccine is at present a dream.

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