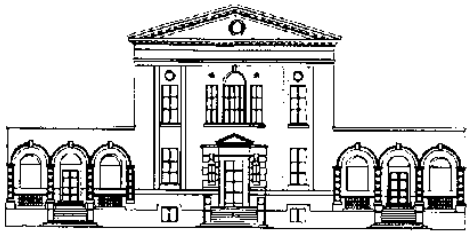
World Health Organization



1 Workshop - La Scuola Medica Salernitana

Zika virus: la nuova epidemia antropologica globale

Giuseppe Ippolito

Emanuele Nicastri

Istituto Nazionale per le malattie Infettive
Lazzaro Spallanzani

16-17 marzo 2016 Vietri sul mare (Salerno)

Fig. 1. Aedes aegypti mosquito.
(Photo by James Gathany, Centers for Disease Control and Prevention. 2006).

Indice

- Impatto politico e mediatico
- Eziologia
- Epidemiologia
- Approccio sindromico e diagnosi differenziale arbovirosi
- Complicanze
- Prospettive future

- Impatto politico e mediatico
- Eziologia
- Epidemiologia
- Approccio sindromico e diagnosi differenziale arbovirosi
- Complicanze
- Prospettive future

2016, February 1
WHO declares global emergency

House Republicans Want Obama to Use Unused Ebola Funding to Fulfill $1.8 Billion Request to Fight Zika Virus

Presidential Proposal and the Congressional Response

President Obama's Request

- President Obama requested **$1.8 billion to combat the Zika Virus**
- Obama wants this money to be new emergency funds to respond to Zika virus health crisis

Congressional Republican Proposal

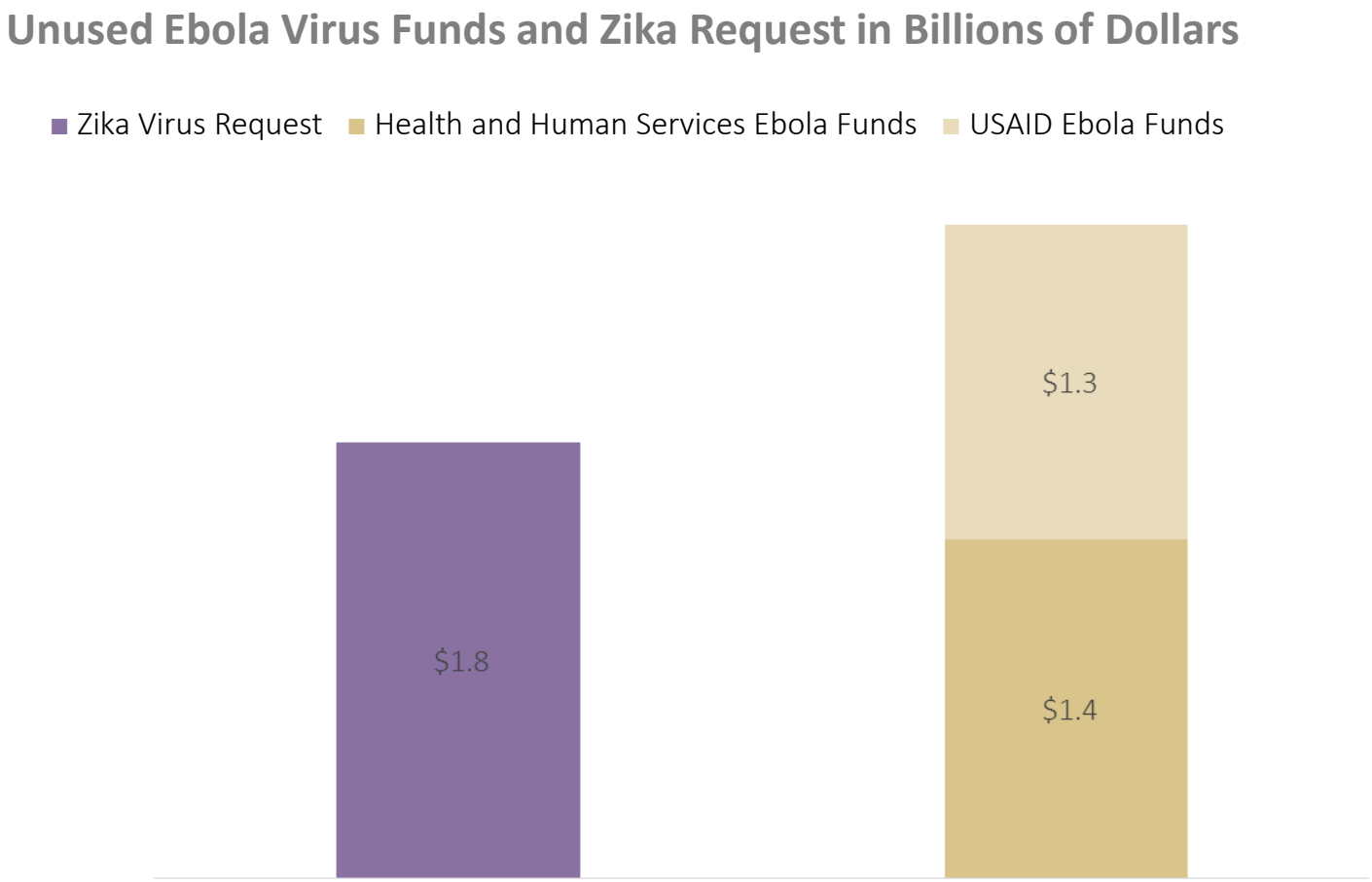
- House Republicans want to use already appropriated funds that were originally meant to fight the Ebola Virus
- Currently, there are **$2.7 billion unused funds set aside to fight the Ebola virus**
- Republicans want to take money from the Ebola fund because they consider it the most expedient way to get the money instead of trying to appropriate new funds
- If the Ebola fund needs to be replenished, Republicans promise to refill it in the FY 2017 budget process

Congressional Democratic Response

- House Democrats call the Republican proposal wanting to "rob Peter to pay Paul" since it is withdrawing money from one health emergency to fix another one
- Democrats agree with the Health and Human Services Secretary Burwell that the unused Ebola funds should not be used for other purposes because Ebola is still being monitored and could reemerge

Source: Will Dobbs-Allsopp and Mary Ellen McIntire, "Ryan Signals Delay on White House's Zika Request," Morning Consult, February 18, 2016; Tom Howell Jr., "Obama Should Use $2.7B Ebola Leftover Funds to Fight Zika," Washington Times, February 18, 2016.

The Department of Health and Human Services and USAID Have $1.4B and $1.3B Respectively to Fight the Ebola Virus



Source: Will Dobbs-Allsopp and Mary Ellen McIntire, "Ryan Signals Delay on White House's Zika Request," Morning Consult, February 18, 2016; Tom Howell Jr., "Obama Should Use $2.7B Ebola Leftover Funds to Fight Zika," Washington Times, February 18, 2016.

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3 More Arrests in Oregon as Protest Leader Says 'Go Home'

Ammon Bundy asked those still occupying an Oregon wildlife refuge to "please stand down." Hours later, the F.B.I. said three protesters had turned themselves in.

- Protester Who Was Killed: 'I'd Rather Die Than Be Caged'
- The Oregon Protest and the Battle Over Western Lands
- Watch: Who Are the Bundys?

Reports of Zika-Linked Defect Rise in Brazil

Health authorities said cases of microcephaly — a condition in which newborns have abnormally small heads — had climbed to 4,180 since October. Brazil typically has 150 cases annually.

ELECTION 2016

Trump's Feud With Fox Steals Spotlight From Debate

The Republican Party is focused on Donald J. Trump's standoff with Fox News rather than on the presidential debate. And neither Fox News nor Mr. Trump show any sign of backing.

- Donald Trump Finds New City to Insult: Brussels

Want Some of This Texas Barbecue? Get in Line.

Waiting for hours in the food line at Franklin Barbecue in Austin, Tex., is a rite of passage for the famished and the faithful from all over.

Schizophrenia Study Offers New Insight Into Its Cause

The finding provides researchers with their first biological handle on a disorder that has confounded modern science. It also helps explain why the schizophrenia often begins in adolescence.

French Official Quits Over Citizenship Plan

The Opinion Pages

The Need for a Tax on Financial Trading

Why Isn't Marco Rubio Winning?

WorldClient -... | Zika Virus As... | margaret Ch... | Zika virus: U... | Factsheet fo... | Dengue, chikungu... | Entry routes ... | Zika Virus Transmi... | zika - An... | ZIKA do Brasil na ...

https://play.google.com/store/search?q=zika&c=apps&docType=1&sp=CAFiBgoEemlrYXoFGADAAQKKAQIIAQ%3D%3D%3D%3AS%3

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EVEV Powered

Xô Aedes
Novetech - Soluções Te

Dengue x Chik x Zik
DVG Gerenciadora de P

Dengue Radar
Seah Ru Hong

AntiZika
Creatixcode

Sem Dengue
Colab S.A.

Zika Virus News +
Appachi

Caça Mosquito
Governo da Bahia

Dengue
Universidad del Norte

Mapa da Dengue
Web Fantastico

Anti Mosquito Sour

Xô Dengue

Dengue - Manejo Cl

Zika Tracker

Zika

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https://play.google.com/store/search?q=zika who&c=apps&hl=en

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Appachi

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ProJaVa

Zika Virus Info New
Hang Klebang

Sem Dengue
Colab S.A.

Zika Vírus e Microc
Shashi kShastri Zone

Zika Virus

AntiZika

Zika Test Prank

Zika Vírus - Minha

Caça Mosquito

- Impatto politico e mediatico
- Eziologia
- Epidemiologia
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- Complicanze
- Prospettive future

Zika virus (ZIKV)

- Arbovirus emergente della famiglia *Flaviviridae, g*enere *flavivirus,* virus a RNA a singola elica .
- Strettamente correlato a Dengue, febbre gialla, Encefalite giapponese e West Nile.
- Trasmesso all'uomo prioritariamente da zanzare della specie *Aedes*
- Quadro clinico simile ai quadri clinini benigni causati dalla febbre di Dengue e di Chikungunya
- Spiccato neurotropismo che giustifica complicanze nell'adulto e nella donna in gravidanza

CURRENT TESTING RECOMMENDATIONS

RT-PCR by serum, urine or saliva within the first 5 or 6 days of illness

highest concentrations found in saliva early in the disease course, but possibly remaining detectable for longer in the urine

SEROLOGY: an acute phase should be drawn from day 6 onward with a convalescent serum drawn 2-3 weeks later

false positives for ZIKV seem more likely if ZIKV is not the first flavivirus that the patient has encountered (*Lanciotti RS.2008)*

If CROSSREACTIVITY is suspected: plaque reduction neutralization testing (PRNT) can help to discriminate between cross-reacting antibodies

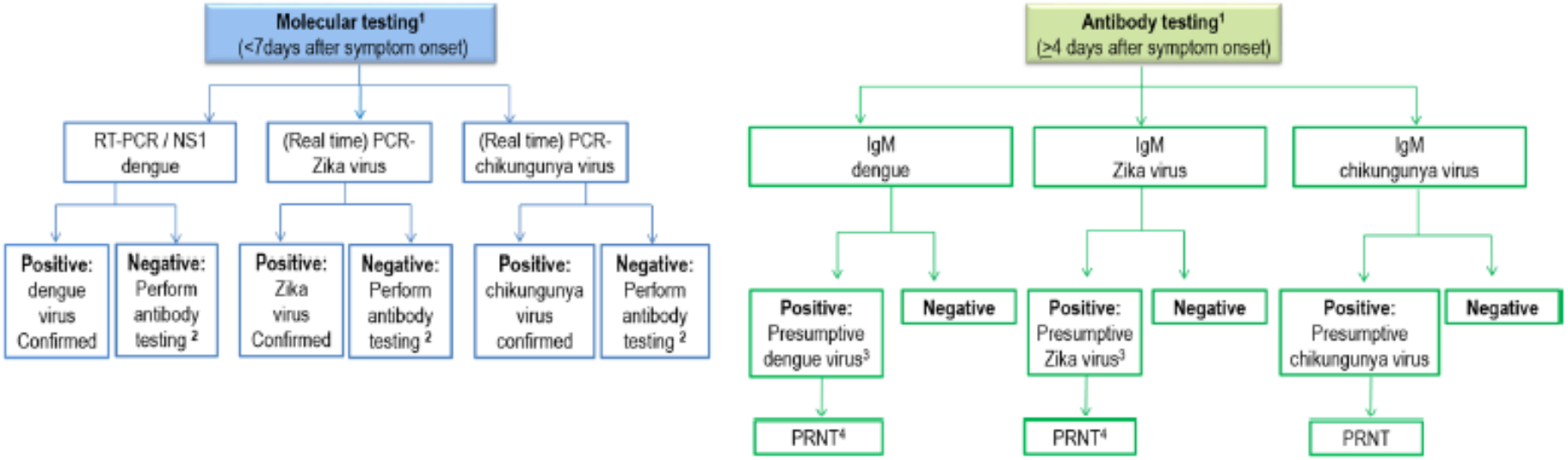
Pan American Health Organization

World Health Organization
Regional Office for the Americas

Memorandum - February 7, 2016
Subject: Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories

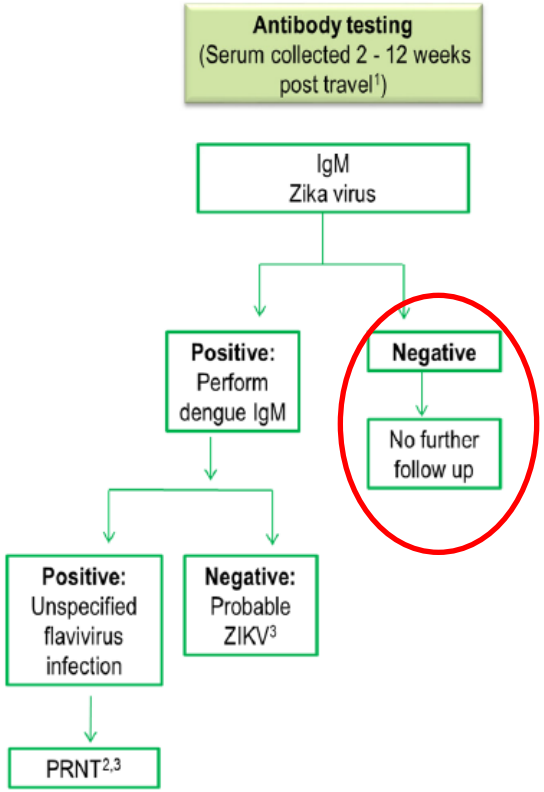
CDC
SAFER·HEALTHIER·PEOPLE

Tiered algorithm for arbovirus detection for suspected cases of chikungunya, dengue, or Zika
(Testing only performed if patient symptomatic and travel history indicates travel to affected area.)



- [1] **Due to extensive cross-reactivity in flavivirus serological assays, for samples collected <7 days post illness onset, molecular detection should be performed first.**
- [2] Perform if sample ≥4 days after symptom onset
- [3] Extensive cross-reactivity would be expected in samples from DENV/ZIKV circulation areas. A positive IgM assay with either antigen should be confirmed by using PRNT against both ZIKV and DENV as well as any other flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas).
- [4] PRNT should include any flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas).

Testing algorithm for Zika virus detection in asymptomatic, pregnant women



- [1] For women living in areas with ongoing transmission, refer to Updated Interim Guidelines for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age During Ongoing Zika Virus Transmission — United States, 2016 for timing of sample collection.
- [2] Extensive cross-reactivity would be expected in samples from DENV/ZIKV circulation areas. A positive IgM assay with both antigens should be followed up by using PRNT against both ZIKV and DENV as well as any other flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas). Depending on previous flavivirus exposure, resolution of infecting flavivirus may not be possible.
- [3] Follow up care should be undertaken as specified in the Interim Guidelines for Pregnant Women During a Zika Virus Outbreak – United States, 2016.

- ZIKV is a mosquito-borne **ssRNA flavivirus** of the Flaviviridae family

subclades reflecting 2 lineages: one African and one Asian

- The Asian lineage particularly seems to have high epidemic potential
- **widespread** occurrence in Africa and Southeast Asia
- **Reservoir**: humans and non-human primates

  antibody in rodents (*Hayes EB 2009*)
- ability to infect multiple species of the **Aedes genus of mosquito**
- **Transmission mode**

Confirmed	**To be confirmed**
mosquito bytes	saliva? Yes
perinatal	breast milk? Yes
transfusion	Tissue/organ donation ? Yes
sexually	

isher: European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Eurosurveillance

Eurosurveillance, Volume 21, Issue 10, 10 March 2016

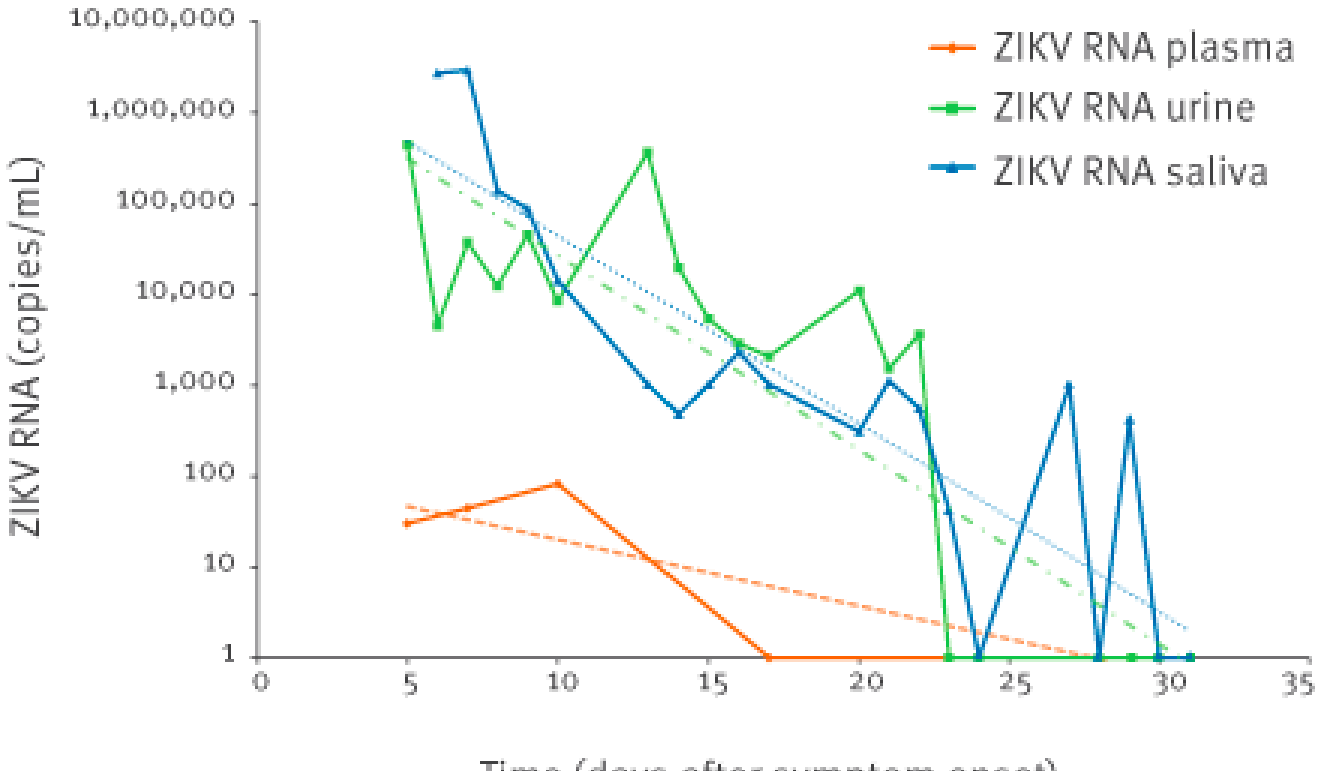
Rapid communication

ISOLATION OF INFECTIOUS ZIKA VIRUS FROM SALIVA AND PROLONGED VIRAL RNA SHEDDING IN A TRAVELLER RETURNING FROM THE DOMINICAN REPUBLIC TO ITALY, JANUARY 2016

L Barzon [1 2], M Pacenti [2], A Berto [1], A Sinigaglia [3], E Franchin [1 2], E Lavezzo [1], P Brugnaro [4], G Palù [1 2]

Figure 1

Kinetics of ZIKV RNA load measured by quantitative real-time RT-PCR in plasma, urine, and saliva samples of a patient with ZIKV infection, Italy, January 2016



The virus was isolated from saliva collected on day 6

Viral isolation is more successful from saliva samples characterised by high viral load and collected during the first week after symptom onset, before the appearance of antibodies.

Infectious Zika viral particles in breastmilk

In New Caledonia, in July, 2015, a 27-year-old febrile woman without any associated symptoms presented at hospital (day zero) at 37 weeks' gestation and naturally delivered a healthy baby (Agpar score 10) who was immediately breastfed. After delivery, the mother was febrile for 2 days, and a maculopapular rash arose which was decreasing on the day of discharge. The mother evolved favourably and clinical examination of the neonate remained normal until discharge.

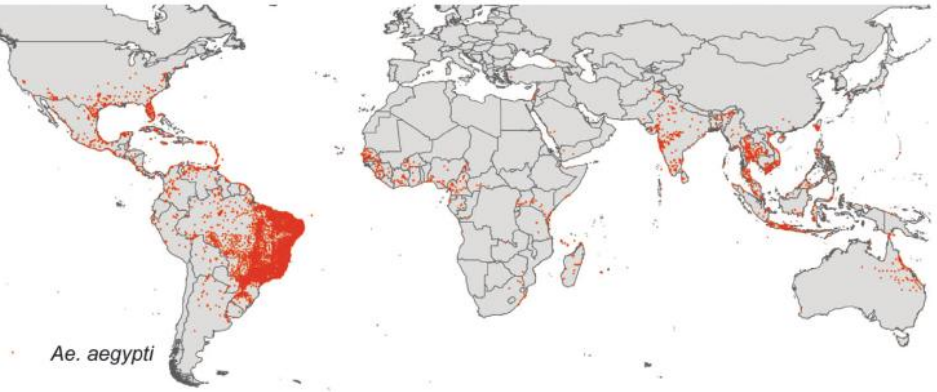
Blood cell count, total protein, and C-reactive protein levels were in the normal range for both mother and neonate. Blood samples from the mother and neonate were collected and tested for Zika virus, dengue virus, and chikungunya virus by RT-qPCR. Breastmilk samples were collected before breastfeeding to avoid possible contamination from the neonate's saliva. Only the mother's serum (day three) and breastmilk (day four) were positive for Zika virus by RT-qPCR (35000 RNA copies per mL in the mother and 850000 RNA copies per mL in the neonate). The only serum from the neonate that was sampled on day three was ambiguous. Breastmilk was inoculated onto Vero cells. Infective viral particles were detected from the breastmilk sample and confirmed by the presence of a cytopathic effect and by RT-qPCR (39 million RNA copies per mL, appendix).

Correspondence

Aedes mosquitoes

- often live around buildings and in urban areas
- active during daylight hours, especially near dawn and dusk
- artificial and natural breeding sites

Distribuzione vettori



igure 2. Map of occurrence points for *Ae. aegypti*.

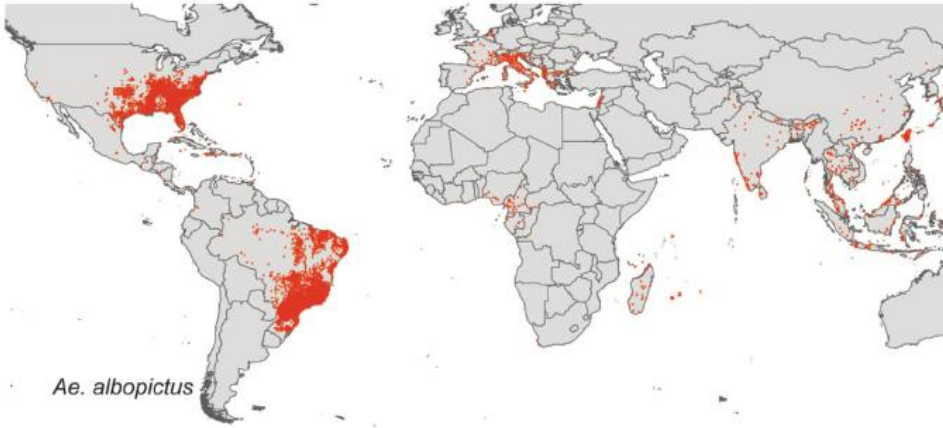
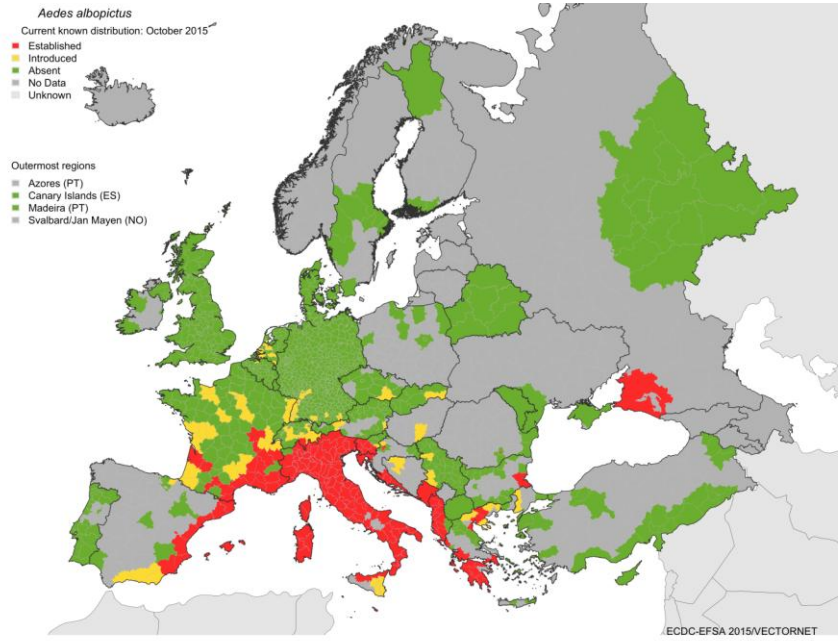


Figure 3. Map of occurrence points for *Ae. albopictus*.



NATURE The global compendium of Aedes aegypti and Ae. Albopictus occurrence +ECDC

PLOS | NEGLECTED TROPICAL DISEASES

Aedes (*Stegomyia*) *albopictus* (Skuse): A Potential Vector of Zika Virus in Singapore

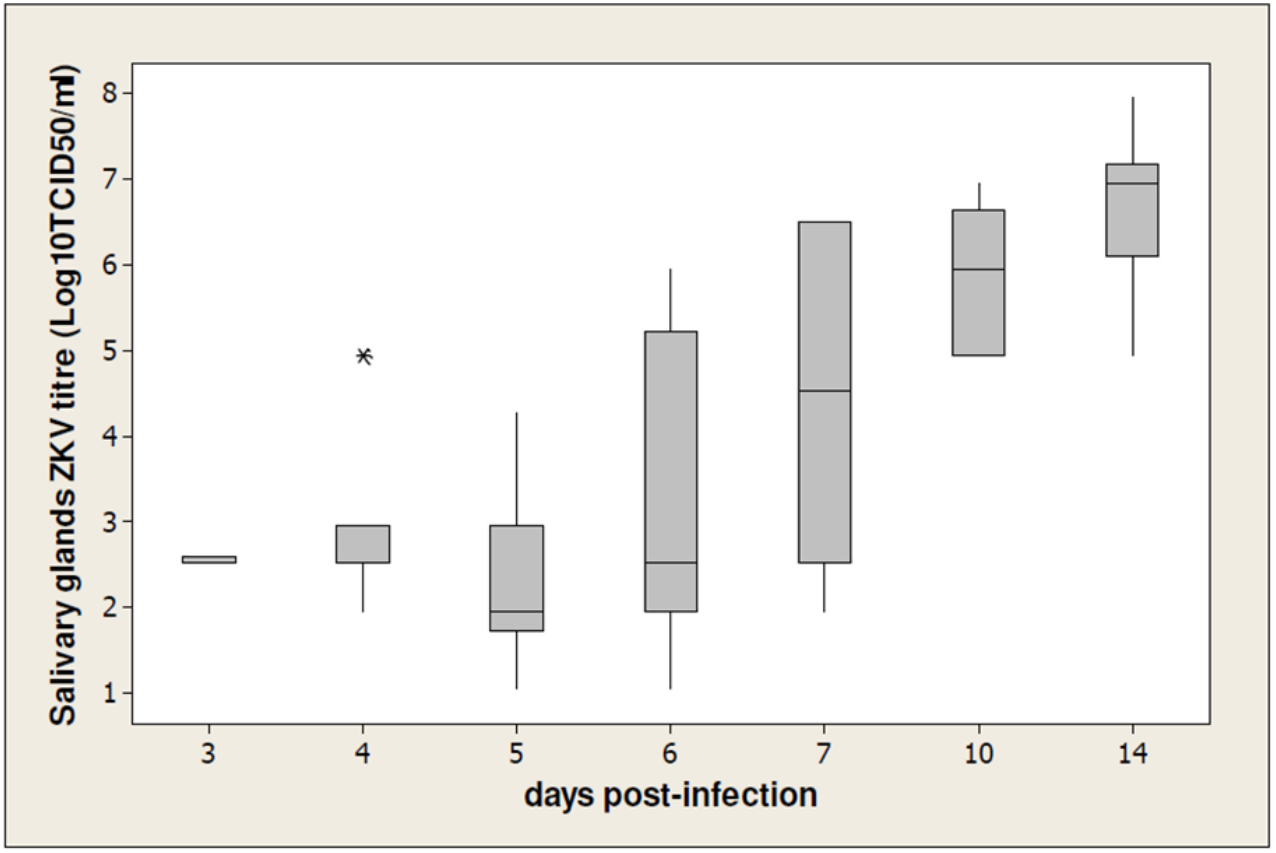


Figure 1. Box plots of ZIKV midgut and salivary glands titres at different days post-infection. Each box represents the median

PLOS | NEGLECTED TROPICAL DISEASES

Aedes (*Stegomyia*) *albopictus* (Skuse): A Potential Vector of Zika Virus in Singapore

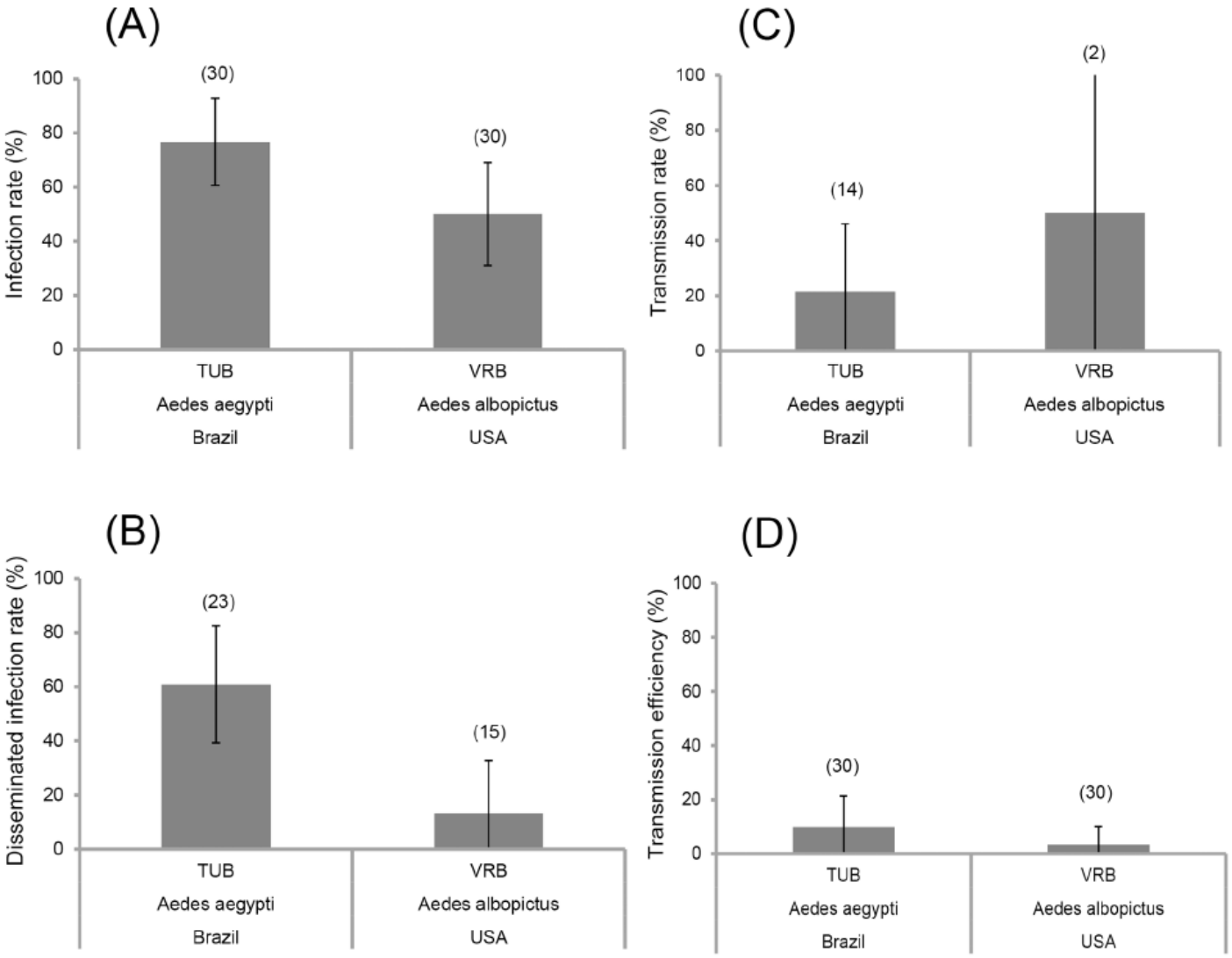
Table 1. Infection, dissemination and transmission rates for *Ae. albopictus* orally fed with ZIKV and held at 29°C at various days post-infection.

Days post- infection	Infection rate		Dissemination rate		Transmission rate	
	No. positive MG (number sampled)	Percent	No. positive SG (number sampled)	Percent	No. positive saliva (number sampled)	Percent
3	12 (12)	100	3 (12)	25	0 (3)	0
4	12 (12)	100	7 (12)	58.3	1 (7)	14.3
5	12 (12)	100	6 (12)	50	2 (6)	33.3
6	12 (12)	100	9 (12)	75	4(9)	44.4
7	11 (12)	91.7	11(11)	100	8 (11)	72.7
10	10 (12)	83.3	10(10)	100	10(10)	100
14	12 (12)	100	12(12)	100	12(12)	100

MG = midgut; SG = salivary gland.

Differential Susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika Virus

PLOS | NEGLECTED TROPICAL DISEASES



A Single Mutation in Chikungunya Virus Affects Vector Specificity and Epidemic Potential

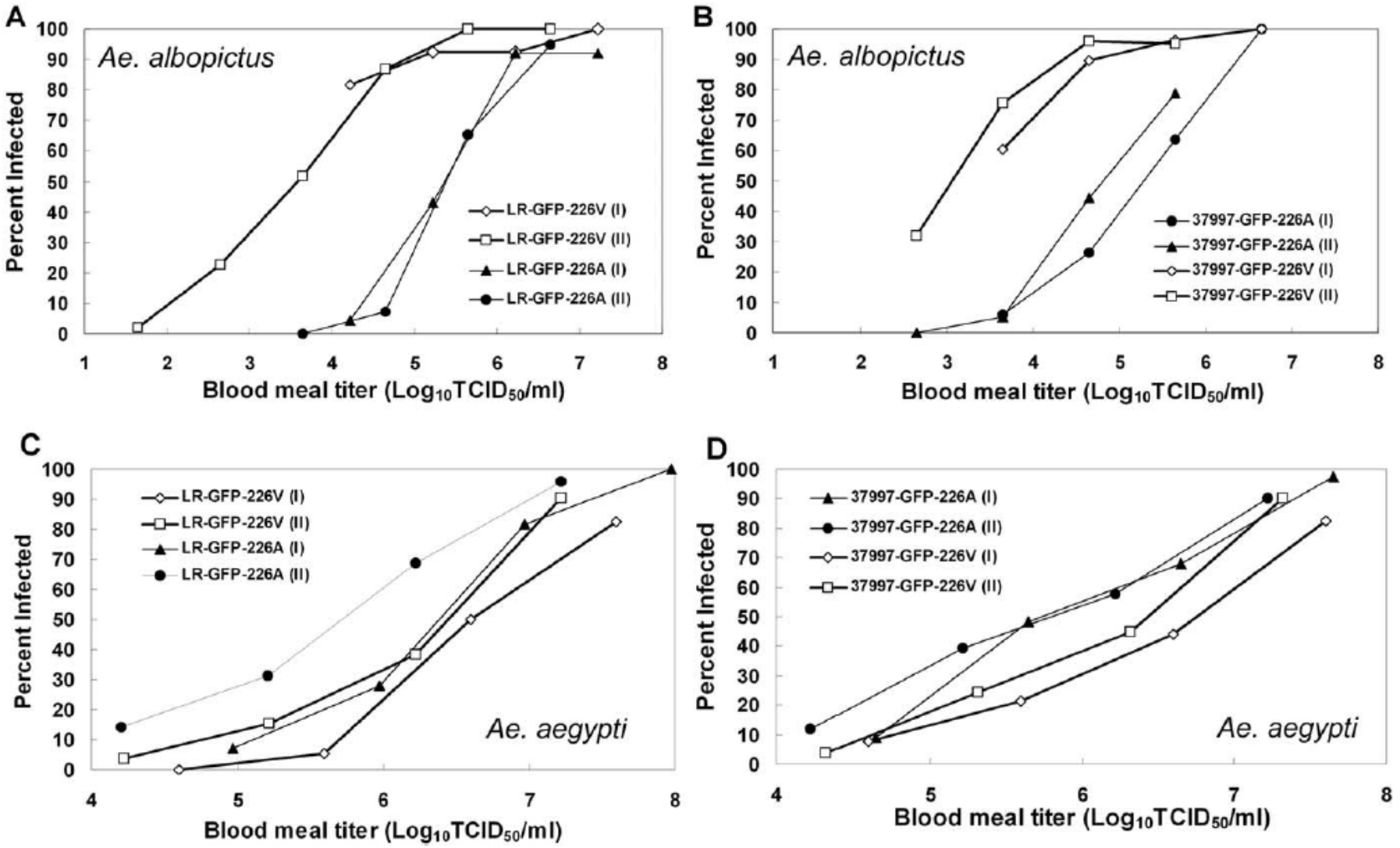


Figure 1. Effect of E1-A226V Mutation on CHIKV-GFP Viruses *Ae. albopictus* and *Ae. aegypti* Midgut Infectivity

Zika Virus: Medical Countermeasure Development Challenges

PLOS | NEGLECTED TROPICAL DISEASES

Robert W. Malone[1,2]*, Jane Homan[3], Michael V. Callahan[4], Jill Glasspool-Malone[1,2], Lambodhar Damodaran[5], Adriano De Bernardi Schneider[5], Rebecca Zimler[6], James Talton[7], Ronald R. Cobb[7], Ivan Ruzic[8], Julie Smith-Gagen[9], Daniel Janies[5‡], James Wilson[10‡], Zika Response Working Group

Table 4. Comparison of predicted to reported cumulative case incidence distribution of primary microcephaly by federated unit (state), Brazil, 2015.

Brazil	Reported cases	Predicted Cases		Brazil	Reported cases	Predicted Cases	
Federated unit with Zika		Lower Limit	Upper Limit	Federated unit with Zika		Lower Limit	Upper Limit
Alagoas	149	32	78	Paraná	No data	334	262
Amazonas	No data	25	93	Pernambuco	1,236	275	220
Bahia	450	153	357	Piauí	No data	26	75
Ceará	192	306	209	Rio de Janeiro	122	127	386
Espírito Santo	No data	52	92	Rio Grande do Norte	181	38	81
Maranhão	No data	12	162	Rondônia	No data	23	42
Mato Grosso	129	65	77	Roraima	No data	12	12
Pará	No data	51	193	São Paulo	No data	1,880	1,043
Paraíba	569	48	93	Tocantins	No data	70	36
				Brazil (18 of 27 states reporting)		3,526	3,515

Zika Virus: Medical Countermeasure Development Challenges

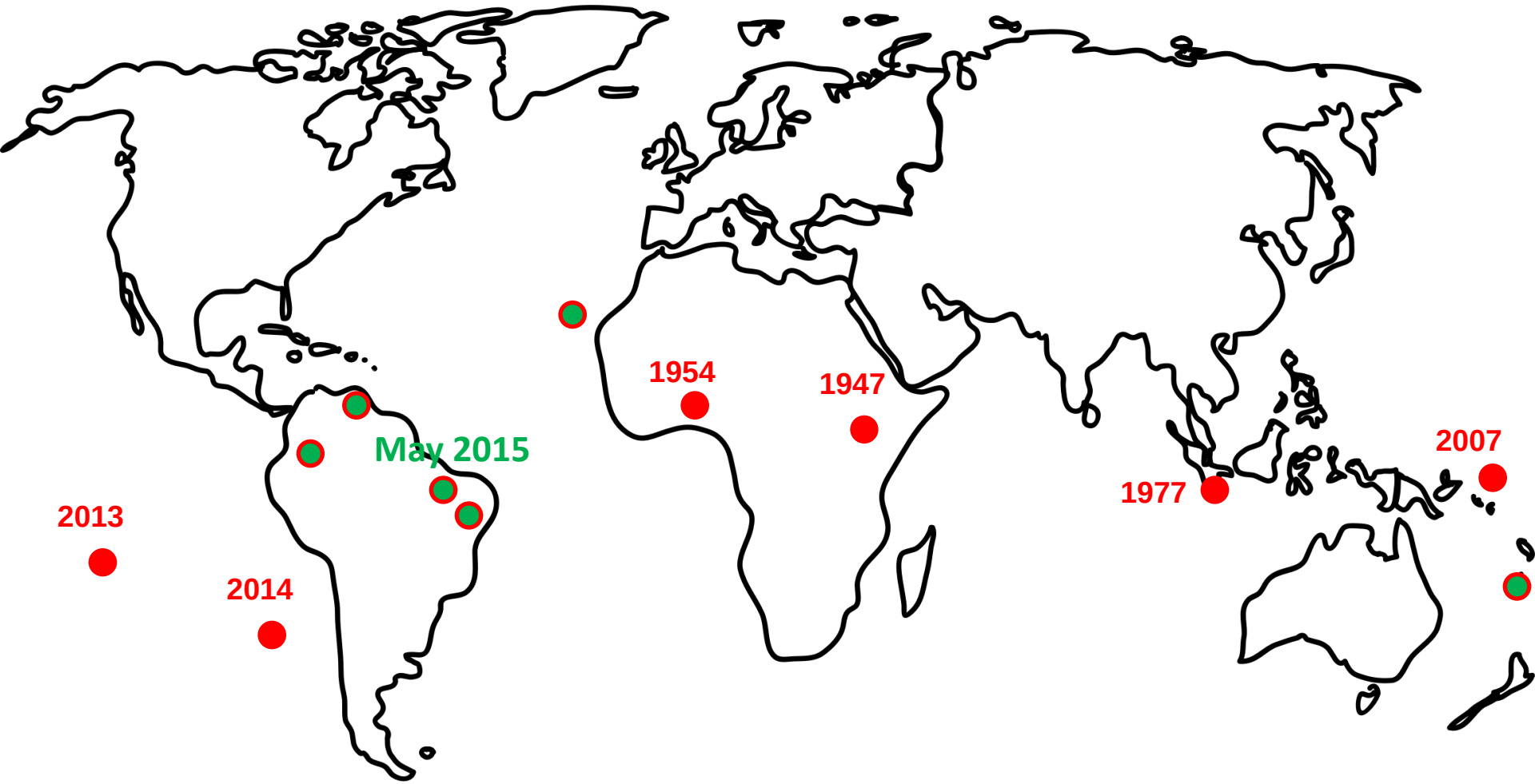
PLOS | NEGLECTED TROPICAL DISEASES

Robert W. Malone[1,2]*, Jane Homan[3], Michael V. Callahan[4], Jill Glasspool-Malone[1,2], Lambodhar Damodaran[5], Adriano De Bernardi Schneider[5], Rebecca Zimler[6], James Talton[7], Ronald R. Cobb[7], Ivan Ruzic[8], Julie Smith-Gagen[9], Daniel Janies[5‡], James Wilson[10‡], Zika Response Working Group

- Considering the average annual birth rate in Brazil of 1.5%, this would indicate 6,600 to 19,500 pregnancies at risk of primary microcephaly from Zika
- In Brazilian states reporting Zika infection during 2015, the attack rate for 2015 is estimated to have been between 0.30% and 0.88%.
- These numbers yield an annual cumulative incidence rate estimate for Brazilian mothers infected with Zika during pregnancy delivering infants with primary microcephaly ranging from 18% to 53%.
- Based on these best estimates of overall Zika incidence, Brazilian mothers infected with Zika during pregnancy are between 3,700 to 11,000 times more likely to deliver infants with primary microcephaly compared to uninfected mothers

History and outbreaks

1947	First identified in a rhesus monkey in the Zika forest of Uganda
1954	First human cases were identified in Nigeria
1977	Small cluster of 7 cases was reported in Java, Indonesia
2007	Outbreak on Yap Island in the Federated States of Micronesia
2013-14	Outbreak in French Polynesia and New Caledonia
Feb 2014	Autochthonous transmission was reported on Easter Island
May 2015	Brazil
Oct 2015	Colombia
Nov 2015	Suriname
Nov 2015	Cape Verde
2015	Vanautu, the Solomon Islands and New Caledonia

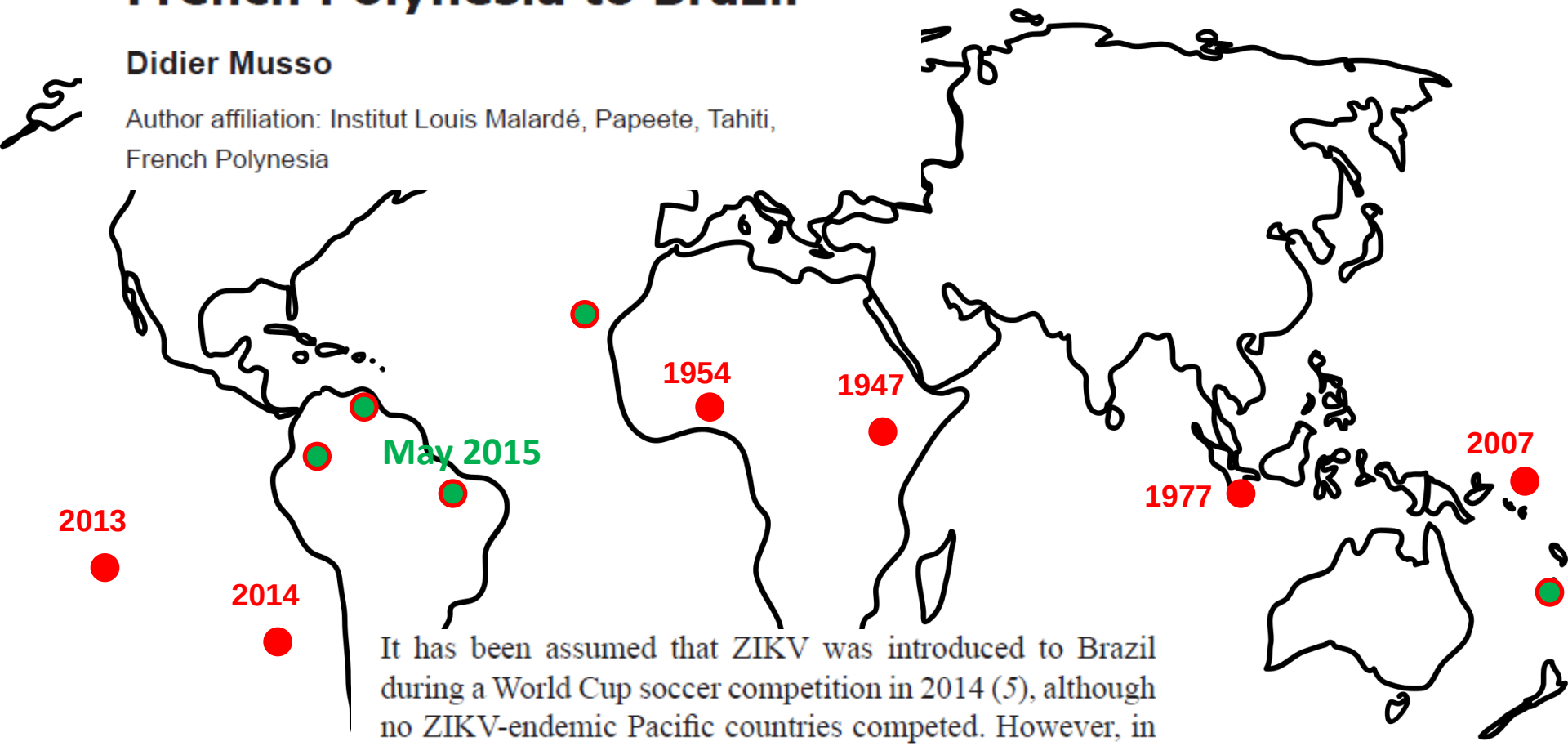

1954
1947
May 2015
2007
1977
2013
2014

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 21, No. 10, October 2015

Zika Virus Transmission from French Polynesia to Brazil

Didier Musso

Author affiliation: Institut Louis Malardé, Papeete, Tahiti, French Polynesia

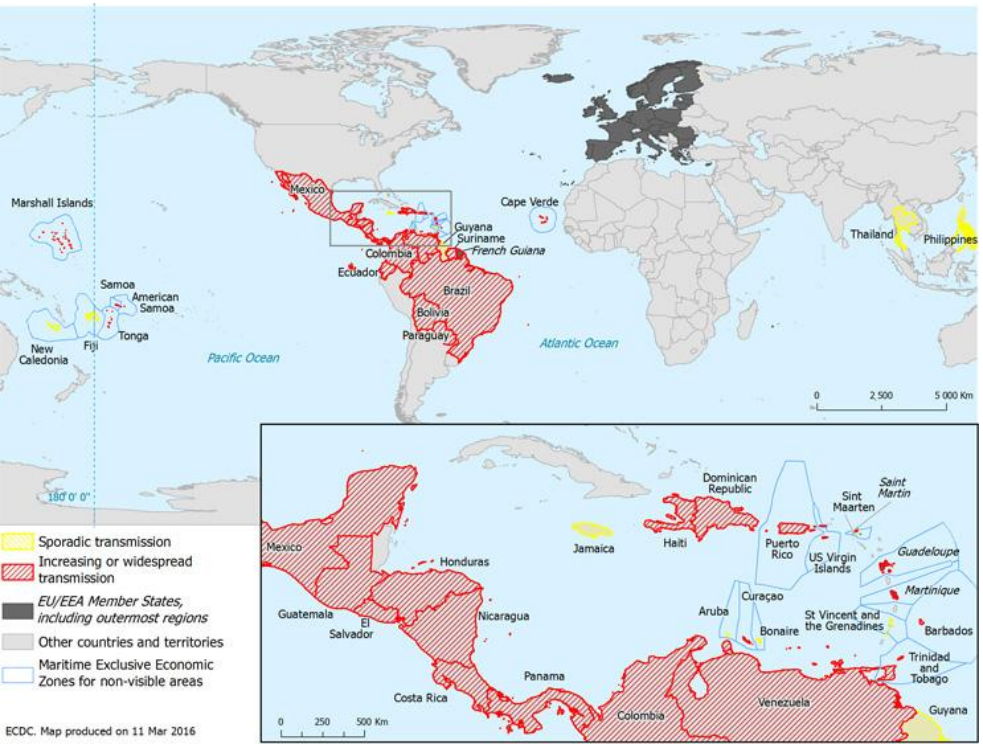


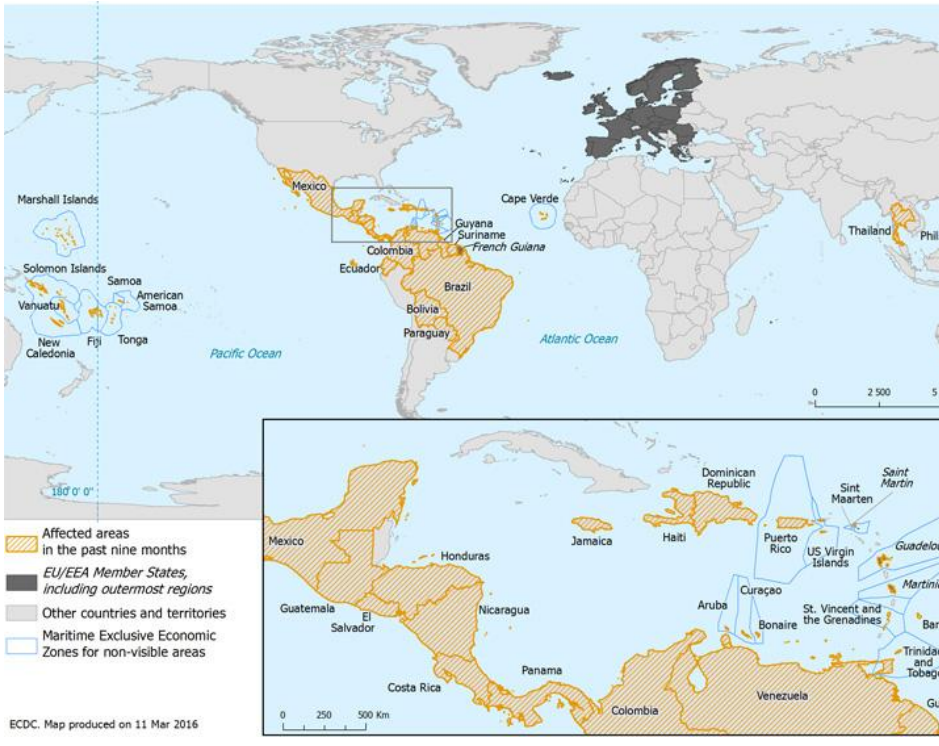
It has been assumed that ZIKV was introduced to Brazil during a World Cup soccer competition in 2014 (*5*), although no ZIKV-endemic Pacific countries competed. However, in August 2014, the Va'a World Sprint Championship canoe race was held in Rio de Janeiro, Brazil. Four Pacific countries (French Polynesia, New Caledonia, Cook Islands, and Easter Island) in which ZIKV circulated during 2014 had teams engaged in this contest in several categories. These data

Paesi che hanno riportato trasmissione autocotona ZIKA al 11 marzo 2016

2 mesi

9 mesi





http://ecdc.europa.eu/en/healthtopics/zika_virus_infection/zika-outbreak/Pages/Zika-countries-with-transmission.aspx


United States
2,767,337
Travellers from Brazil, Sep 2014-Aug 2015
France
404,525
Portugal
411,407
Italy
419,955
New York
Orlando
Miami
Cancun
China
84,332
PERNAMBUCO
BRAZIL
Lima
Risk of local transmission of Zika virus
Seasonal
Year-round
• Cities with >1,000 travellers from Brazil, Sep 2014-Aug 2015, '000
Angola
82,838
Santiago
Montevideo
Buenos Aires
Source: Dr K Khan, St Michael's Hospital, Toronto
Chile
614,687
Argentina
1,314,694

- Impatto politico e mediatico
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Quadro clinico

- Nessun sintomo sino al 75-80% dei casi, generalmente di lieve entità e di breve durata (2-7 giorni) si manifesta dopo un periodo di incubazione di 3-12 giorni (3-7gg)
-
- Febbricola (< 38.5 °C, <50%), artriti e artralgie con possibile tumefazione articolare (piccole articolazioni), rash maculo-papulare cranio caudale, iperemia congiuntivale non purulenta, cefalea retrobulbare e mialgia, astenia e cefalea.

- L'associazione con le complicanze neurologiche quali la sindrome di Guillain-Barré e con la microcefalia nel feto è tuttora oggetto di studio.

- La maggior parte dei pazienti guarisce completamente senza complicanze neurologiche e i tassi di ospedalizzazione sono bassi. Non vi sono vaccini a disposizione ed il trattamento è sintomatico.
- Decessi estremamente rari

http://www.who.int/mediacentre/factsheets/zika/en/
Ioos S, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. Medecine et maladies infectieuses. 2014 Jul;44(7):302-7
http://www.cdc.gov/mmwr/volumes/65/wr/mm6503e1.htm

Diagnosi diferenziale in returning traveller

- Dengue
- Chikungunya
- Leptospirosis
- Malaria
- Rickettsia
- Parvovirus
- Group A Streptococcus
- Rubella
- Measles
- Adenovirus
- Enterovirus

Co-distribution and co-infection of chikungunya and dengue viruses

Furuya-Kanamori *et al. BMC Infectious Diseases* (2016) 16:84

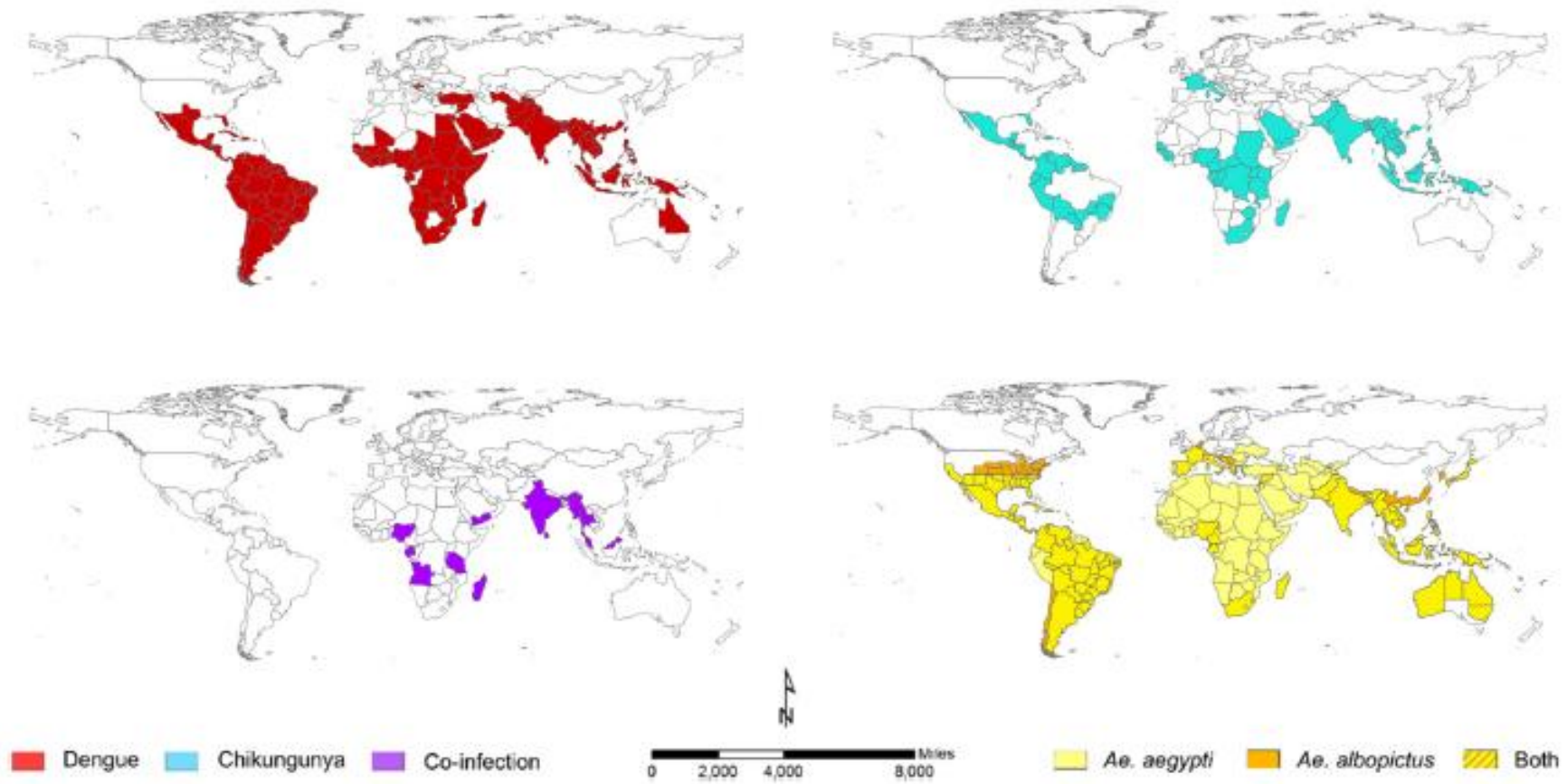
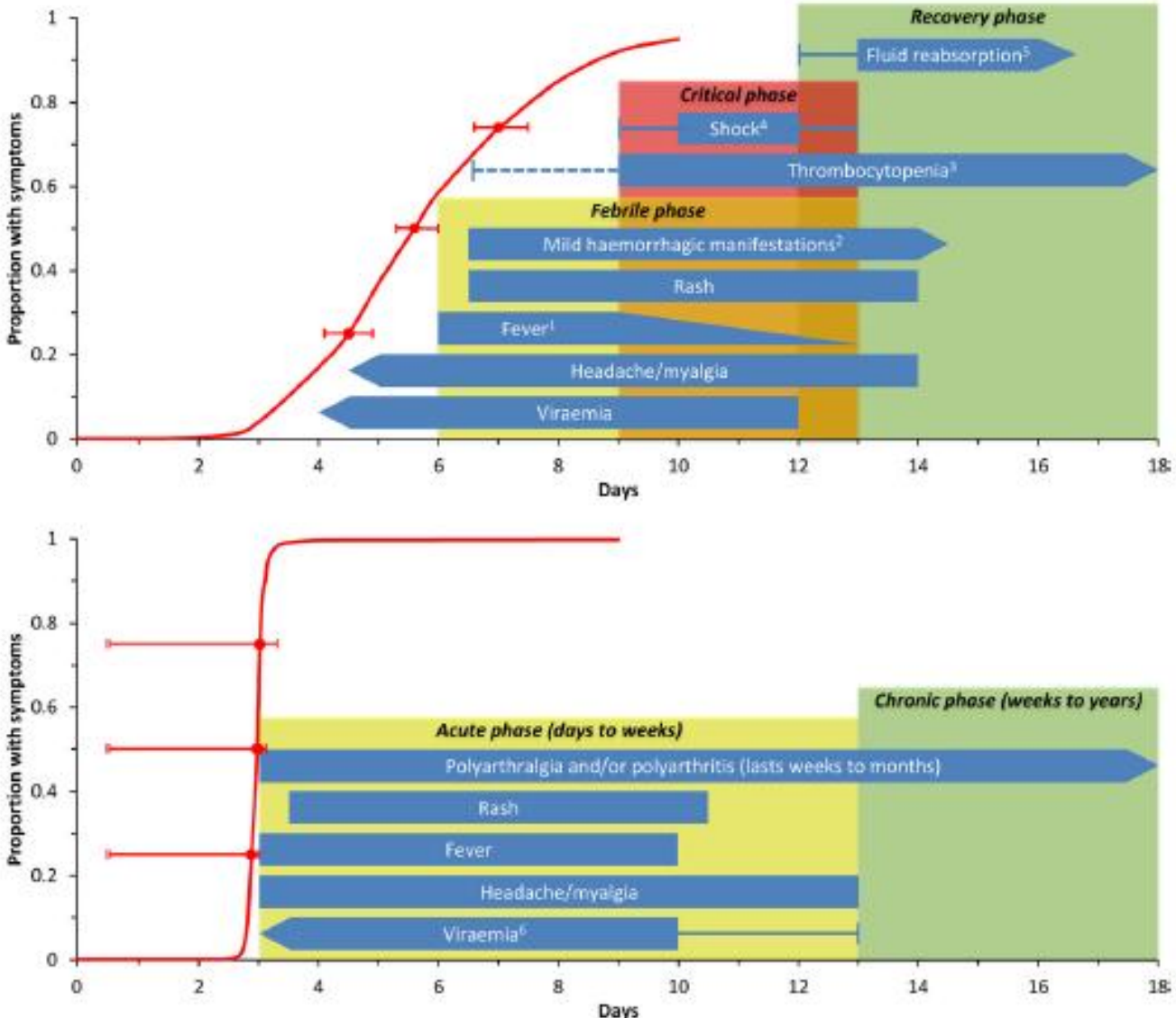


Fig. 1 legend. The global distributions of endemic/epidemic dengue (*top left*) and chikungunya (*top right*) and reports of co-infection (*bottom left*) as well as the principal vectors of both arboviruses, *Aedes aegypti* and *Aedes albopictus* (*bottom right*)

Co-distribution and co-infection of chikungunya and dengue viruses

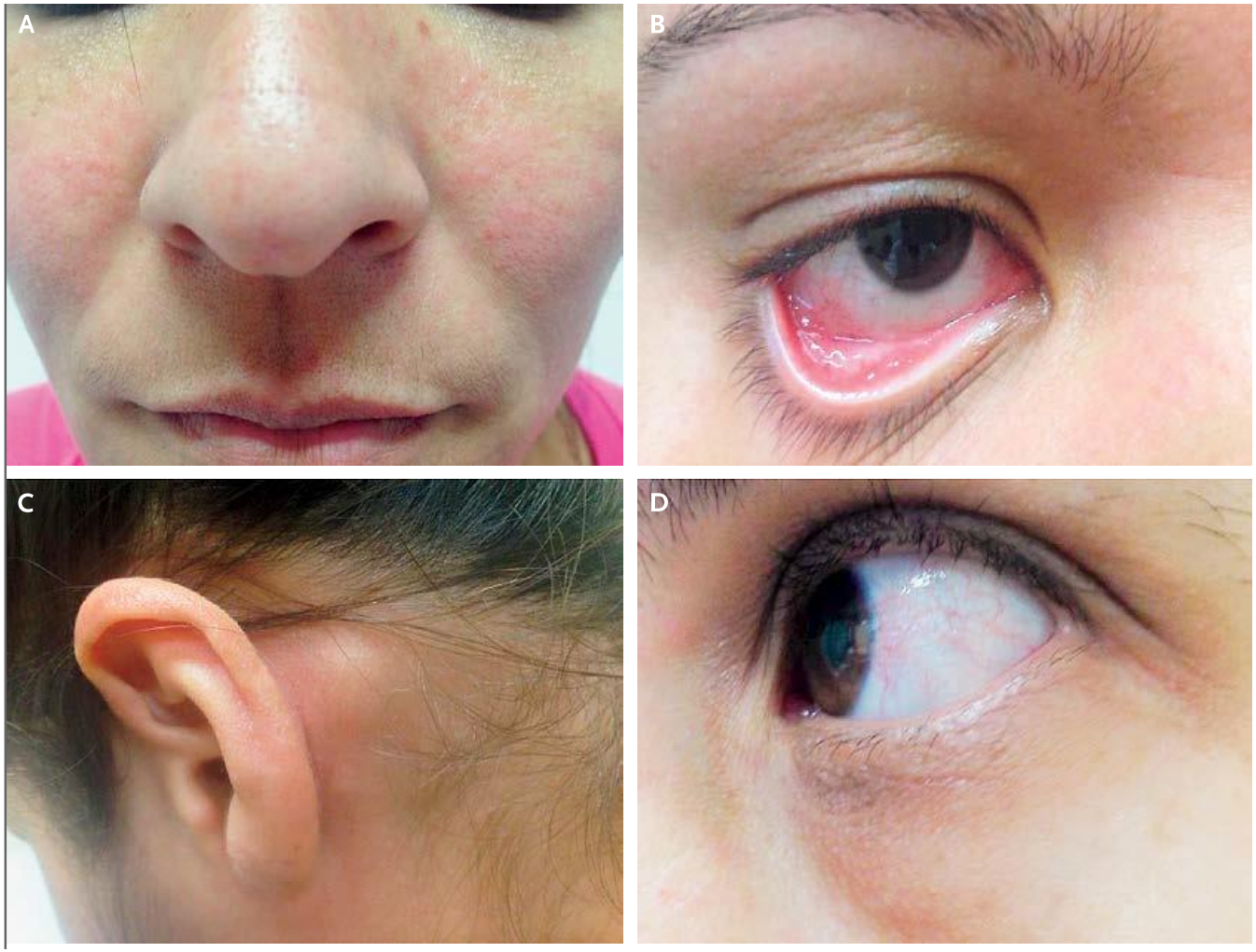
Furuya-Kanamori *et al. BMC Infectious Diseases* (2016) 16:84



Segni/sintomi	Dengue	Zika	Chikungunya
Febbre (durata)	>38° C (4 - 7 gg)	Assente o di lieve entità (1 – 2 gg)	>38° C (2 - 3 gg)
Rash (frequenza)	Dal 4° giorno nel 30-50% dei casi	Dal 1 o 2° giorno nel 90-100% dei casi	Dal 2° al 5° giorno nel 50% dei casi
Mialgia (frequenza)	***/***	**/***	*/***
Artralgie (frequenza)	*/***	**/***	***/***
Artralgie (intensità)	Lievi	Lievi/moderate	Moderate/severe
Edema articolare	Rara	Frequente; di lieve entità	Frequente; di intensità moderata o severa
Congiuntivite	Rara	50 – 90% dei casi	30% dei casi
Cefalea	***	**	**
Prurito	Lieve	Moderato/severo	Lieve
Linfoadenomegalia	Lieve	severa	Moderata

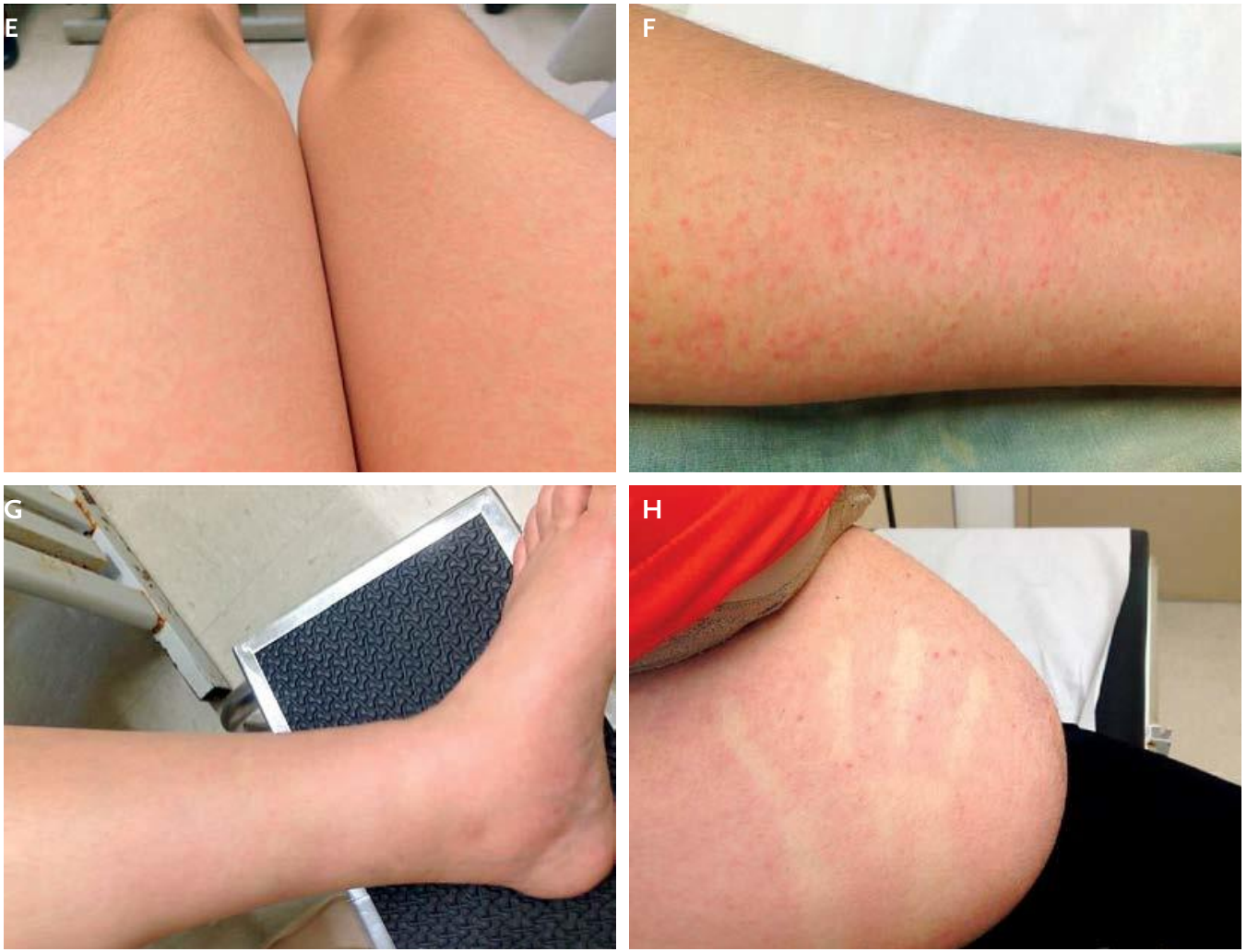
http://www.sciencedirect.com/science/article/pii/S0399077X14001085

Zika Virus Infection in Pregnant Women in Rio de Janeiro — Preliminary Report



Brasil P et al. N Engl J Med 2016. DOI: 10.1056/NEJMoa1602412

Zika Virus Infection in Pregnant Women in Rio de Janeiro — Preliminary Report



Brasil P et al. N Engl J Med 2016. DOI: 10.1056/NEJMoa1602412

- Impatto politico e mediatico
- Eziologia
- Epidemiologia
- Approccio sindromico e diagnosi differenziale arbovirosi
- Complicanze neurologiche
- Prospettive future

ORIGINAL ARTICLE

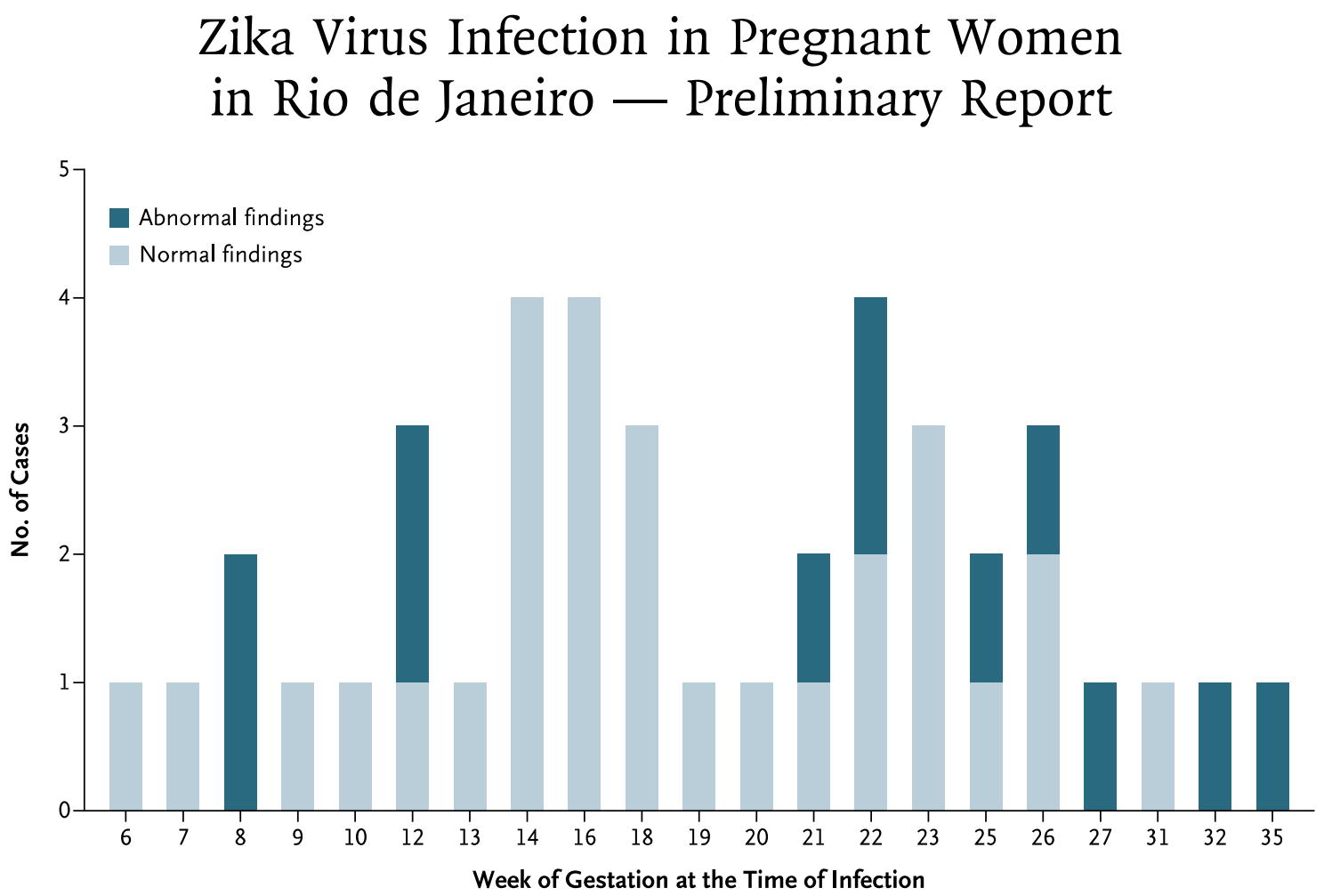
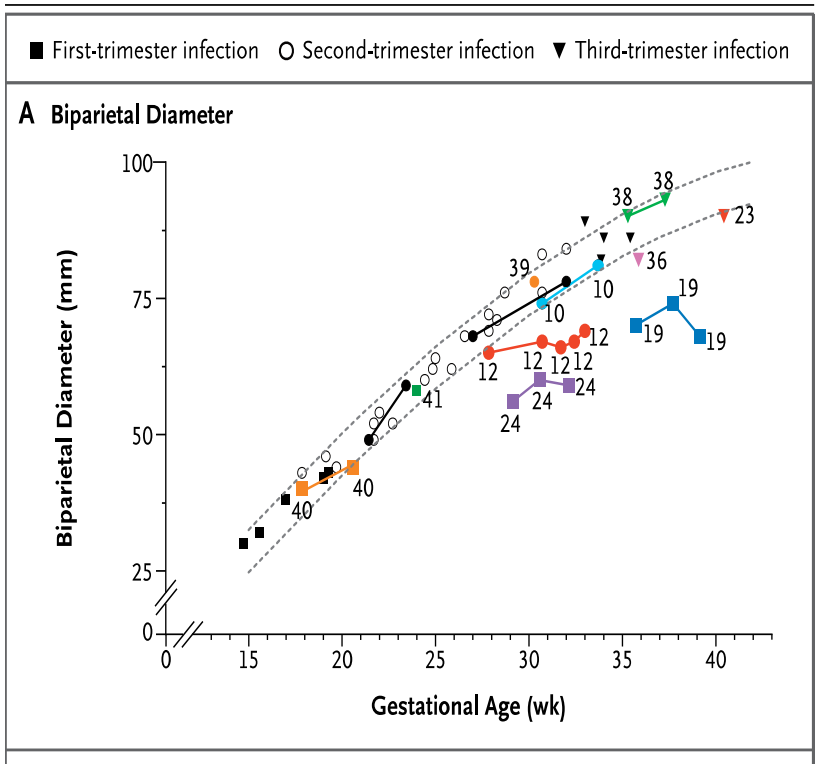
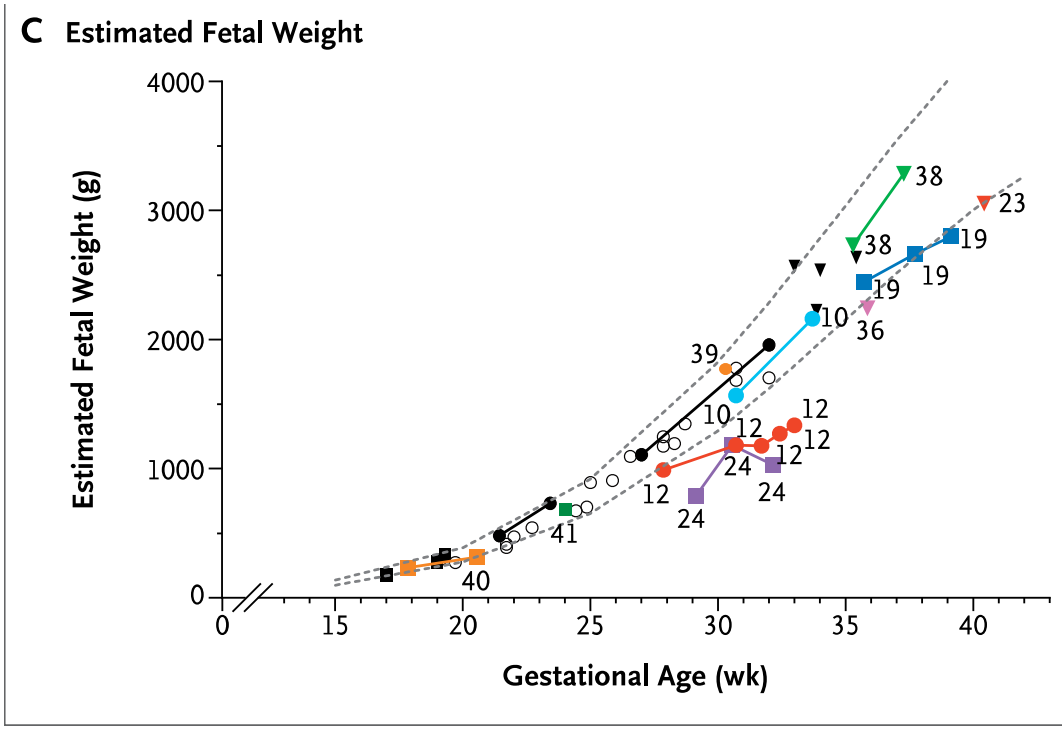


Figure 2. Week of Gestation at the Time of ZIKV Infection and Abnormal Ultrasonographic and Doppler Findings.
Twelve of 42 women (29%) in whom fetal ultrasonography was performed had abnormal findings.

ORIGINAL ARTICLE

Zika Virus Infection in Pregnant Women in Rio de Janeiro — Preliminary Report





1952 Dick GW→ the virus could cross the blood brain barrier

First description of pathological properties of Zika: viral tropism to the brain in intraperitoneally infected mice and an increase in viral titres over several days.

Dick GW. Trans R Soc Trop Med Hyg 1952;46:521e34.

1971 Bell TM→ the virus infected both neurons and glia, producing a variety of intracytoplasmic inclusions, which they termed, "virus factories."

These factories originated from the endoplasmic reticulum and associated with other organelles including the nucleus and the mitochondria.

Bell TM. Arch Gesamte Virusforsch 1971;35:183e93.

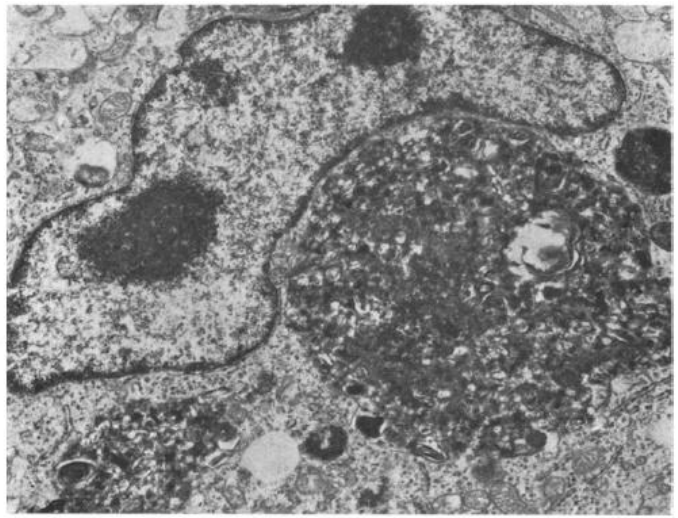


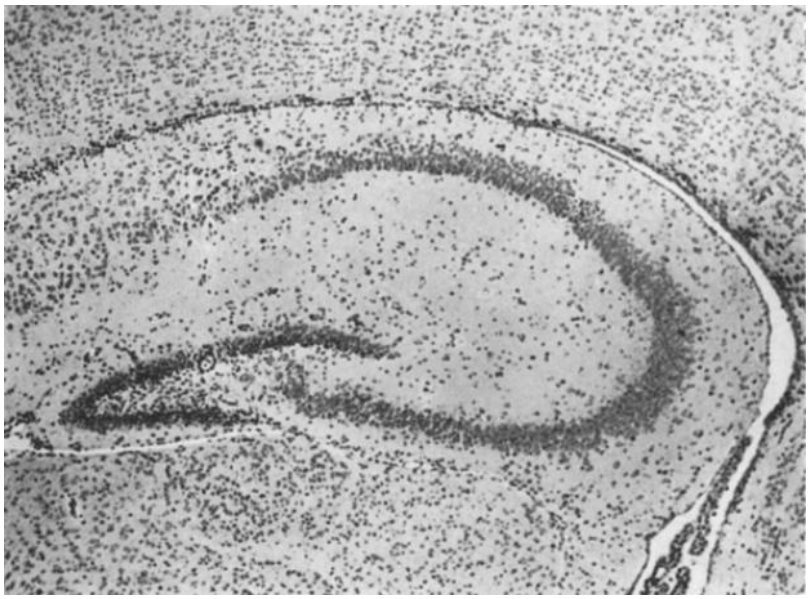
Fig. 6. Virus factory (more advanced stage than fig. 5) in cytoplasm of astroglial cell. × 11,500

Those microscopic observations describe what we now know as **autophagy**

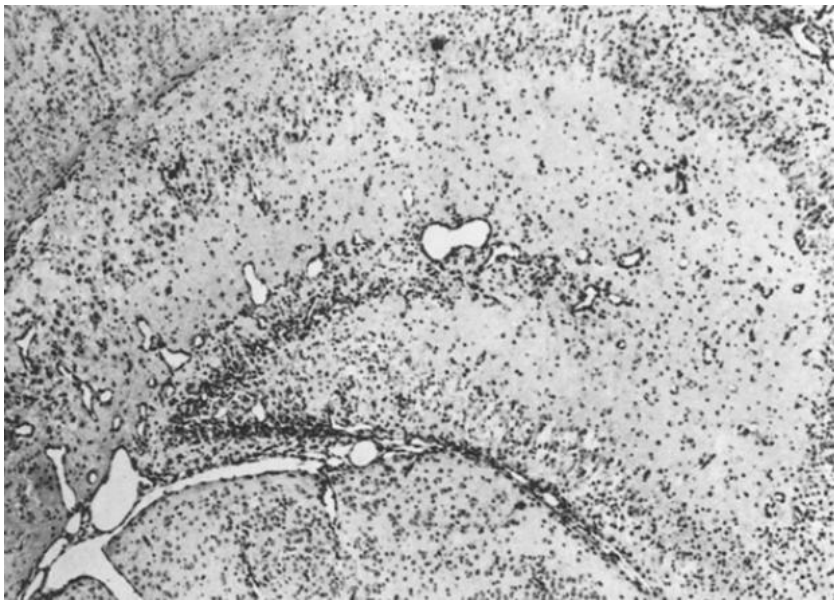
Zika Virus Infection of the Central Nervous System of Mice

By

T. M. Bell, E. J. Field, and H. K. Narang



Normal Ammon's horn of mouse 7-days-old.



Ammon's horn of Zika infected mouse 7-days-old.

Archiv für die gesamte Virusforschung 35, 183—193 (1971)

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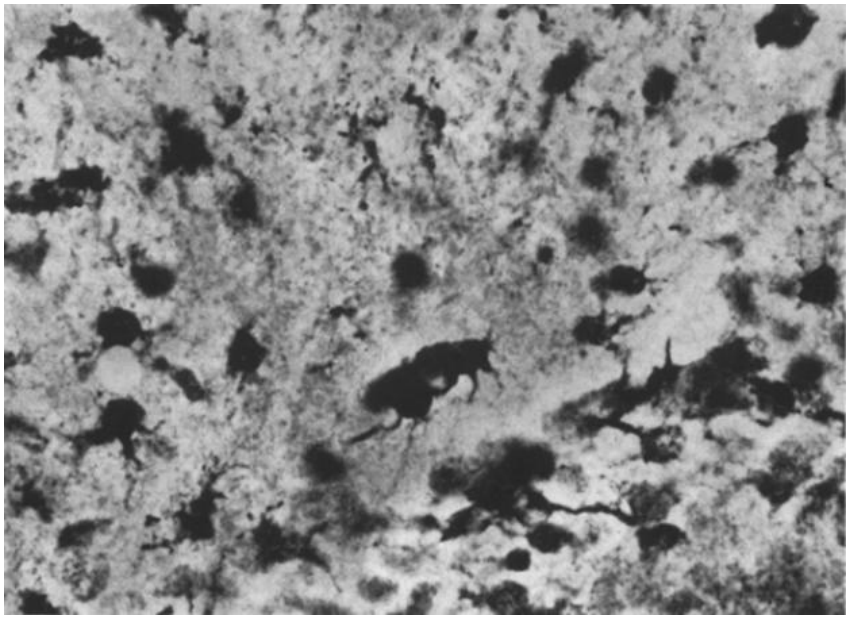
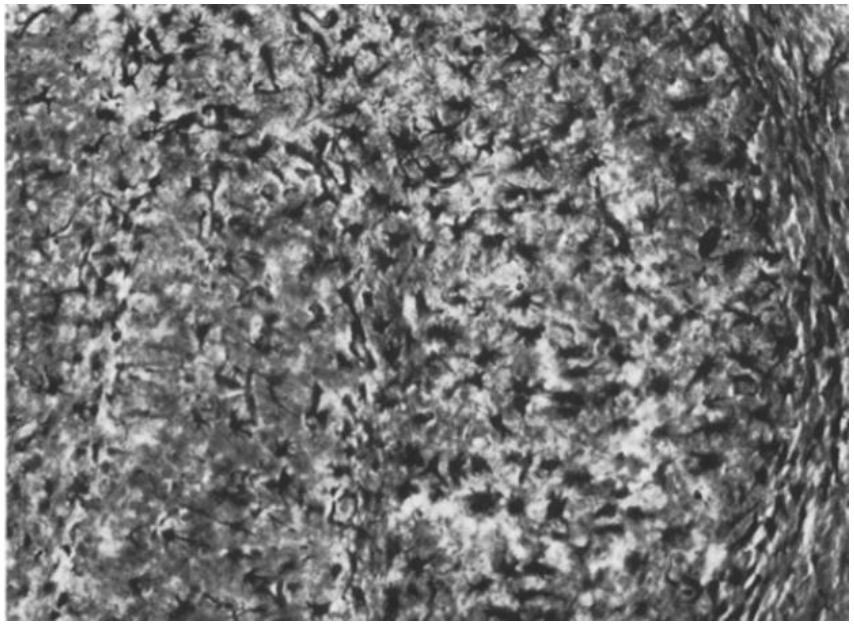


Astrocytes in Ammon's horn of normal 7-days-old mouse.



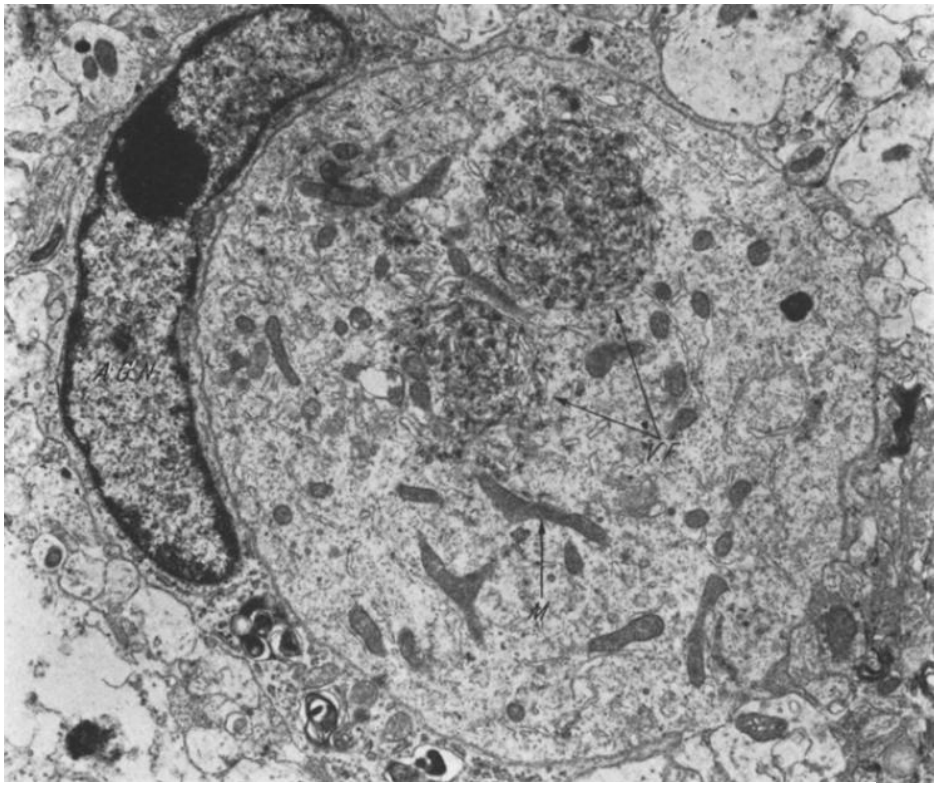
Ammon's horn of 50-day-old mouse with Zika infection.

Archiv für die gesamte Virusforschung 35, 183—193 (1971)

Zika Virus Infection of the Central Nervous System of Mice

By

T. M. Bell, E. J. Field, and H. K. Narang



Ammon's horn of 7-day-old mouse infected with Zika virus.

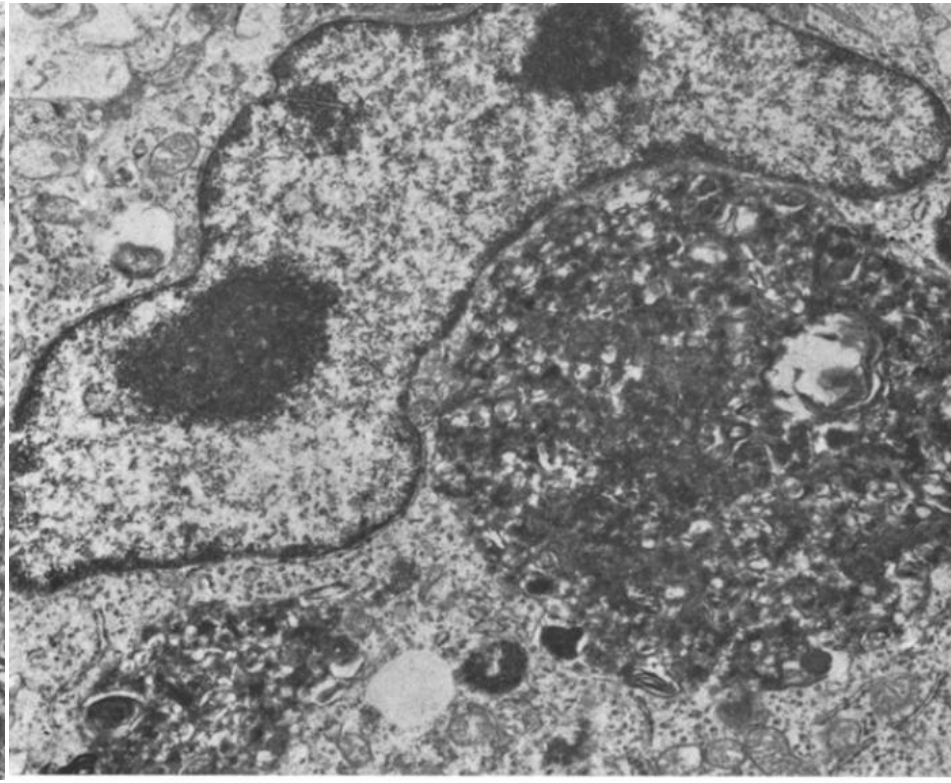


Fig. 6. Virus factory (more advanced stage than fig. 5) in cytoplasm of astroglial cell, × 1

Archiv für die gesamte Virusforschung 35, 183—193 (1971)

Zika Virus Infection of the Central Nervous System of Mice

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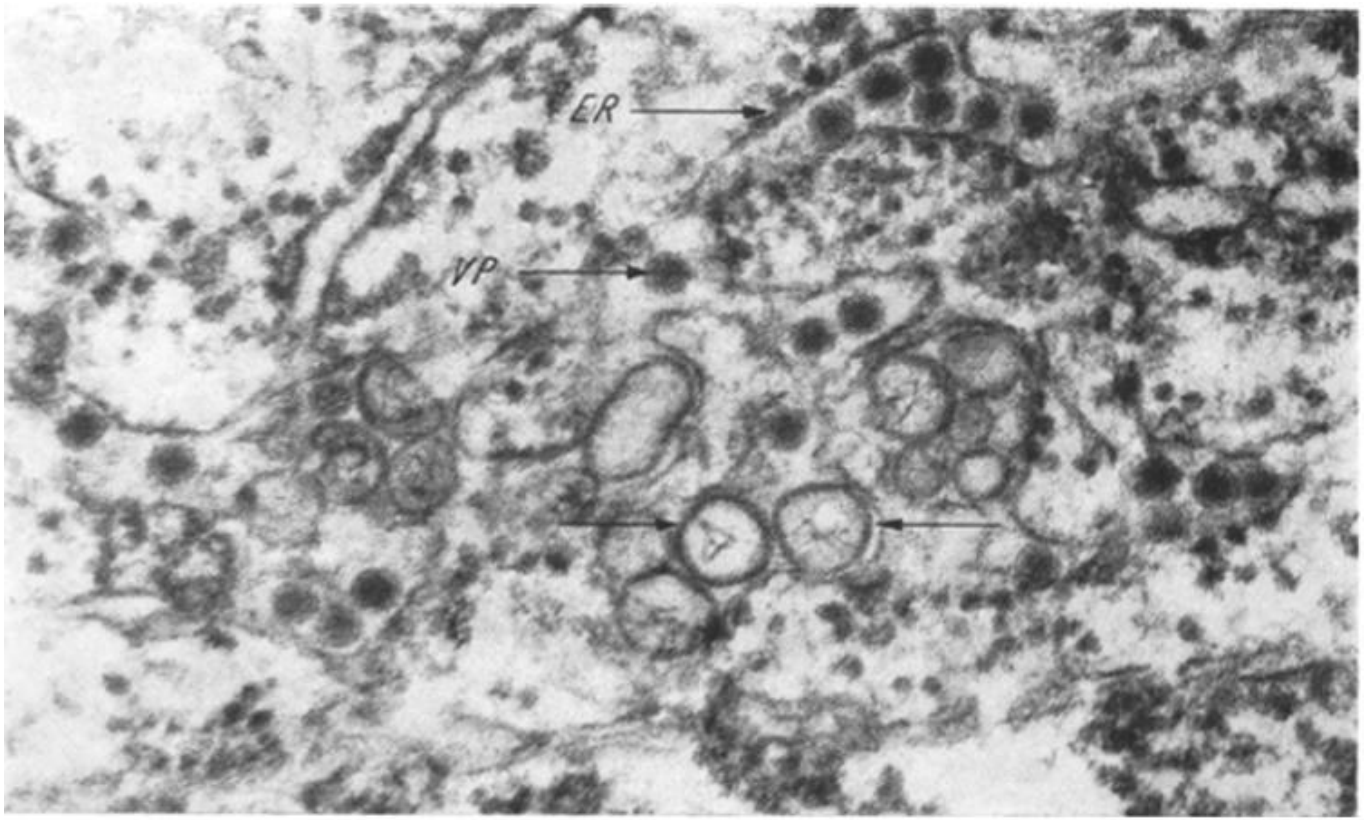


Fig. 8. Thalamus neurone of 7-day-old mouse with Zika infection. Endoplasmic reticulum (ER) contains

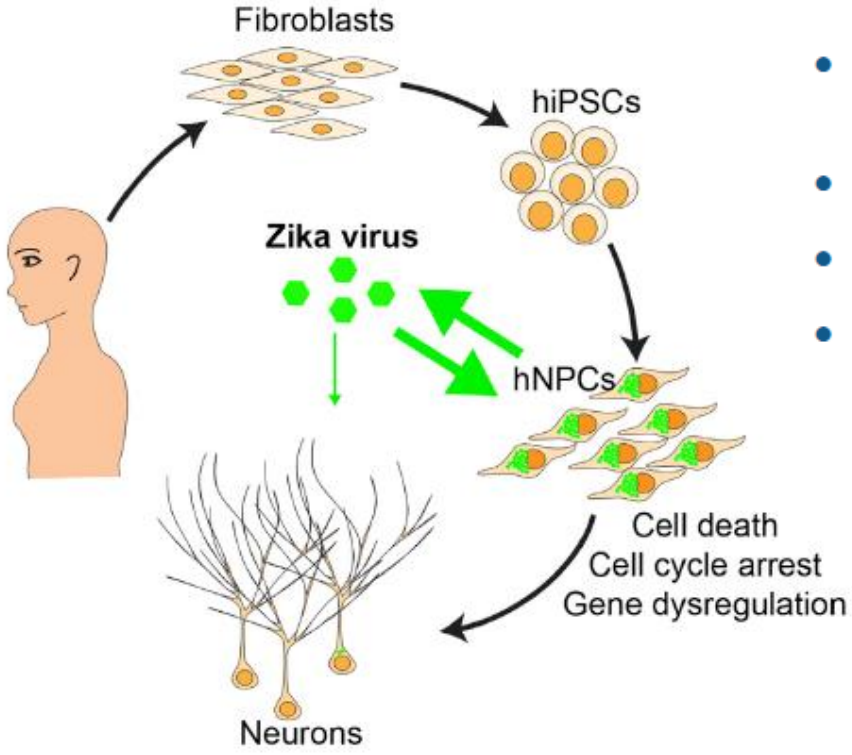
Archiv für die gesamte Virusforschung 35, 183—193 (1971)

Brief Report

Cell Stem Cell

Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth

Hengli Tang, Christy Hammack, Sarah C. Ogden, ..., Peng Jin, Hongjun Song, Guo-li Ming



- Zika virus (ZIKV) infects human embryonic cortical neural progenitor cells (hNPCs)
- ZIKV-infected hNPCs produce infectious ZIKV particles
- ZIKV infection leads to increased cell death of hNPCs
- ZIKV infection dysregulates cell cycle and transcription in hNPCs

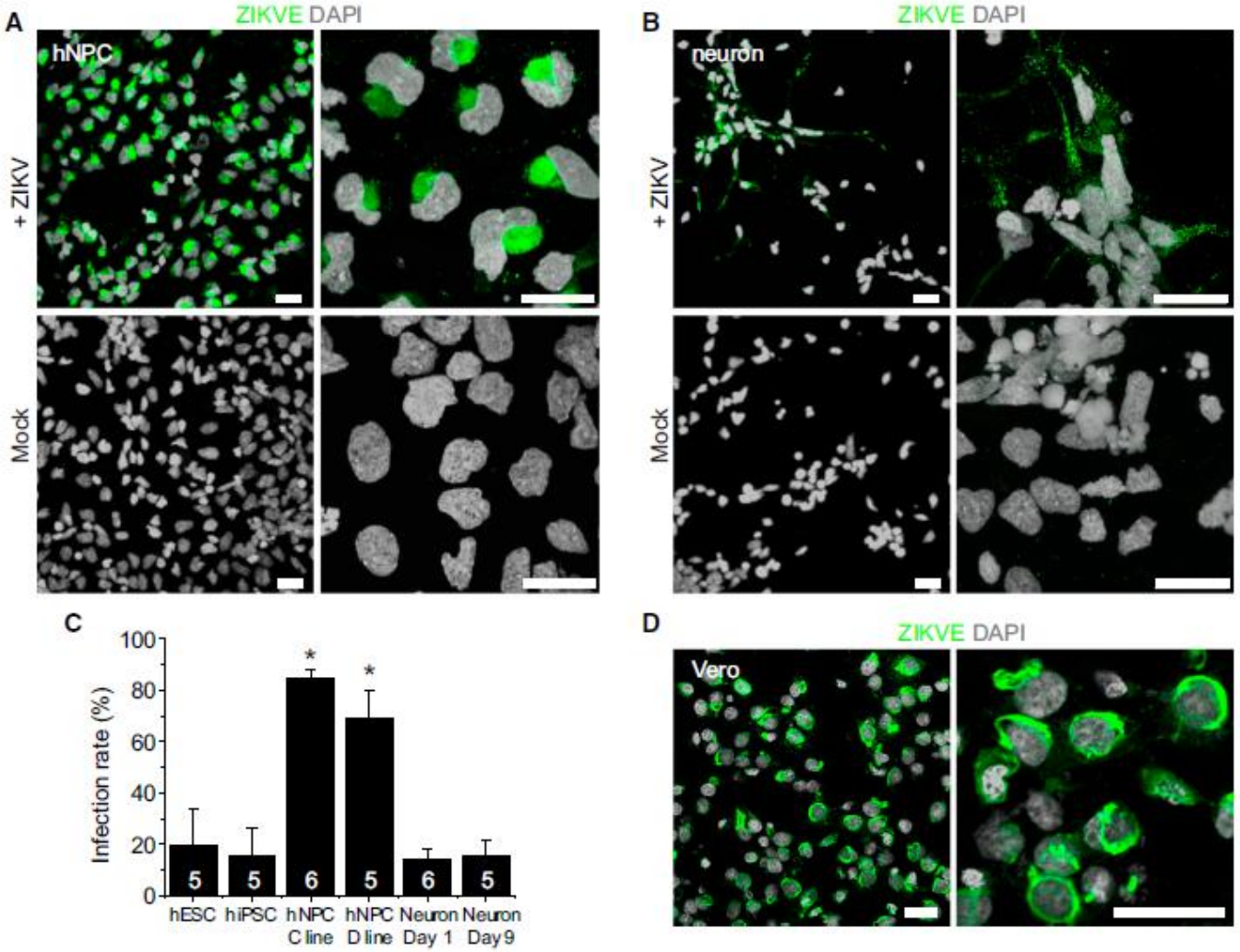


Figure 1. ZIKV Infects hiPSC-Derived Neural Progenitor Cells with High Efficiency

(A and B) Sample confocal images of forebrain-specific hNPCs (A) and immature neurons (B) 56 hr after infection with ZIKV supernatant, immunostained for ZIKV envelop protein (ZIKVE; green) and DAPI (gray). Cells were differentiated from the C1-2 hiPSC line. Scale bars, 20 μm.

(C) Quantification of infection efficiency for different cell types, including hESCs, hiPSCs, hNPCS derived from two different hiPSCs, and immature neurons 1 or 9 days after differentiation from hNPCs. Both hESCs and hiPSCs were analyzed 72 hr after infection, whereas all other cells were analyzed 56 hr after infection. Numbers associated with bar graphs indicate numbers of independent experiments. Values represent mean ± SD (*$p < 0.01$; Student's t test).

(D) Production of infectious ZIKV particles by infected hNPCs. Supernatant from hNPC cultures 72 hr after ZIKV infection was collected and added to Vero cells for 2 hr. The Vero cells were further cultured for 48 hr. Shown are sample images of ZIKVE immunostaining (green) and DAPI (gray). Scale bars, 20 μm.

See also Figure S1 and Table S1.

Autophaghy and arbovirus

- ✓Interactions between the Flavivirus and the Endoplasmic Reticulum induce autophagy.

- ✓Yet these viruses prevent completion of the autophagy process, providing a perfect environment for the creation of "viral factories" to maximize viral replication and amplification.

- ✓To date autophagy has not been described in Zika infected neural cells

CAUSES OF MICROCEPHALY

Amplification of **CENTROSOME** number has been revealed to be one of the inducers of this condition.

- delay in mitosis
- an increase in apoptosis
- improper neural stem cell orientation
- premature neuronal differentiation
- decrease in progenitor cells.

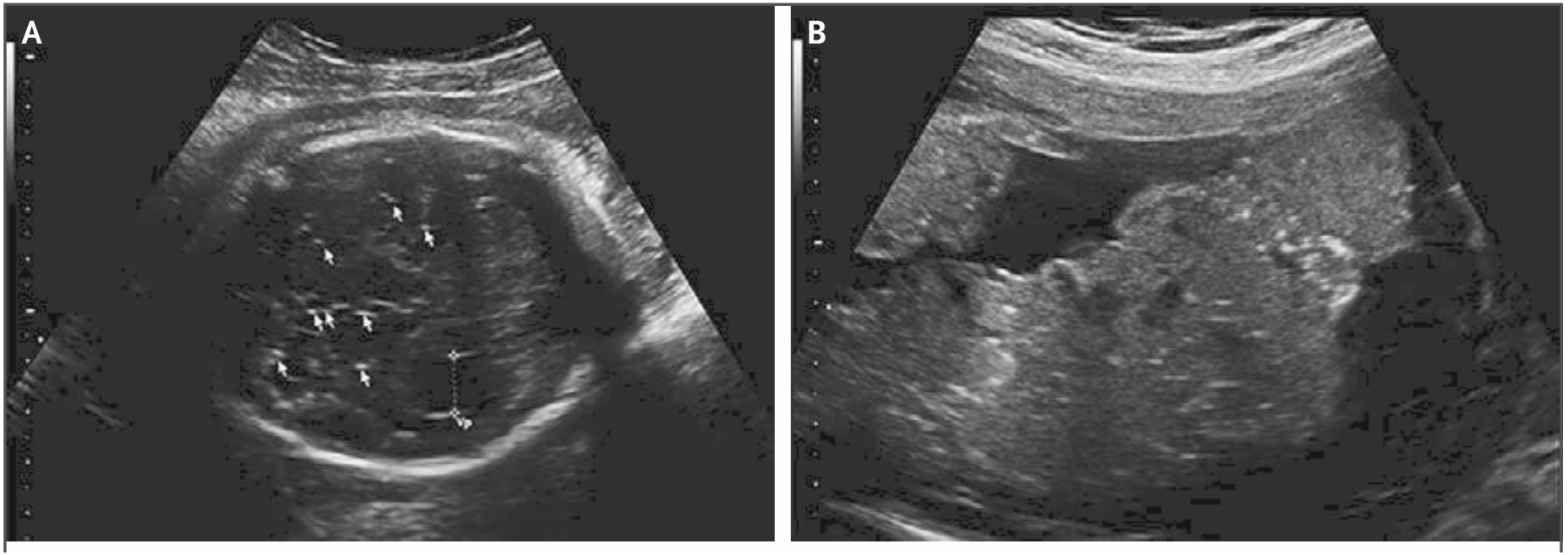
The overall effect reduces the formation of brain matter leading to the reduced brain size indicative of **microcephaly.**

Marthiens V. Nat Cell Biol 2013;15:731e40

Zika Virus Associated with Microcephaly

The NEW ENGLAND JOURNAL of MEDICINE

Jernej Mlakar, M.D., Misa Korva, Ph.D., Nataša Tul, M.D., Ph.D.,



Panel A shows numerous calcifications in various parts of the brain (some marked with arrows) and the dilated occipital horn of the lateral ventricle (Vp, marked with a measurement bar) as seen on transverse ultrasonography.

Panel B shows numerous calcifications in the placenta

Zika Virus Associated with Microcephaly

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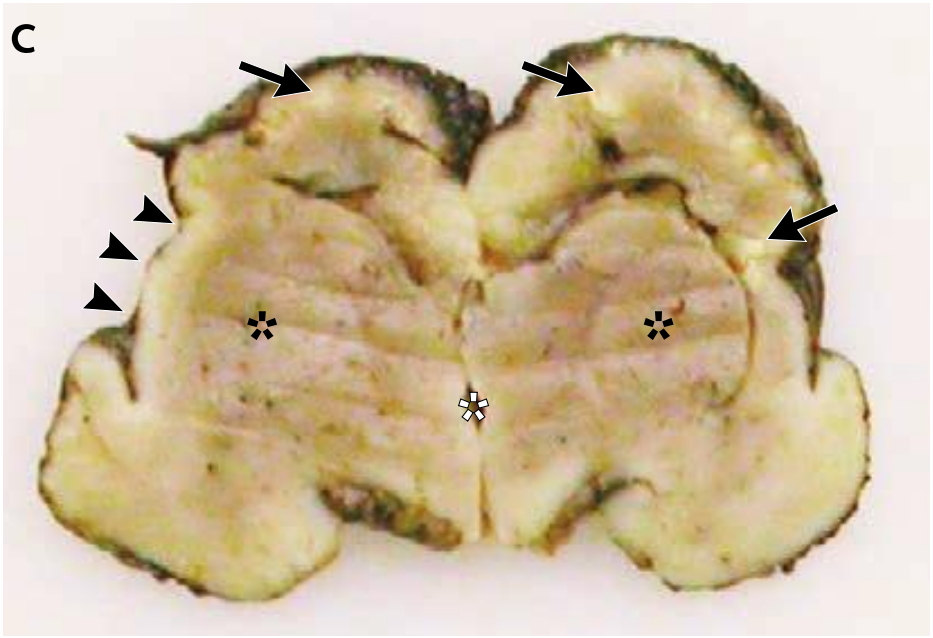

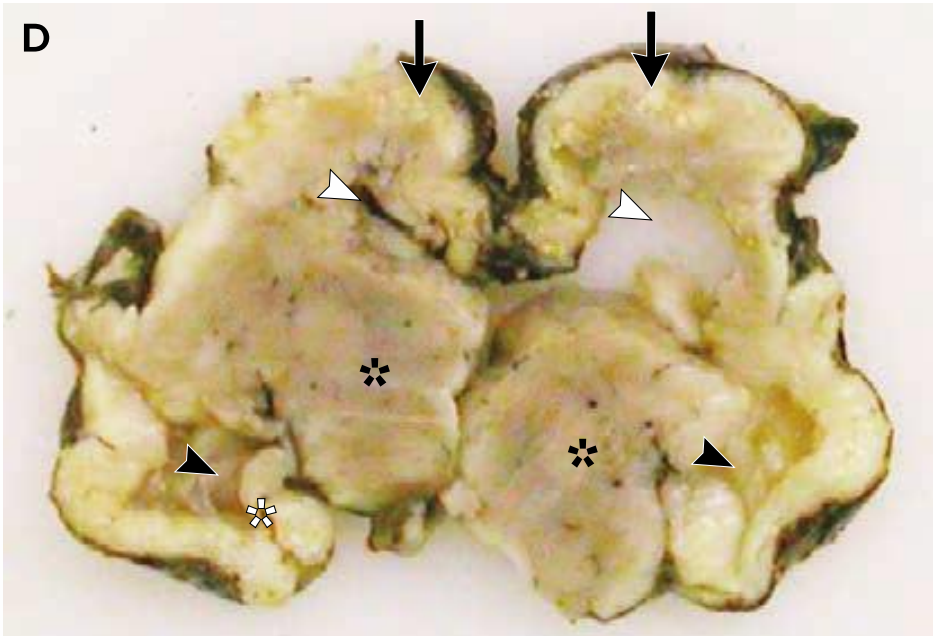

Panel C shows multifocal cortical and subcortical white calcifications (arrows) and almost complete loss of gyration of the cortex. The basal ganglia are developed but poorly delineated (black asterisks), and the sylvian fissures are widely open on both sides (arrowheads on the left). The third ventricle is not dilated (white asterisk). Panel D shows dilated body of the lateral ventricles (white arrowheads); the left is collapsed. Temporal horns of the lateral ventricles (black arrowheads) are also dilated. The thalami (black asterisks) and the left hippocampus (white asterisk) are well developed, whereas the contralateral structure is not recognizable owing to autolysis.

Zika Virus Associated with Microcephaly

The NEW ENGLAND JOURNAL of MEDICINE

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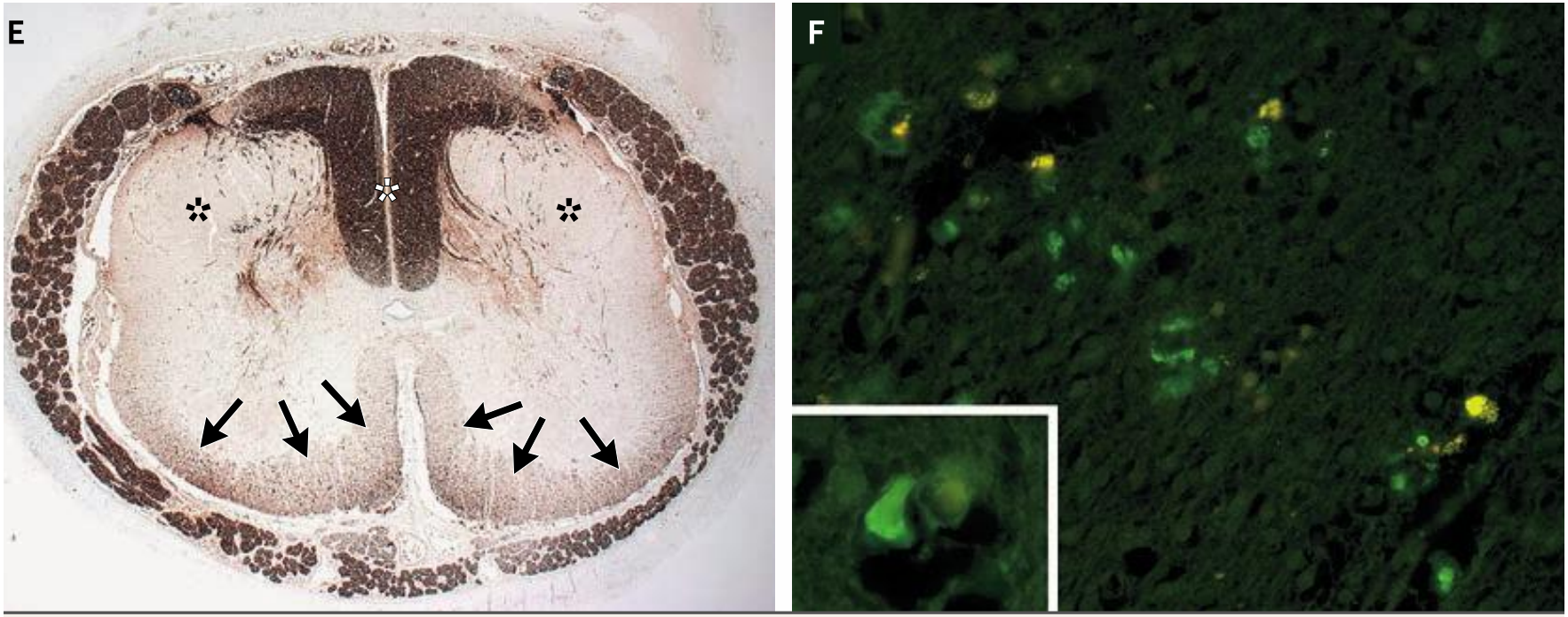

Panel E shows neurofilament immunohistochemical staining of axons in a cross-section of the lumbar spinal cord with severe Wallerian degeneration of the lateral corticospinal tracts (black asterisks), moderate involvement of other descending tracts (arrows), and well-preserved ascending tracts in the dorsal columns (white asterisk) (neurofilament, clone 2F11 [Dako]).
Panel F shows indirect immunofluorescence of fetal brain tissue, revealing a green granular intracytoplasmic reaction (see also inset). The yellow signals adjacent to the green granules indicate auto- fluorescence of lipofuscin, suggesting that viral particles are located in the cytoplasm of neurons.

Zika Virus Associated with Microcephaly

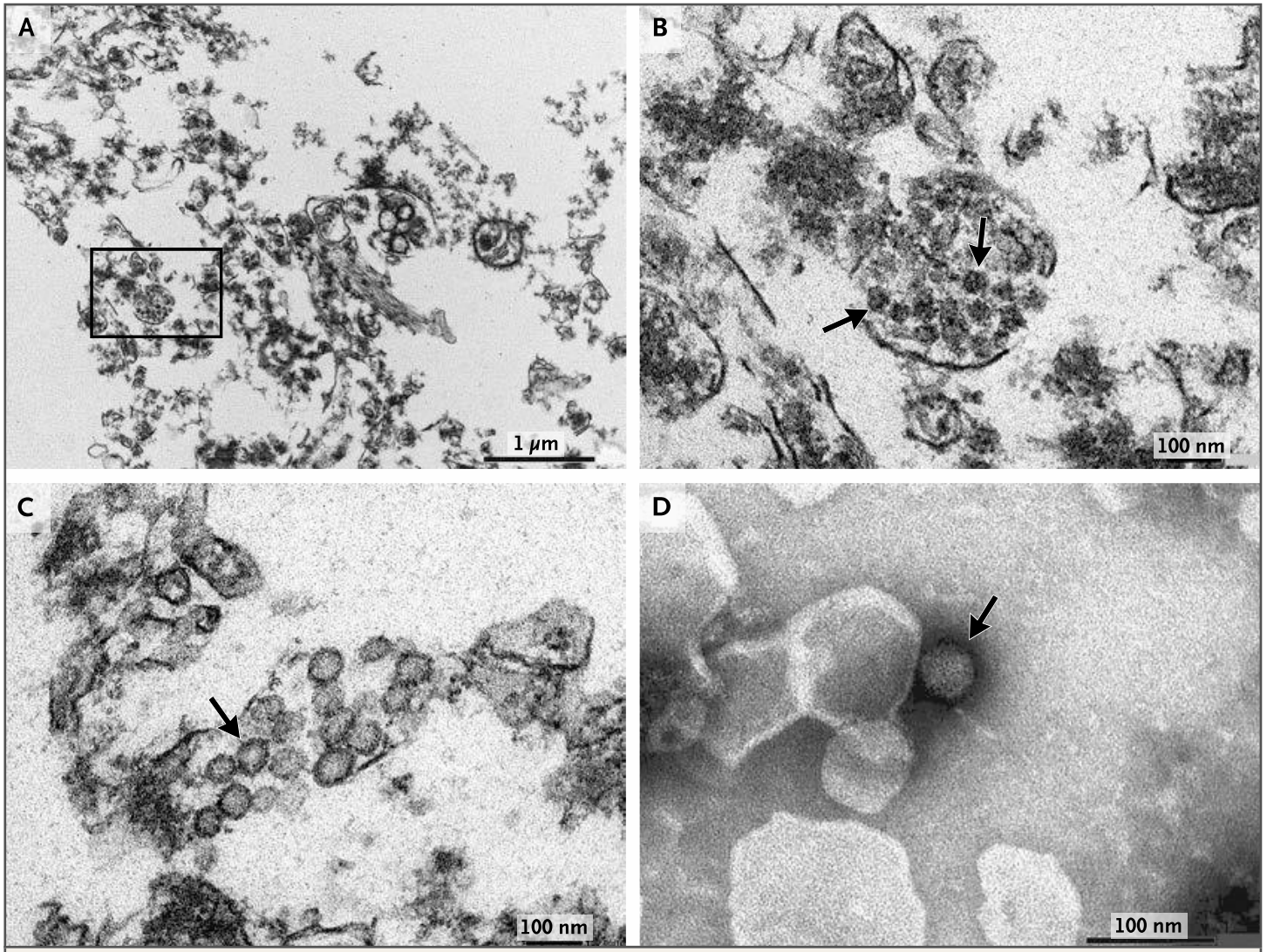

Figure 3. Electron Microscopy of Ultrathin Sections of Fetal Brain and Staining of a Flavivirus-like Particle.

Panel A shows a damaged brain cell with a cluster of dense virions located in the disrupted endoplasmic reticulum. Remains of membranes derived from different cellular compartments and filamentous structures are also seen. A magnified view of the boxed area with virions clearly visible (arrows) is shown in Panel B. Panel C shows a group of enveloped structures with a bright interior, presumably indicating viral replication (arrow). Panel D shows a negatively stained viral particle with morphologic characteristics consistent with those of Flaviviridae viruses (arrow).

Zika Virus Associated with Microcephaly

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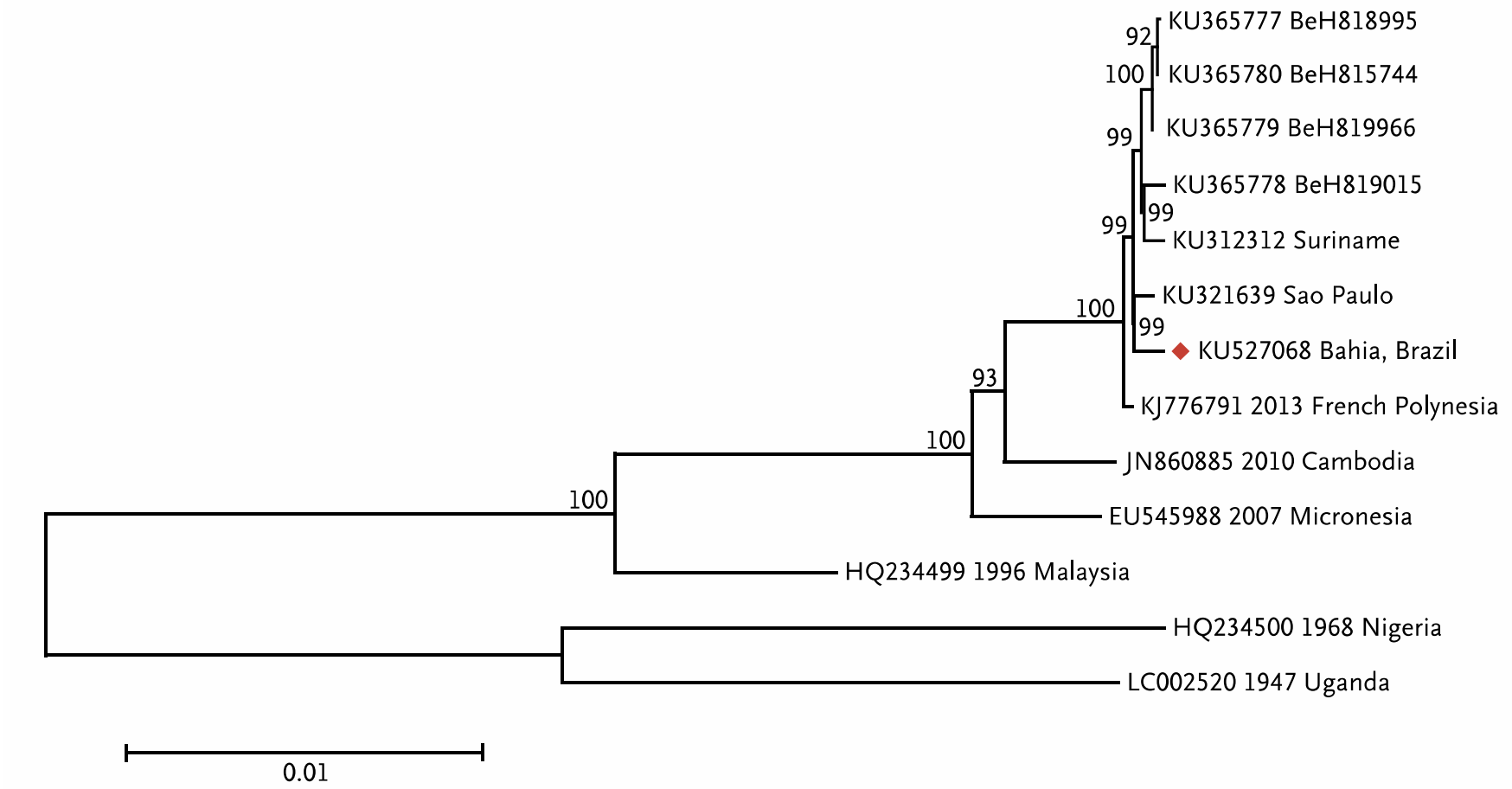


Figure 4. **Phylogenetic Analysis of the Complete Genome of Zika Virus.**

Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study

Guilherme Calvet, Renato S Aguiar*, Adriana S O Melo, Simone A Sampaio, Ivano de Filippis, Allison Fabri, Eliane S M Araujo, Patricia C de Sequeira, Marcos C L de Mendonça, Louisi de Oliveira, Diogo A Tschoeke, Carlos G Schrago, Fabiano L Thompson, Patricia Brasil, Flavia B dos Santos, Rita M R Nogueira, Amilcar Tanuri†, Ana M B de Filippis†*

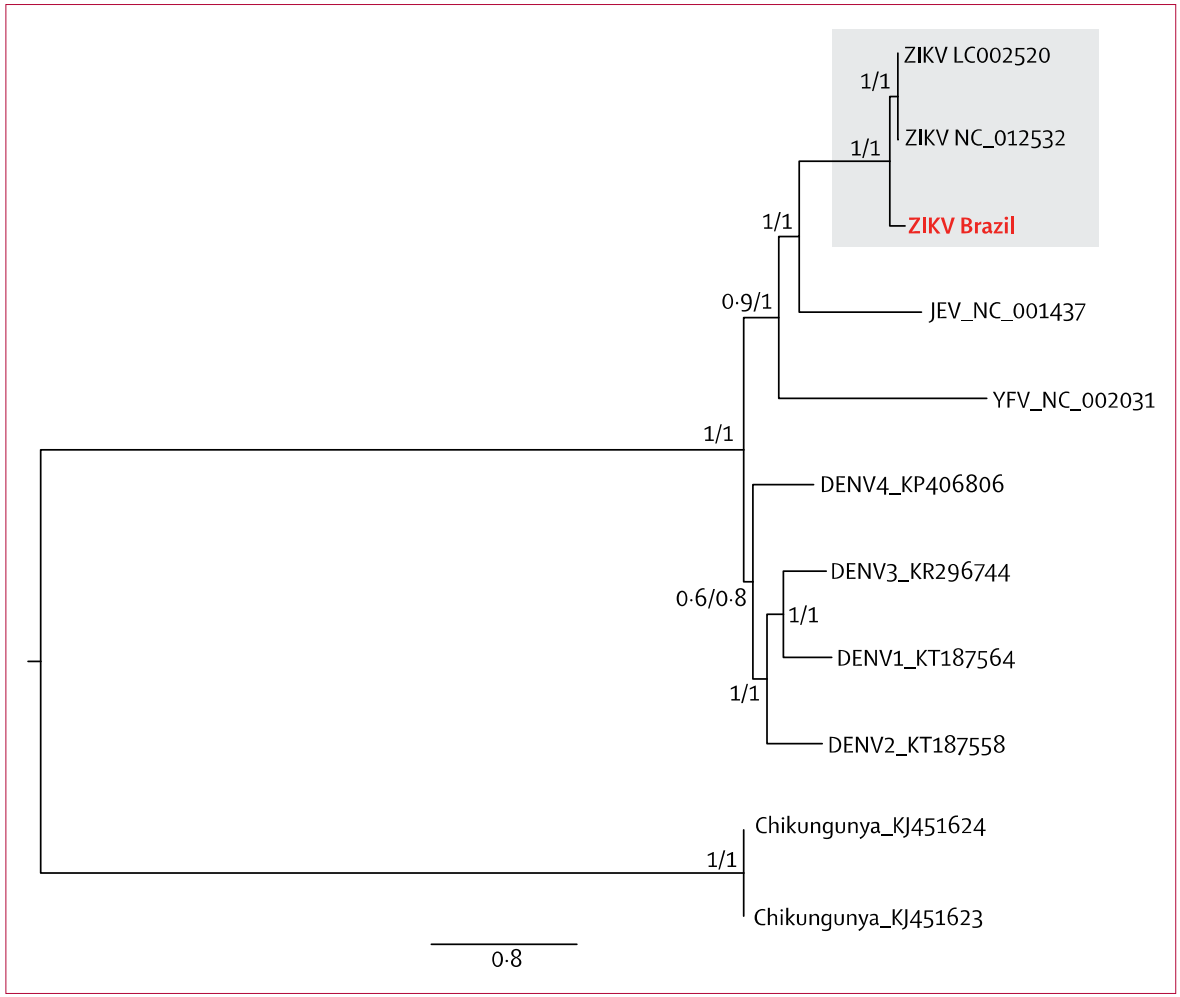


Figure 4: **Maximum likelihood phylogeny of Brazilian Zika virus, other Flaviviridae genomes, and an alphavirus genome**

Argentine and Brazilian doctors suspect mosquito insecticide as cause of microcephaly

Claire Robinson / GMWatch

10th February 2016

With the proposed connection between the Zika virus and Brazil's outbreak of microcephaly in new born babies looking increasingly tenuous, Latin American doctors are proposing another possible cause: Pyriproxyfen, a pesticide used in Brazil since 2014 to arrest the development of mosquito larvae in drinking water tanks. Might the 'cure' in fact be the poison?

Since 2014, the insecticide Pyriproxyfen has been use to kill mosquitos in water tanks in Brazil. Water tank in Bahia state, northeast Brazil. Photo: Francois Le Minh via Flickr (CC BY-NC-ND).

"Malformations" The World Health

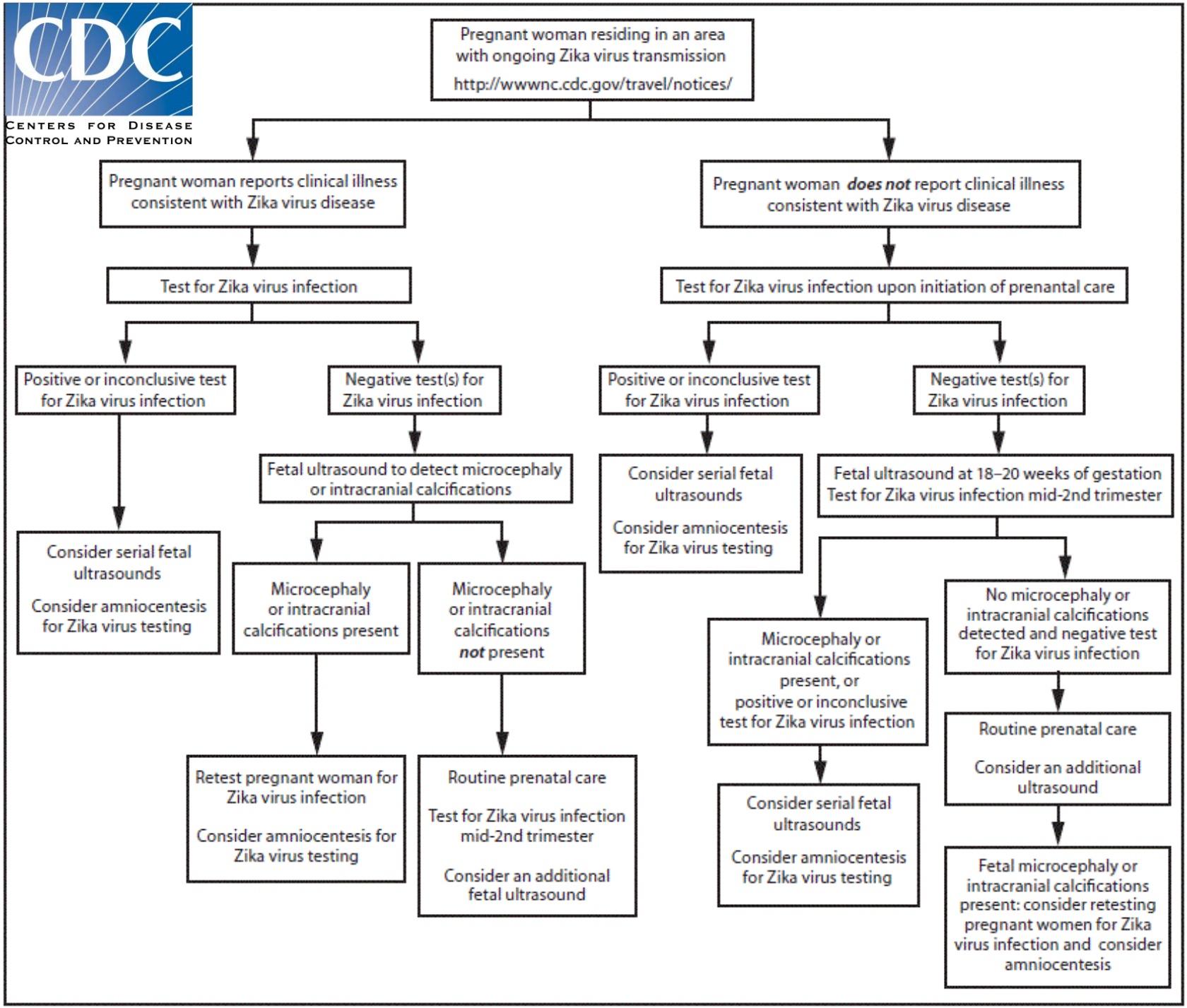
THE LANCET

Zika virus infection in French Polynesia

Published Online

	Gestational age at maternal viral infection (weeks)	Gestational age at first ultrasonography anomalies (weeks)	Gestational age at diagnosis of fetal microcephaly (weeks)	Neuroimagery findings		
				Gestational age (weeks)	MRI	Anomalies
Case 1	9^{+3}	25^{+6}	30^{+1}	28^{+4}	Yes	Ventricular dilatation Thin corpus callosum
Case 2	8	22	22	25	Yes	Microcephaly Absent corpus callosum Abnormal gyration Cerebral calcifications
Case 3	Unknown	29^{+2}	29^{+2}	30^{+1}	Yes	Microcephaly Absent corpus callosum Ventricular dilatation Abnormal gyration Vermian hypoplasia
Case 4	12	21	21	Unknown	No	Microcephaly Absent corpus callosum Ventricular dilatation Vermian agenesis Abnormal gyration Intrauterine growth restriction

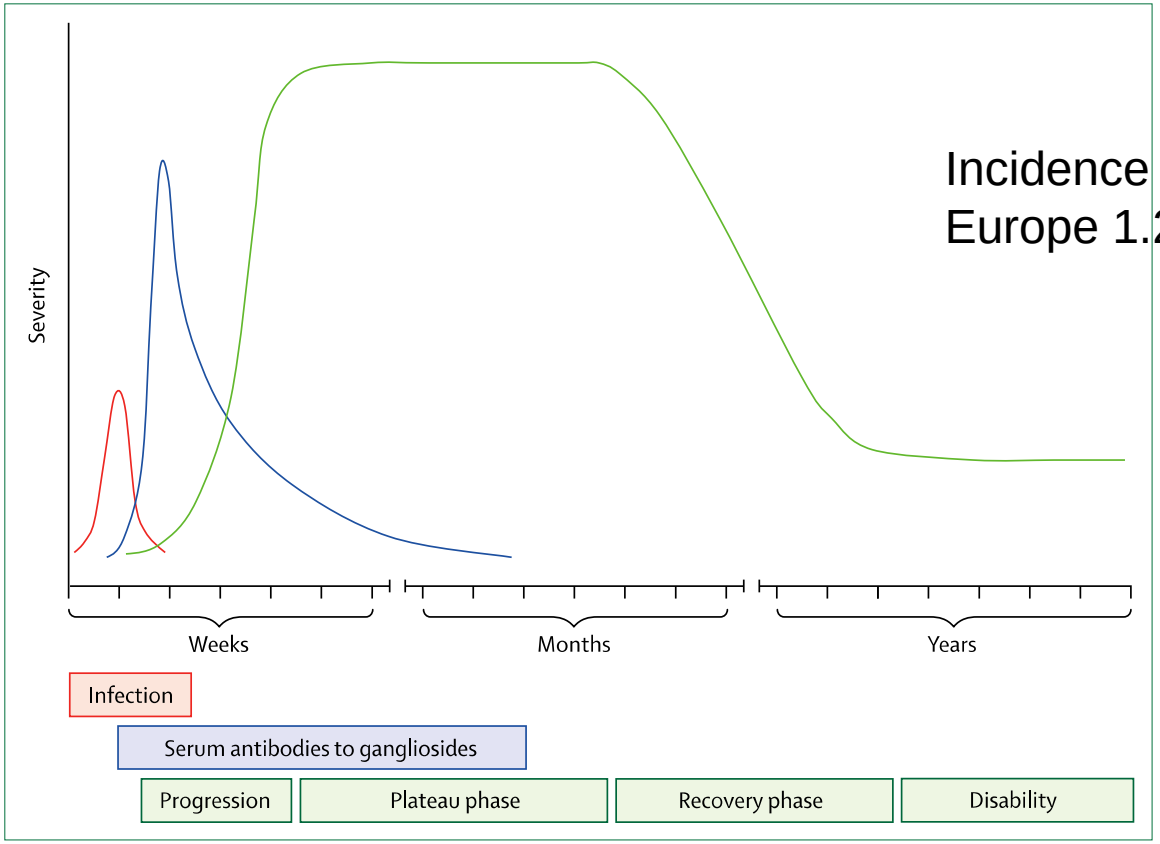
Table: **Fetal imagery findings in cases of proven congenital Zika infection**


CDC
Centers for Disease Control and Prevention
Pregnant woman residing in an area with ongoing Zika virus transmission
http://wwwnc.cdc.gov/travel/notices/
Pregnant woman reports clinical illness consistent with Zika virus disease
Pregnant woman does not report clinical illness consistent with Zika virus disease
Test for Zika virus infection
Test for Zika virus infection upon initiation of prenatal care
Positive or inconclusive test for Zika virus infection
Negative test(s) for Zika virus infection
Positive or inconclusive test for Zika virus infection
Negative test(s) for Zika virus infection
Consider serial fetal ultrasounds
Consider amniocentesis for Zika virus testing
Fetal ultrasound to detect microcephaly or intracranial calcifications
Consider serial fetal ultrasounds
Consider amniocentesis for Zika virus testing
Fetal ultrasound at 18–20 weeks of gestation
Test for Zika virus infection mid-2nd trimester
Microcephaly or intracranial calcifications present
Microcephaly or intracranial calcifications not present
Microcephaly or intracranial calcifications present, or positive or inconclusive test for Zika virus infection
No microcephaly or intracranial calcifications detected and negative test for Zika virus infection
Retest pregnant woman for Zika virus infection
Consider amniocentesis for Zika virus testing
Routine prenatal care
Test for Zika virus infection mid-2nd trimester
Consider an additional fetal ultrasound
Consider serial fetal ultrasounds
Consider amniocentesis for Zika virus testing
Routine prenatal care
Consider an additional ultrasound
Fetal microcephaly or intracranial calcifications present: consider retesting pregnant women for Zika virus infection and consider amniocentesis

Guillain-Barré syndrome

Hugh J Willison, Bart C Jacobs, Pieter A van Doorn

Guillain-Barré syndrome is the most common and most severe acute paralytic neuropathy, with about 100 000 people developing the disorder every year worldwide. Under the umbrella term of Guillain-Barré syndrome are several



Incidence 0.6-4 x 100.000
Europe 1.2-1.9 x 100.000

Figure 1: **Guillain-Barré syndrome time course**

www.thelancet.com **Published online February 29, 2016**

Guillain-Barre Syndrome

Outbreak in French Polynesia (2013-2014)

- 74 patients presented with neurological or autoimmune illness who had symptoms consistent with ZIKV in the previous days;
- 42 of these patients were diagnosed with GBS
- This was a 20-fold increase over baseline

?

- genetic evolution of the virus to a more pathogenic genotype
- a particular susceptibility in the Polynesian population
- Previous DENV illness may predispose patients to GBS via sequential arboviral immune stimulation

Oeler E. 2014

Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study

Van-Mai Cao-Lormeau, Alexandre Blake*, Sandrine Mons, Stéphane Lastère, Claudine Roche, Jessica Vanhomwegen, Timothée Dub, Laure Baudouin, Anita Teissier, Philippe Larre, Anne-Laure Vial, Christophe Decam, Valérie Choumet, Susan K Halstead, Hugh J Willison, Lucile Musset, Jean-Claude Manuguerra, Philippe Despres, Emmanuel Fournier, Henri-Pierre Mallet, Didier Musso, Arnaud Fontanet*, Jean Neil*, Frédéric Ghawché**

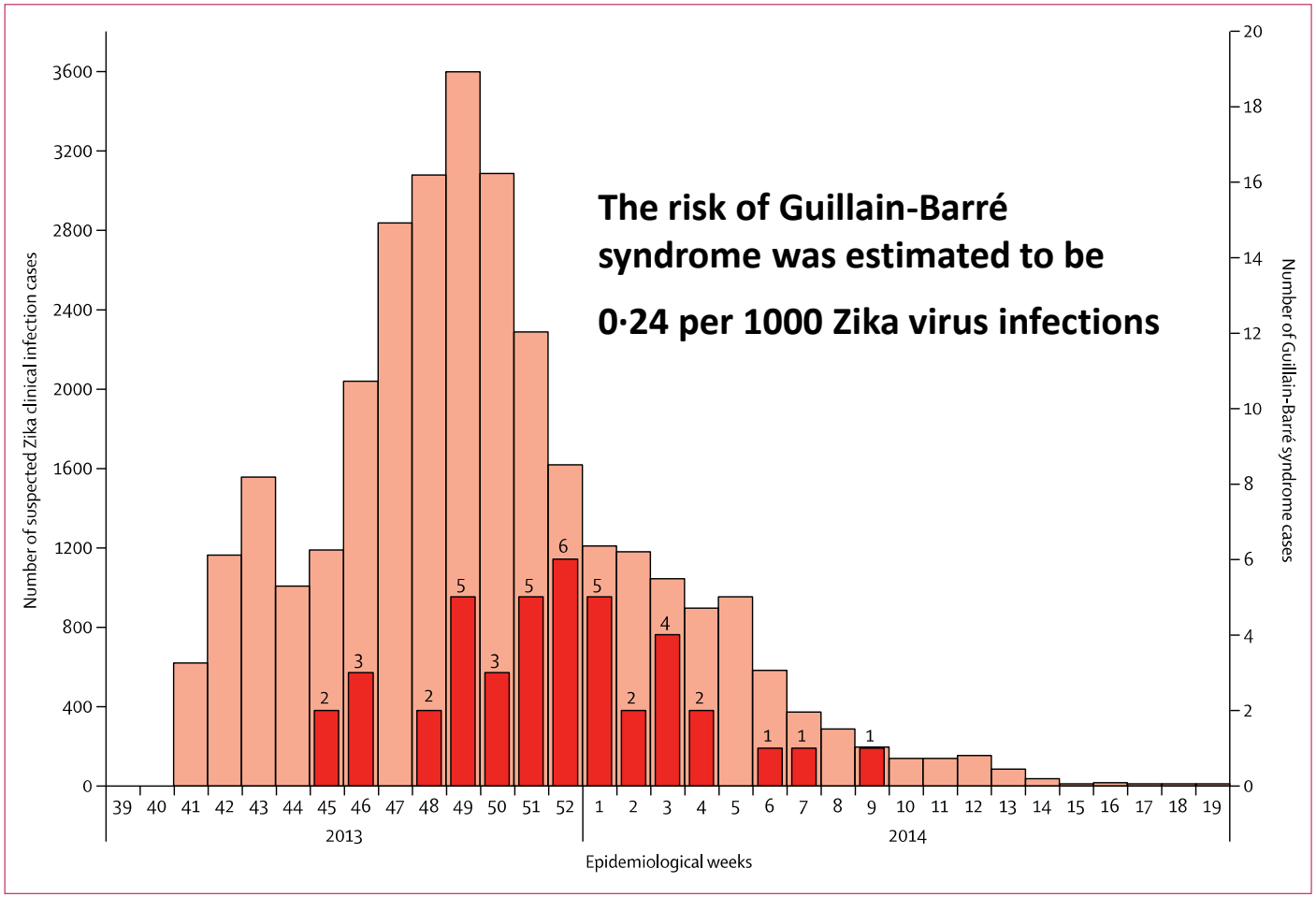


Figure: **Weekly cases of suspected Zika virus infections and Guillain-Barré syndrome in French Polynesia between October, 2013, and April, 2014**

Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study

Van-Mai Cao-Lormeau, Alexandre Blake*, Sandrine Mons, Stéphane Lastère, Claudine Roche, Jessica Vanhomwegen, Timothée Dub, Laure Baudouin, Anita Teissier, Philippe Larre, Anne-Laure Vial, Christophe Decam, Valérie Choumet, Susan K Halstead, Hugh J Willison, Lucile Musset, Jean-Claude Manuguerra, Philippe Despres, Emmanuel Fournier, Henri-Pierre Mallet, Didier Musso, Arnaud Fontanet*, Jean Neil*, Frédéric Ghawché**

	viral RNA	IgM	IgG	Zika IgM/IgG					Neutralising antibodies	IgM Zika/IgM dengue			
				+/+	+/–	–/+	–/–	Zika virus positive		+/+	+/–	–/+	–/–
Guillain-Barré syndrome (N=42*)	0 (0)	39 (93%)	29 (69%)	27	12	2	1	41 (98%)	42 (100%)	8 (19%)	31 (74%)	0	3 (7%)
Control group 1 (N=98)	ND	17 (17%)	25 (26%)	7	10	18	63	35 (36%)	54 (56%)	6 (6%)	11 (11%)	8 (8%)	73 (75%)
Control group 2 (N=70)	70 (100%)	ND	5 (7%)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Data are n (%) or n. *RT-PCR was only done for 41 patients with Guillain-Barré syndrome; tested samples for patients with Guillain-Barré syndrome are late samples (around 3 months after admission), except for the RT-PCR (admission sample). ND=not done. IFA=immunofluorescent assay. MIA=microsphere immunoassay.

Table 2: **Detection of Zika RNA (by RT-PCR), Zika and dengue IgM (by IFA), Zika IgG (MIA), and neutralising antibodies**

	Guillain-Barré syndrome*(n=42)	Control group 1 (n=98)	OR (95% CI)	OR† (95% CI)	Control group 2 (n=70)	OR (95% CI)	OR† (95% CI)
Zika virus IgM and/or IgG positivity	41 (98%)	35 (36%)	59·7 (10·4–+∞)	..	..	..	..
Positive Zika virus seroneutralisation	42 (100%)	54 (56%)	34·1 (5·8–+∞)	..	..	..	..
Dengue virus IgG positivity	40 (95%)	87 (89%)	2·0 (0·4–19·9)	1·0 (0·2–11·5)	58 (83%)	6·0 (0·8–269·5)	4·0 (0·5–184·7)

Data are n (%), unless otherwise shown. *Tested samples for patients with Guillain-Barré syndrome are late samples (around 3 months after admission). †Adjusted for Zika virus IgG positivity. OR=odds ratio.

Table 4: **Zika virus and dengue virus serological patterns associated with Guillain-Barré syndrome**

Thrombocytopenia and subcutaneous bleedings in a patient with Zika virus infection

Karimi et al Lancet 2016

Laboratory examination showed slight microcytic anaemia (haemoglobin concentration 7·2 mmol/L) but a normal haematocrit, and a profound thrombocytopenia of 20×10^9 platelets per L, with normal coagulation parameters. A watch-and-wait decision was made. On day 29, she developed gum bleeding, at a thrombocyte count of 10×10^9 platelets per L. At this point, we decided to treat her presumed immune-mediated thrombocytopenia with intravenous immunoglobulins, upon which her thrombocyte count increased to 80×10^9 platelets per L within a few days. Concentrations of thrombocyte antibodies and thrombopoietin have been measured, and results are still pending.

In follow-up PCR analyses at the AMC on days 17 and 18, her blood sample was negative for Zika virus and her urine sample was positive.

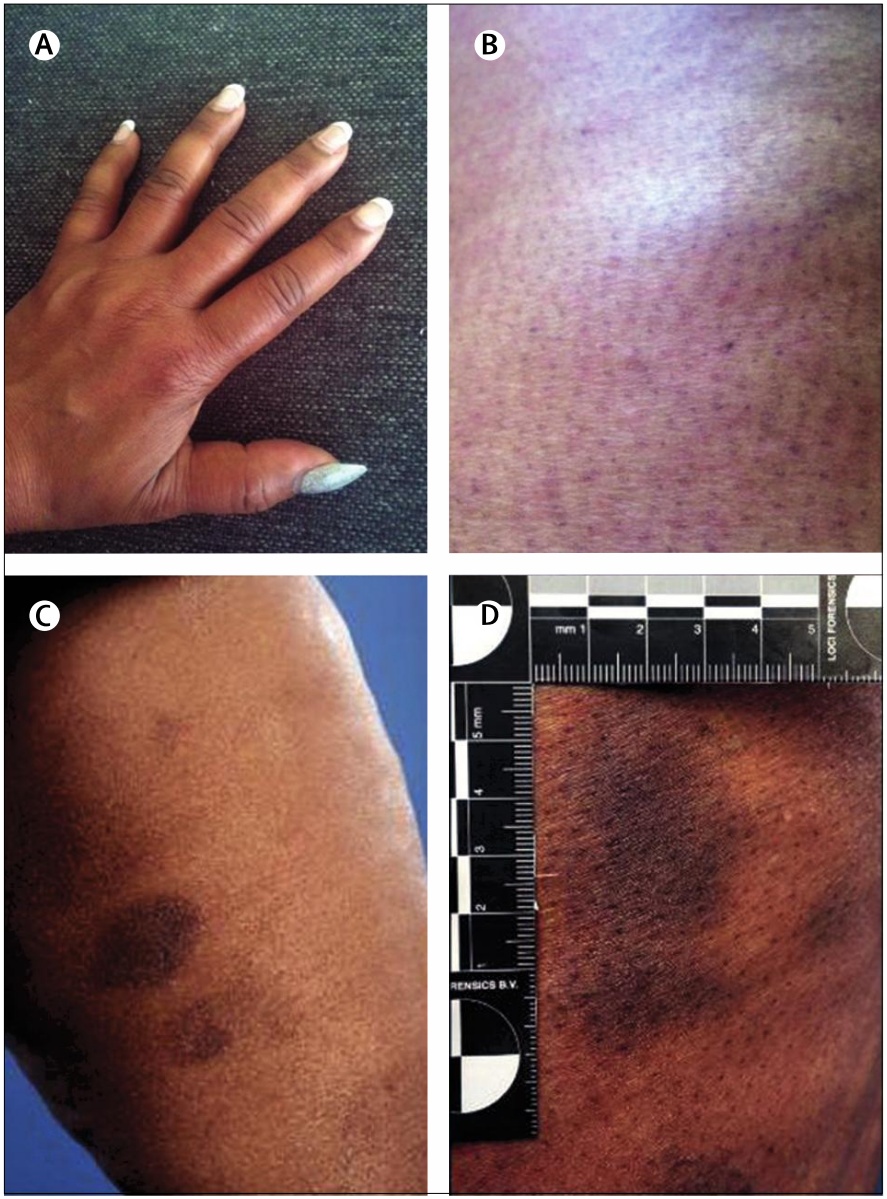


Figure: **Skin and tissue symptoms**
(A) Swelling of the hand and wrists, and (B) skin rash during acute illness (day 2). Photos courtesy of the patient. (C,D) Subcutaneous haematomas on day 18, when the patient was recovering from acute illness. Photos taken at the Academic Medical Center, Amsterdam, Netherlands.

THE LANCET

Acute myelitis due to Zika virus infection

Sylvie Mécharles, Cécile Herrmann, Pascale Poullain, Tuan-Huy Tran, Nathalie Deschamps, Grégory Mathon, Anne Landais, Sébastien Breurec, Annie Lannuzel

On day 2, she developed dysuria and urinary retention needing catheterisation, but no abnormal urinary frequency or urgency. The left-sided hemiparesis and pain worsened, and we noted loss of temperature sensation below the T2 dermatome on the left and T4 on the right, and bilateral Hoffman signs. Spinal MRI showed lesions of the cervical and thoracic spinal cord. The cervical lesion was enlarged, suggesting oedema (figure). Conus medullaris and lumbar

We started methylprednisolone 1 g daily for 5 days. On the seventh day of admission her neurological condition improved and we could remove the catheter. 1 month after admission she had moderate weakness in both legs but was able to walk unaided. Repeat MRI showed reduced cervical spinal oedema (appendix).

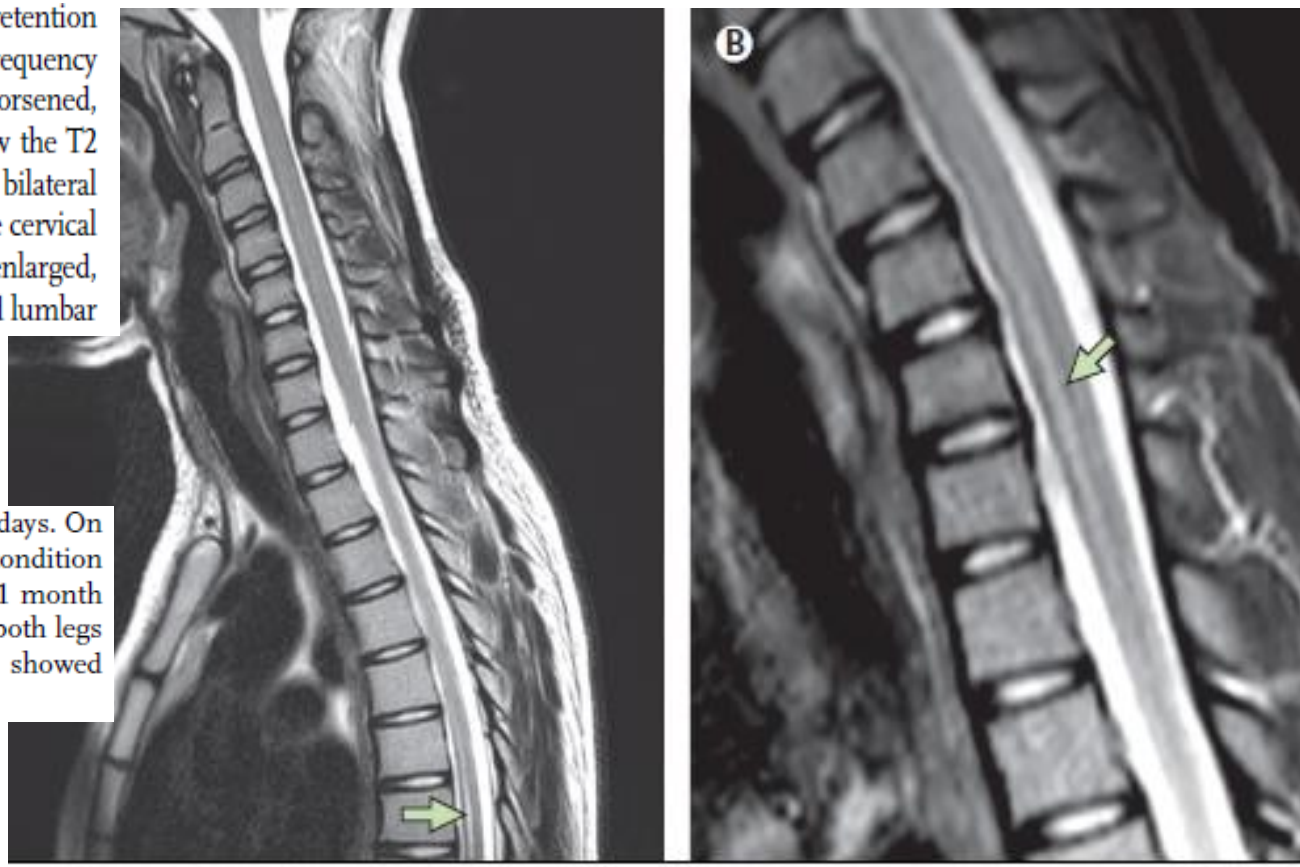
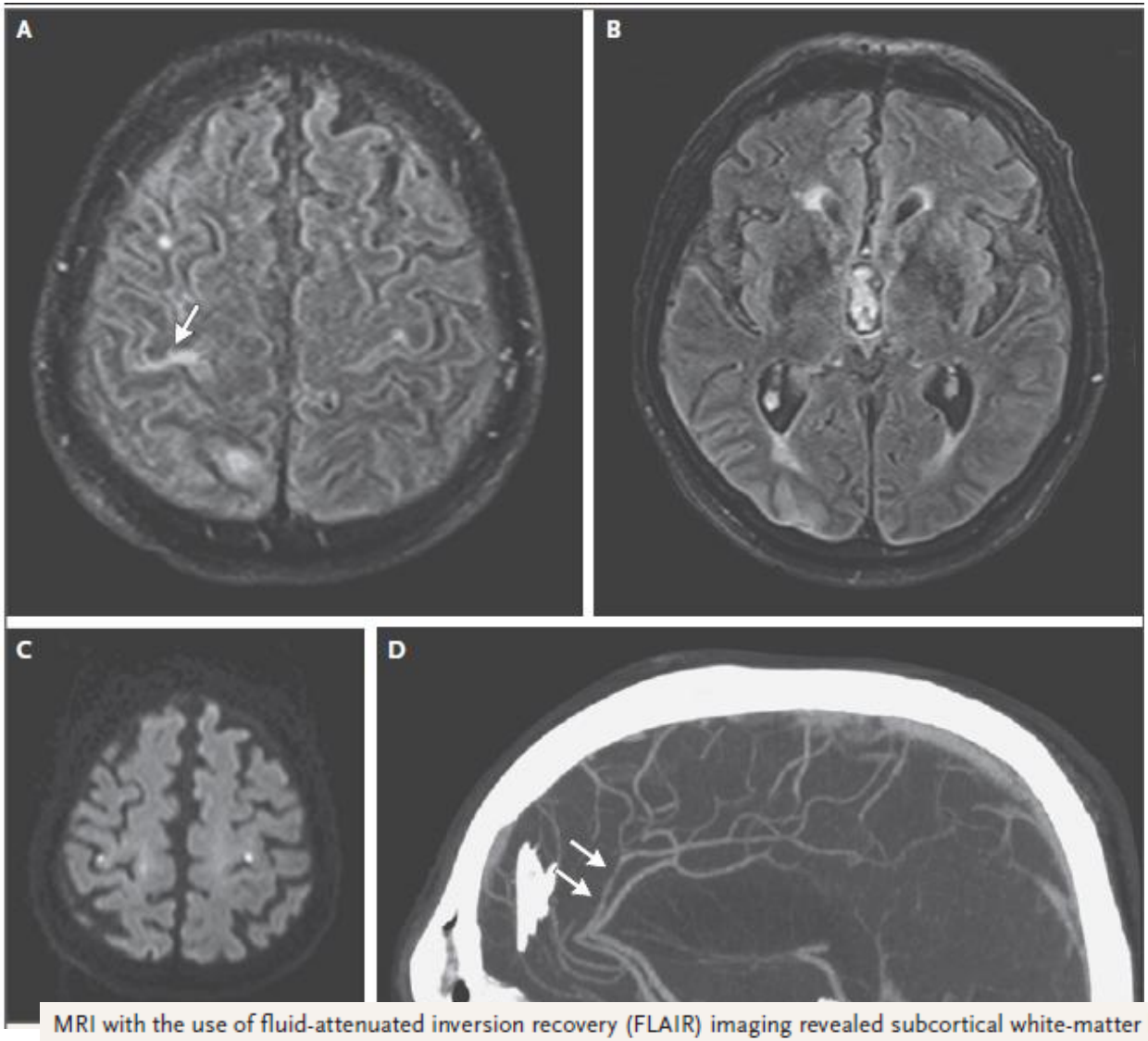


Figure: **Magnetic resonance imaging (MRI) showing myelitis in Zika virus infection**
(A) T2 sequences showing hypersignal in the thoracic cord T5–T8 (arrow) and enlargement of the cervical spinal cor
(B) Sagittal short time inversion recovery (STIR) sequences showing hypersignal in the cervical spinal cord C4–C7 (a

Zika Virus Associated with Meningoencephalitis

The NEW ENGLAND JOURNAL of MEDICINE



MRI with the use of fluid-attenuated inversion recovery (FLAIR) imaging revealed subcortical white-matter hyperintensities in the right frontal region, the right parietal region (Panel A), the right temporo-occipital region (Panel B), and bilateral rolandic regions (Panel A). The slight hyperintensity of the right rolandic fissure (Panel A, arrow) is suggestive of meningitis. The multiple punctuated hyperintensities on diffusion-weighted sequences are suggestive of ischemic foci (Panel C). The MRI with FLAIR imaging and diffusion-weighted sequences were performed with the use of a 3T MRI unit (Magnetom Verio, Siemens). The computed tomographic angiogram shows an irregular narrowing of the right callosomarginal artery (Panel D, arrows). Angiography was performed with the use of a Discovery CT750 HD scanning system (GE Medical Systems).

An 81-year-old man was admitted to ICU in France, 10 days after he had been on a 4-week cruise in the area of New Caledonia

Liquor: 41 cells (98% PMN), protein level 76 mg/dl, CSF/blood glucose ratio 0.75.

Discharged from ICU on day 17, fully recovered by day 38, residual weakness (4/5) of the left arm.

- Impatto politico e mediatico
- Eziologia
- Epidemiologia
- Approccio sindromico e diagnosi differenziale arbovirosi
- Complicanze
- Prospettive future

WHO Emergency Group Zika open questions

Zika pregnancy, microcephaly, other neurological disorders

- Case definition for congenital Zika infection
- If a woman has had Zika but recovered, are future pregnancies safe?
- Is Zika also causing other types of damage in unborn babies, which will only show up later in development?
- is this really just the tip of the iceberg?
- What is the best diagnostic approach for a pregnant women returnig home from a Zika endemic countries ?
- Surveillance register of neurological defects in adults and neonates

WHO Emergency Group Zika open questions

Vector control

- Vector surveillance, including the determination of mosquito vector species and their sensitivity to insecticides, should be enhanced to strengthen risk assessments and vector control measures.
- Vector control measures and appropriate personal protective measures should be aggressively promoted and implemented to reduce the risk of exposure to Zika virus.

WHO Emergency Group Zika open questions

Risk communication

- to be enhanced in Zika countries to address population concerns, enhance community engagement, improve reporting, and ensure application of vector control and personal protective measures.
- To ensure women of childbearing age and particularly pregnant women have the necessary information and materials to reduce risk of exposure.
- To provide Information on the risk of sexual transmission, and measures to reduce that risk, should be available to people living in and returning from areas of reported Zika virus transmission.

WHO Emergency Group Zika open questions

Clinical care

- Pregnant women who have been exposed to Zika virus should be counselled and followed for birth outcomes,
- In areas of known Zika virus transmission, health services should be prepared for increases in neurological syndromes and/or congenital malformations.

Travel measures

- There should be no general restrictions on travel or trade with countries, areas and/or territories with Zika virus transmission, apart from pregnant women;
- Pregnant women whose sexual partners live in or travel to Zika areas should ensure safe sexual practices or abstinence

WHO Emergency Group Zika open questions

- **Research & product development**
- New diagnostics for Zika virus infection, especially for pregnancy.
- Research, development and evaluation of novel vector control measures.
- Research on vaccines and therapeutics for Zika virus in the medium term.
- Zika persistence in infected persons

Future perspective & Ethics consideration

- What is the next ongoing scenario in the Mediterranean basin with the local presence of Zika competent vector *Aedes* ?
- What is the Zika demographic and antropologic impact on birth rate in South America, and in case, in Europe?

THE LANCET

A crucial time for public health preparedness: Zika virus and the 2016 Olympics, Umrah, and Hajj

The potential role of scheduled international mass gatherings in 2016 could exacerbate the spread of Zika virus beyond the Americas. In Brazil, the Rio Carnival on Feb 5–10 attracts more than 500 000 visitors, and on Aug 5–21 more than 1 million visitors are expected to go to the summer Olympics followed by Paralympic Games on Sep 7–18. Meanwhile, Saudi Arabia expects to host more than 7 million pilgrims from over 180 countries for the Umrah, between June and September, and the Hajj pilgrimage on Sept 8–13.[4,5] Saudi Arabia receives about 7000 pilgrims from Latin America annually.