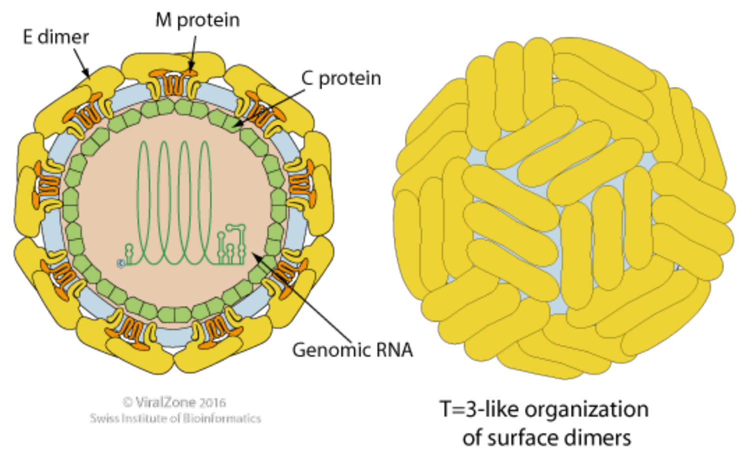
Univerza *v Ljubljani*
Medicinska fakulteta

Zika: star virus z novim obrazom

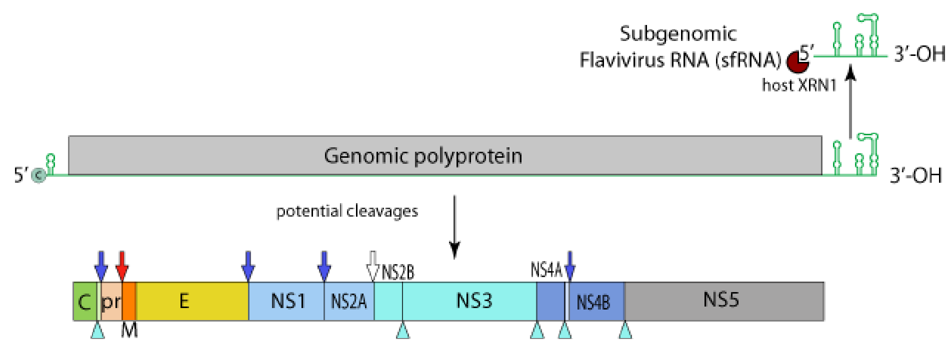
TATJANA AVŠIČ ŽUPANC
Inštitut za mikrobiologijo in imunologijo
Medicinska fakulteta, Univerza v Ljubljani

Zika virus

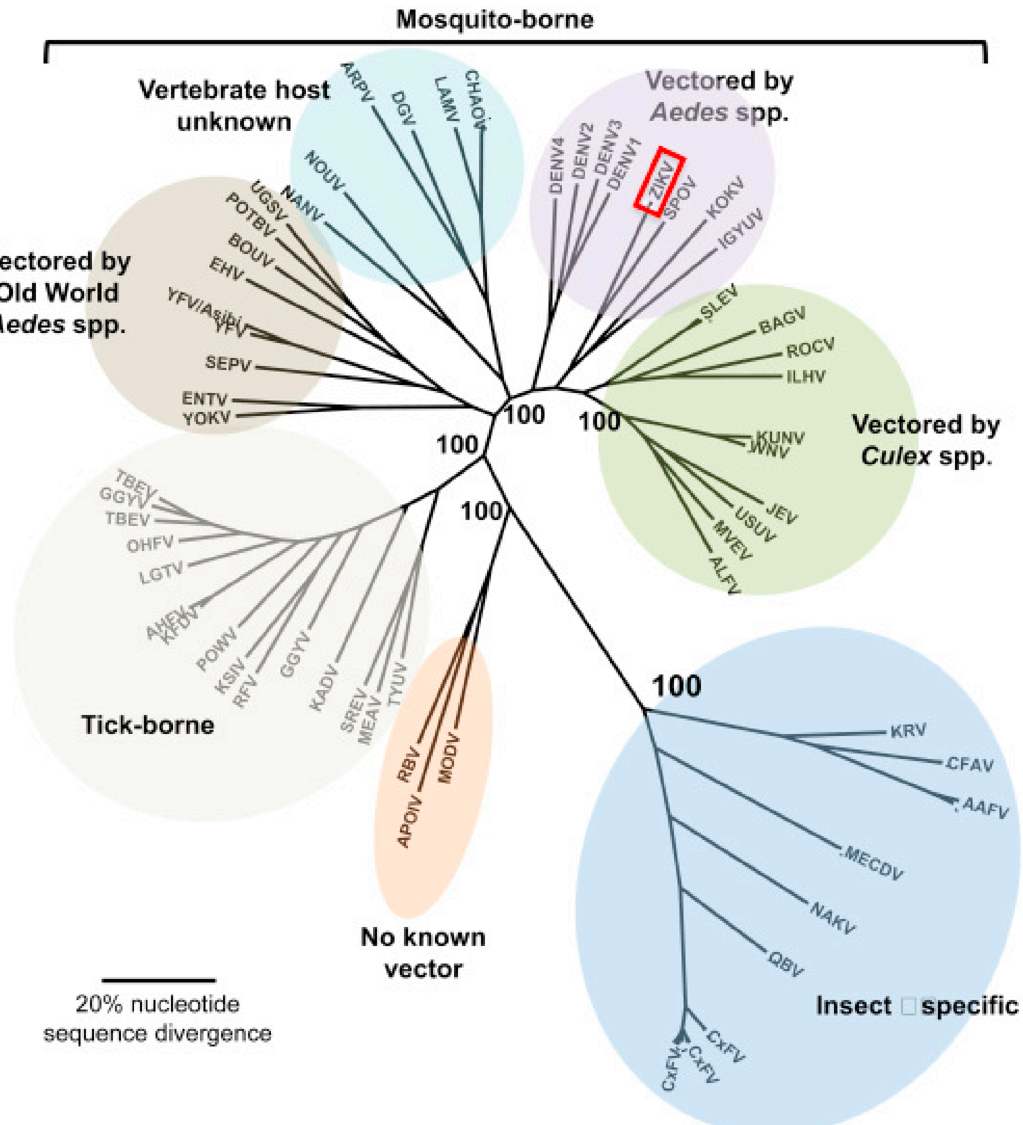
- Flavivirus (*Flaviviridae*)
- Enveloped, spherical 50nm;



- ssRNA (+) genome
- 3'-end loop structure – forms a sfRNA
- sfRNA is essential for pathogenicity – inhibits host RIG-I

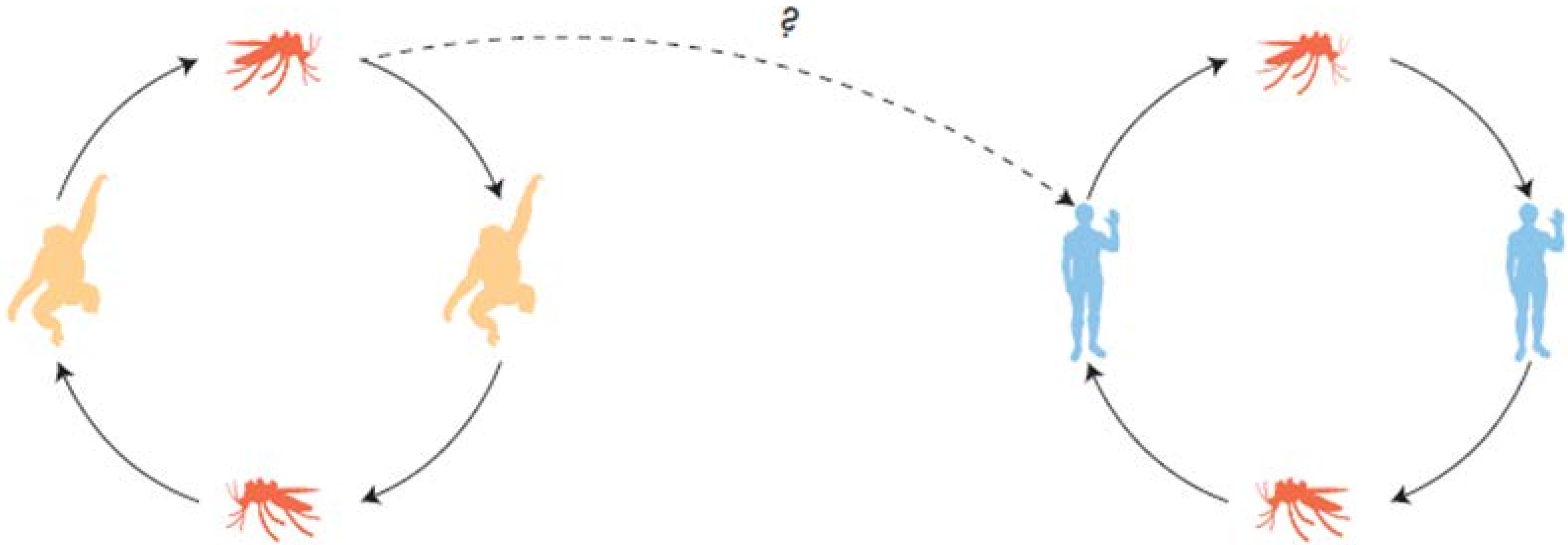


Zika virus – phylogenetic relationship within Flaviviruses



Zika virus transmission cycles

- vectors: mosquitoes – principal *Aedes aegypti*



Sylvatic cycle

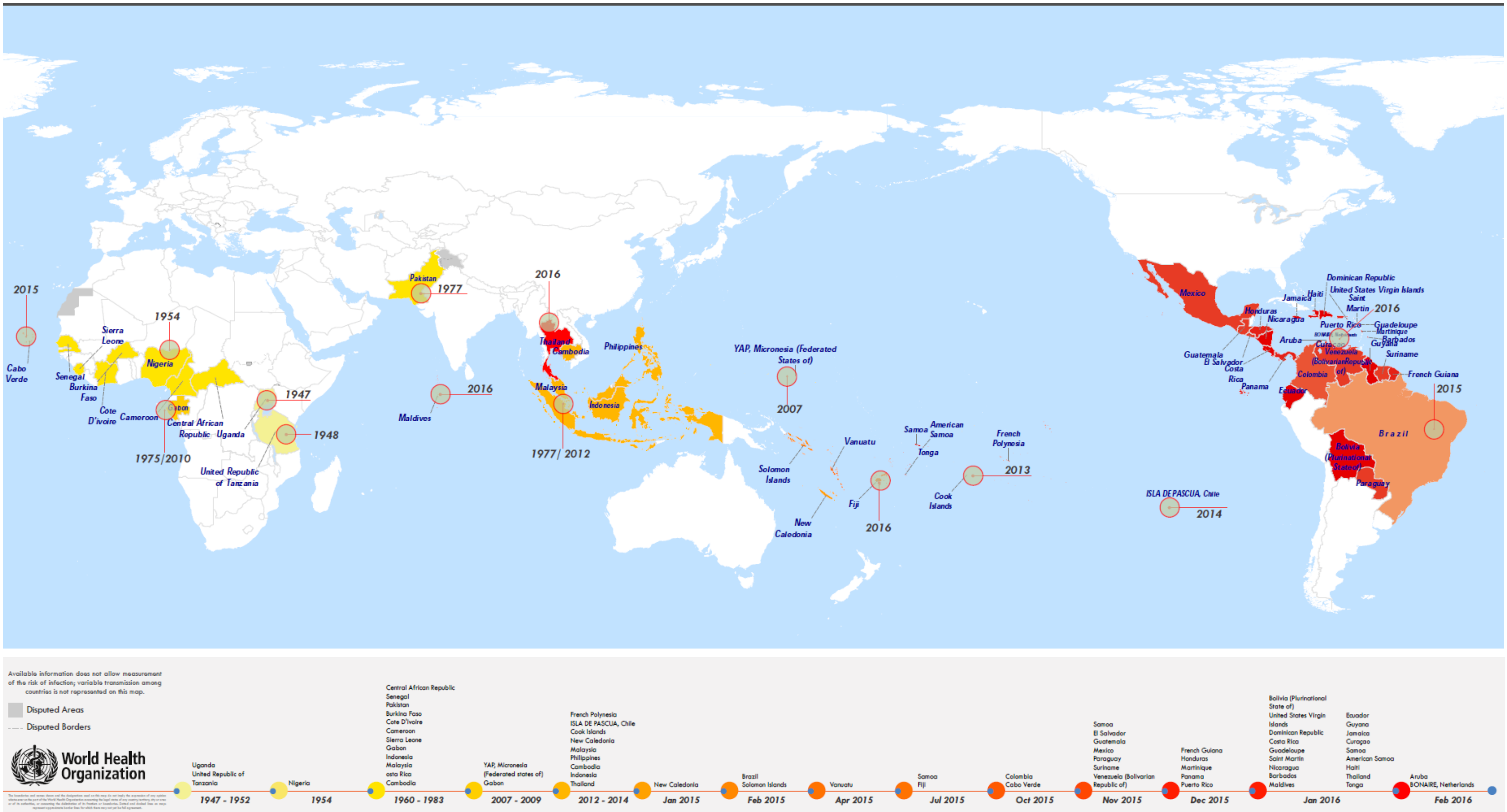
Urban cycle

Zika fever – ZIKV disease

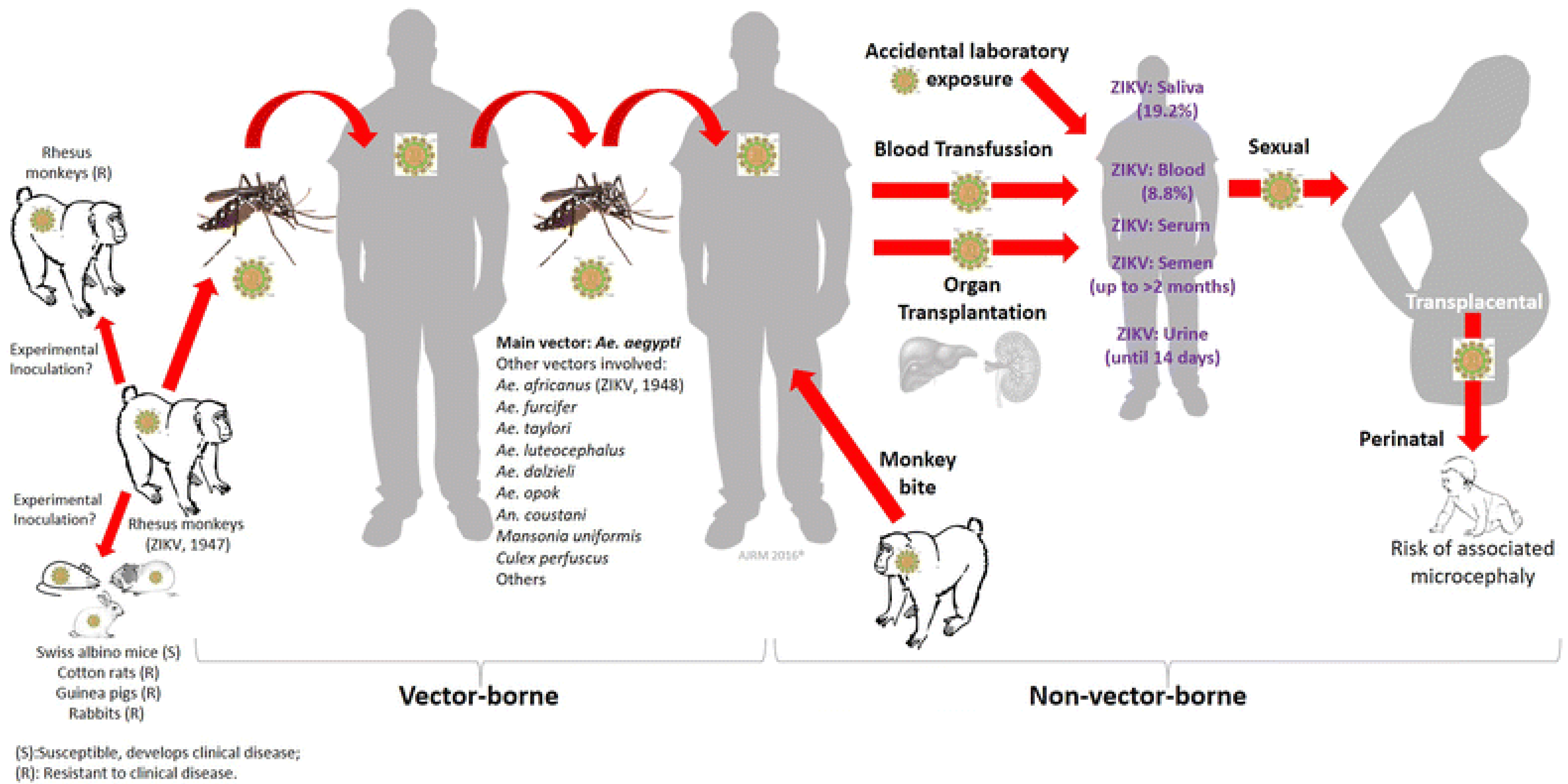
- Fever
- Conjuctivitis
- Joint pain
- Maculopapular rash

- Postinfectious GBS
- Microcephaly and other severe brain defects

Countries and territories showing historical distribution of ZIKV, 1947-2016

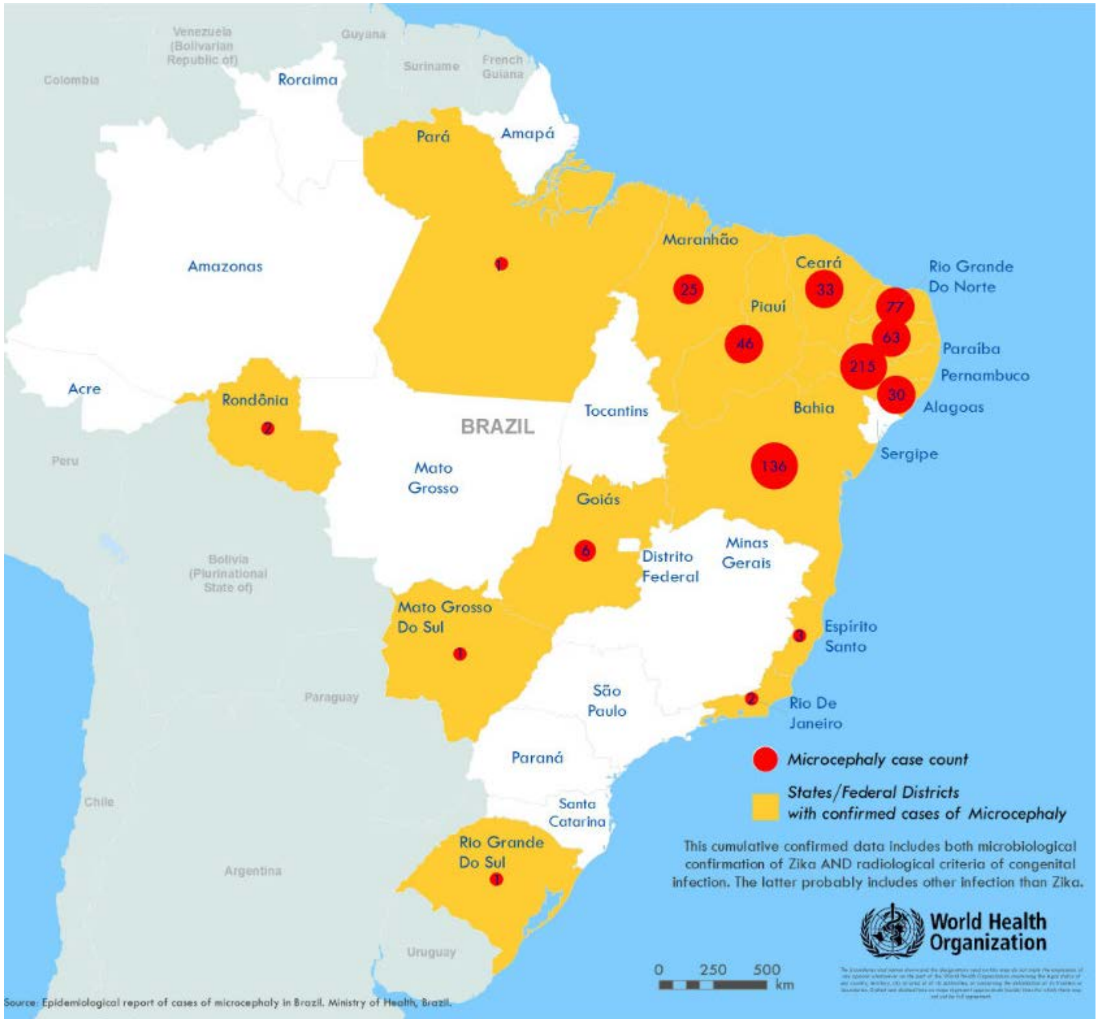


Reported forms of ZIKV transmission

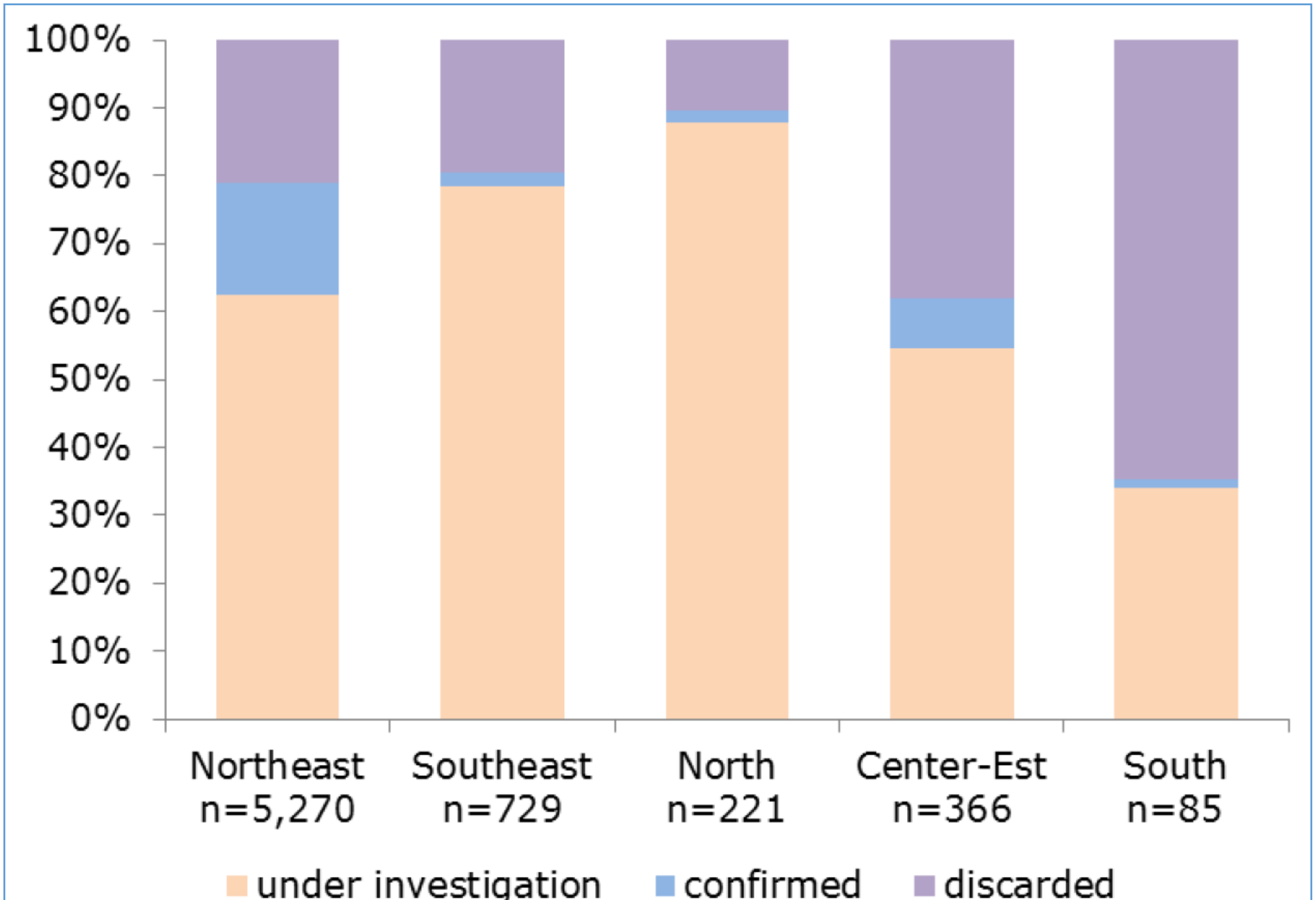


Rodriguez-Morales et al. Ann Clin Microbiol Antimicrob 2016

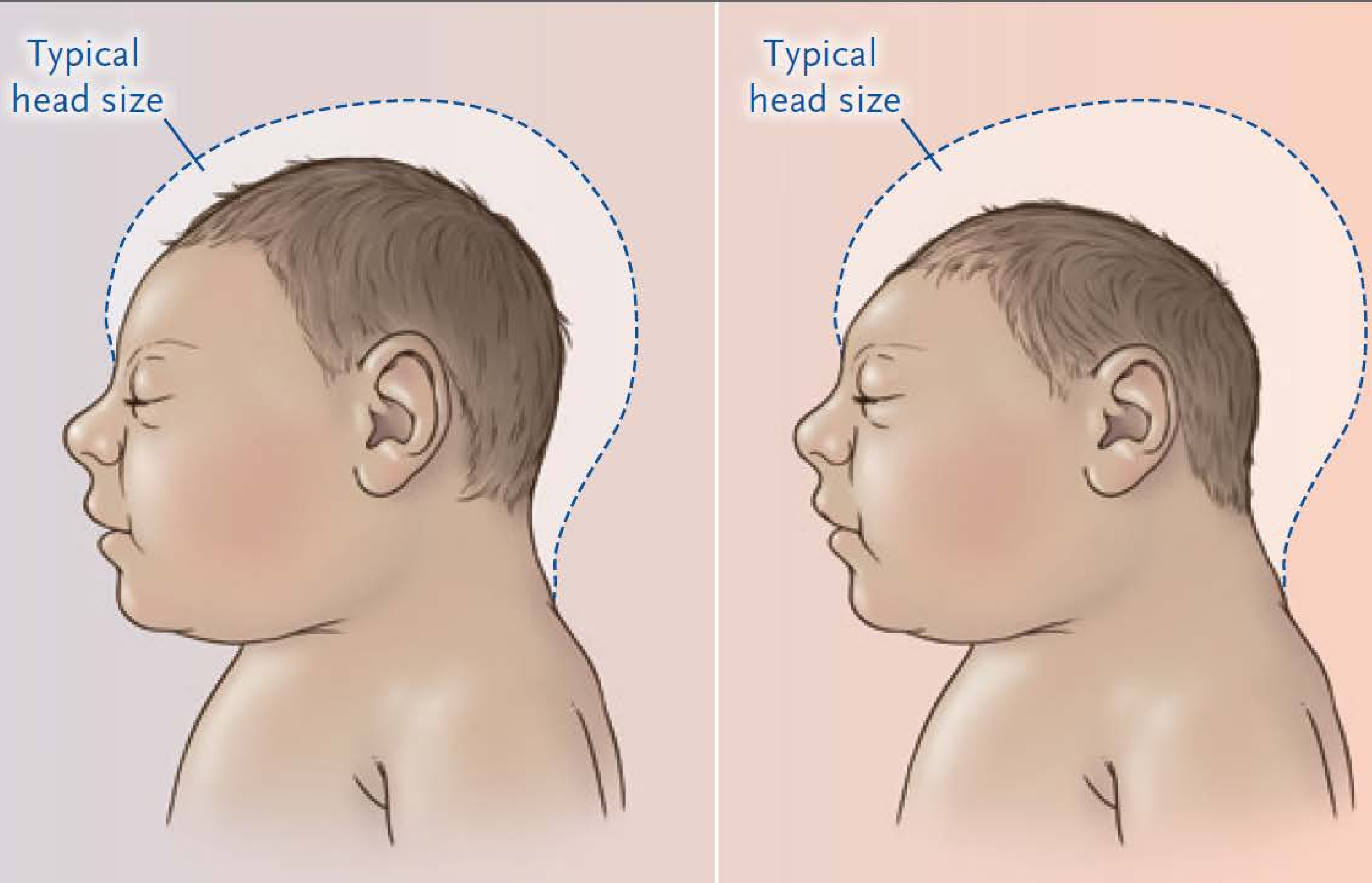
Distribution of cumulative confirmed cases of microcephaly, Brazil (27th February 2016)



907 confirmed cases of microcephaly or other nervous system malformation among newborns have been reported in Brazil since 22 October 2015.



PAHO (March 2016)



Baby with
Moderate Microcephaly

Baby with Severe
Microcephaly

Causes of microcephaly

There are many potential causes of microcephaly, but often the cause remains unknown. The most common causes include:

- infections: toxoplasmosis, rubella, herpes, syphilis, cytomegalovirus and HIV;
- exposure to toxic chemicals: maternal exposure to heavy metals like arsenic and mercury, alcohol, radiation, and smoking;
- genetic abnormalities such as Down syndrome;
- severe malnutrition during fetal life.

Case definition of Zika virus-related fetal microcephaly

Fetal microcephaly with a molecular or epidemiological link to Zika virus in the absence of other conditions that are known to cause microcephaly.

- The pregnant woman is a confirmed case of Zika virus disease; **or**
- The pregnant woman had sexual contact with a confirmed case, or a history of symptoms or signs consistent with Zika virus infection and residing/travelling in an area with ongoing Zika virus transmission during her pregnancy; **or**
- Presence of Zika virus in amniotic fluid (identified through amniocentesis and RT-PCR assay); **or**
- Presence of Zika virus in fetal brain tissue (identified postmortem through RT-PCR assay).

WHO, Interim guidance, 2 March 2016
WHO/ZIKV/MOC/16.2

Case definition of Zika virus-related fetal microcephaly

Fetal microcephaly with a molecular or epidemiological link to Zika virus in the absence of other conditions that are known to cause microcephaly.

- Other congenital infections: toxoplasmosis, rubella, herpes, syphilis, cytomegalovirus and HIV;
- Exposure to toxic drugs, chemicals and radiation;
- Genetic abnormalities e.g. Down syndrome;
- Fetal malnutrition and placental insufficiency.

WHO, Interim guidance, 2 March 2016
WHO/ZIKV/MOC/16.2

ORIGINAL ARTICLE
BRIEF REPORT

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Zika Virus Associated with Microcephaly

Jernej Mlakar, M.D., Misa Korva, Ph.D., Nataša Tul, M.D., Ph.D.,
Mara Popović, M.D., Ph.D., Mateja Poljšak-Prijatelj, Ph.D., Jerica Mraz, M.Sc.,
Marko Kolenc, M.Sc., Katarina Resman Rus, M.Sc., Tina Vesnaver Vipotnik, M.D.,
Vesna Fabjan Vodušek, M.D., Alenka Vizjak, Ph.D., Jože Pižem, M.D., Ph.D.,
Miroslav Petrovec, M.D., Ph.D., and Tatjana Avšič Županc, Ph.D.

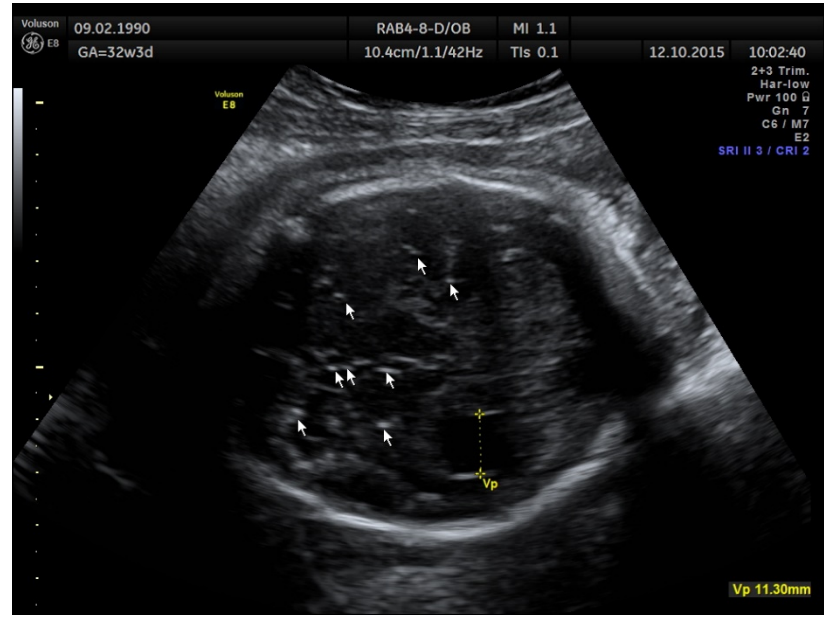
N Engl J Med 2016; 374:951-958 March 10, 2016 DOI: 10.1056/NEJMoa1600651

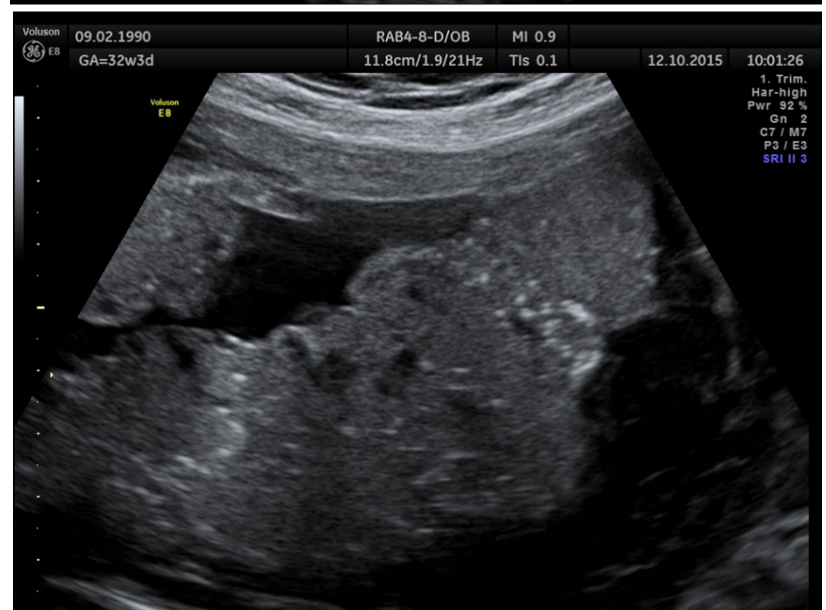
Case history

- In mid October 2015, a 25-year-old previously healthy female came to the Department of Perinatology, UCC of Ljubljana because of assumed fetal anomalies.
- She has lived and worked as a volunteer in Natal, Rio Grande do Norte, Brazil since December 2013 .
- During the 13^{th} week of gestation she became ill: high fever, severe musculoskeletal and retroocular pain, itching generalized maculopapular rash. Infection with ZIKV was highly suspected - no virological diagnostic was performed.
- Ultrasound visits at 14 and 20 weeks of gestation showed normal fetal growth and anatomy.
- The patient returned to EU, where US examination performed at 29 weeks of gestation, showed the signs of fetal anomalies.

Case history

- Ultrasonography performed at 32 weeks of gestation confirmed intrauterine growth retardation (estimated third percentile of fetal weight) with normal amniotic fluid
- a placenta measuring 3.5 cm in thickness with numerous calcifications
- a head circumference below the second percentile for gestation (microcephaly), moderate ventriculomegaly, and a transcerebellar diameter below the second percentile
- Brain structures were blurred, and there were numerous calcifications in various parts of the brain
- There were no other obvious fetal structural abnormalities.





Case history

- Due to severe brain disease and microcephaly, the fetus was given a poor prognosis for neonatal health.
- The mother requested medical termination of pregnancy which was approved by national and hospital ethics committees.
- At the delivery, the only morphologic anomaly was the prominent microcephaly. An autopsy was performed, as is mandatory in all cases of termination of pregnancy.

Male fetus 1470g

Kariotyping

- 46 XY b.p.

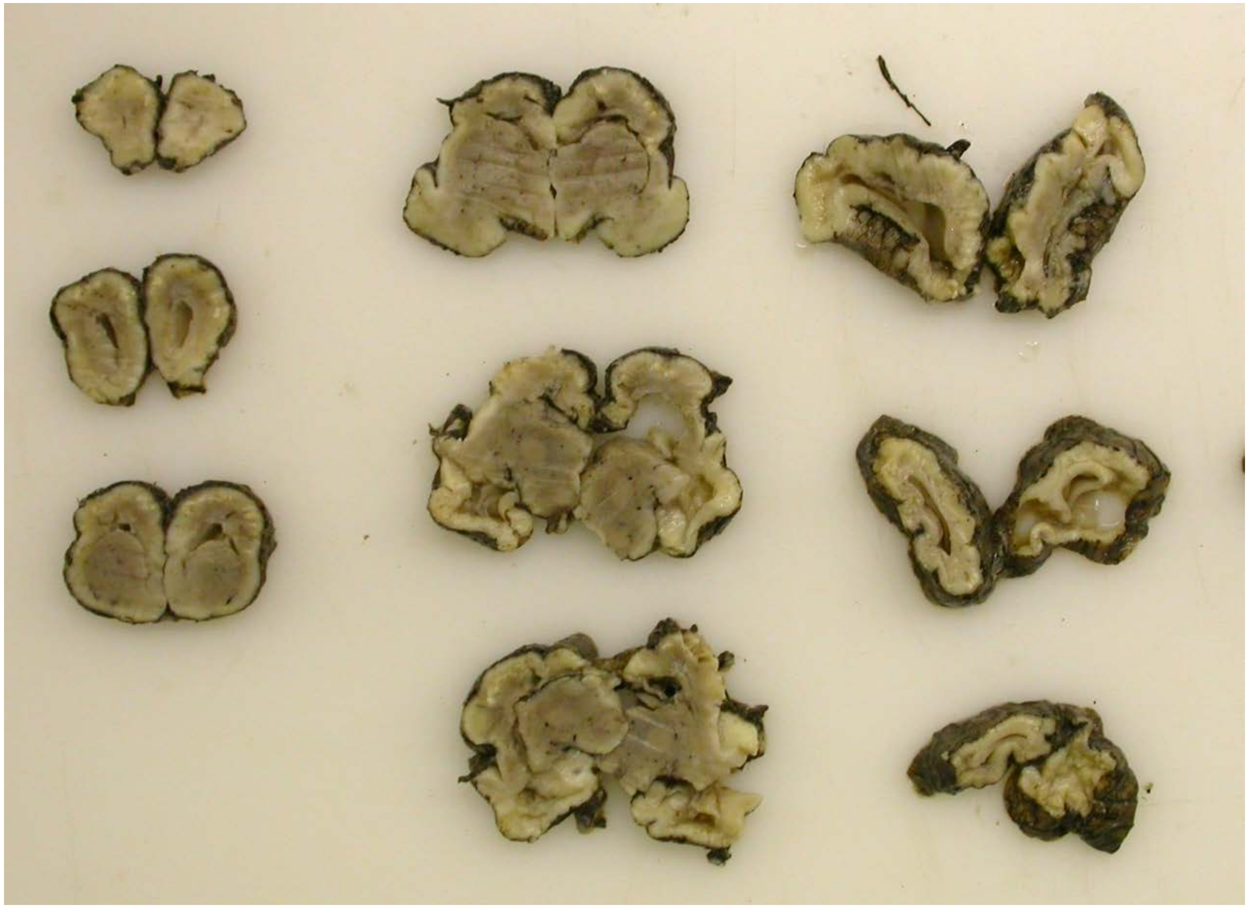
- After the termination of pregnancy microarray analysis of the fetal tissue was performed: no genetic factors for microcephaly

Autopsy results

Microcephaly (1st percentile)
low placental-fetal weight ratio (< 3rd percentile)
calcifications of the placenta
Micrencephaly 84 g (4 SD below average)

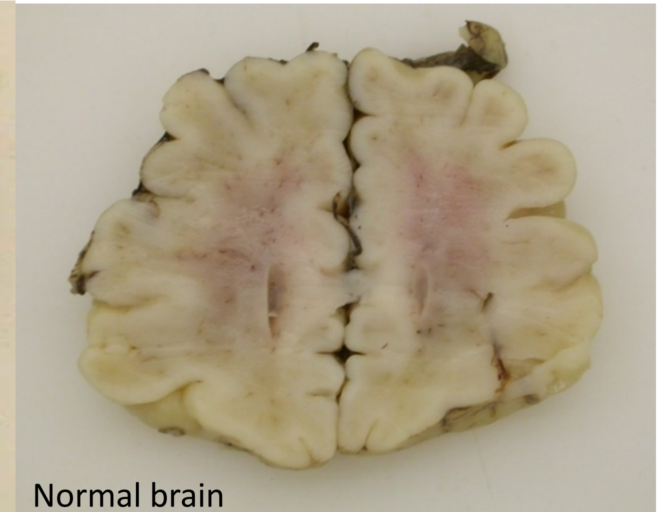
32 weeks of gestation

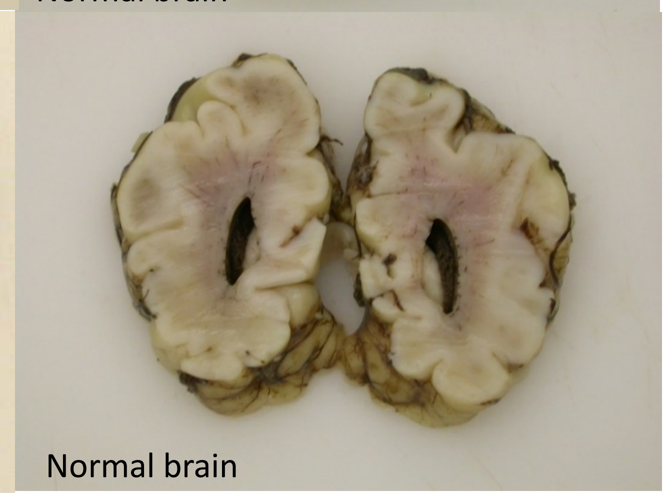
Autopsy results



- Widely open sylvian fissures
- Small cerebellum and brain stem
- Almost complete agyria
- Internal hydrocephalus of lateral ventricules

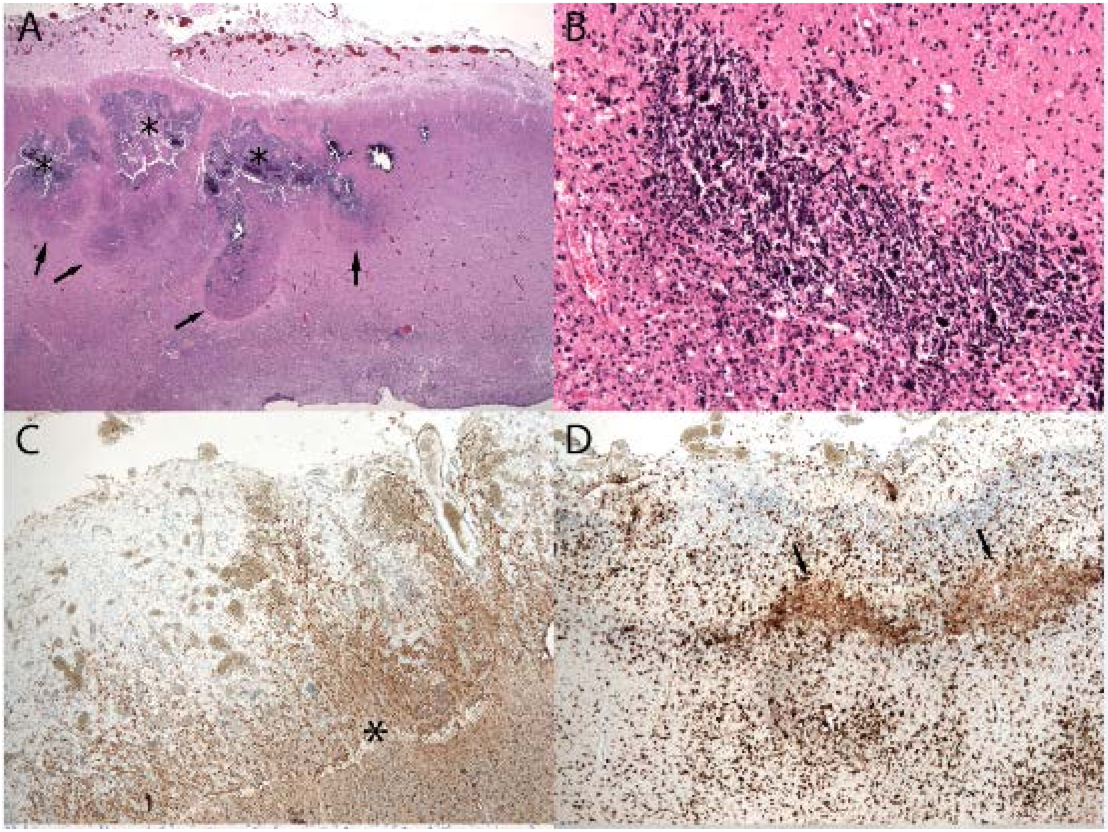
Autopsy results





- Complete agyria in frontal lobe
- Numerous variable-size calcifications
- Hydrocephalus due to demaged brain cortex and white matter

Microscopic analysis of brain tissue

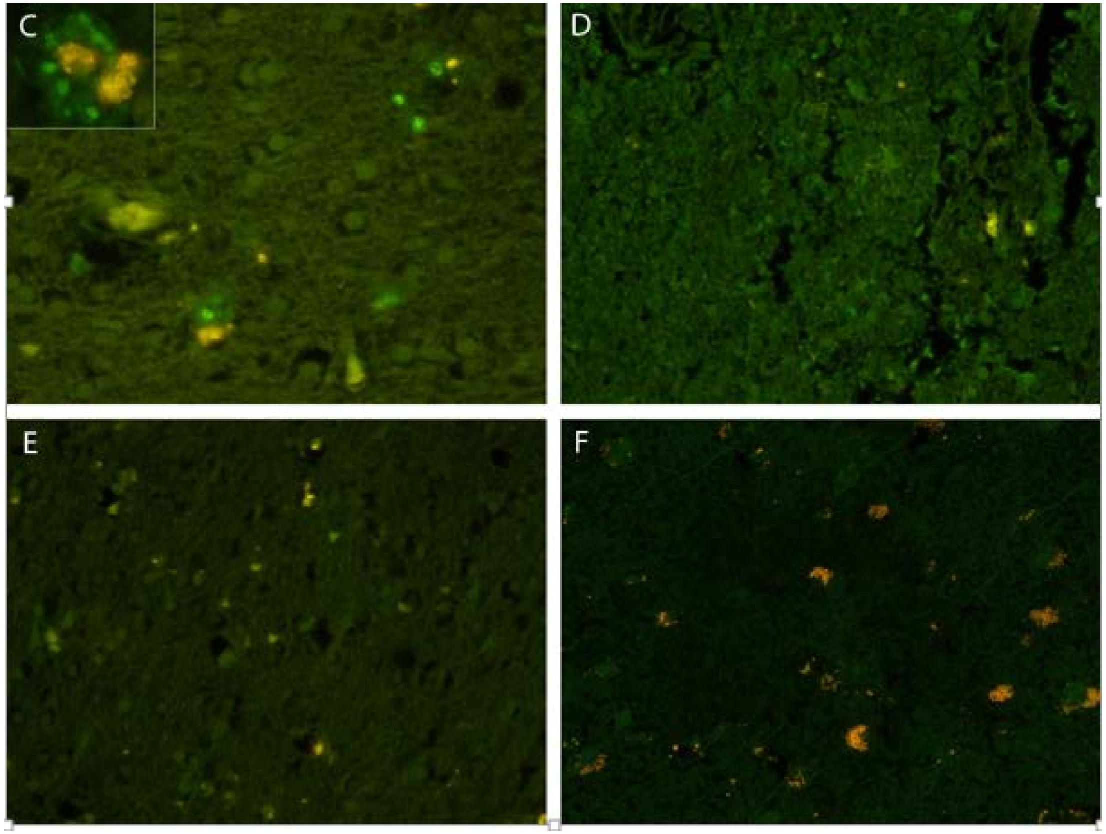


A. Calcifications (cortical, subcortical) and absence of gyration
B. Calcifications with filamentous structures – demaged axons and dendrites
C. IHC labeling of proliferate activated astrocytes – extend into subarachnoid space
D. IHC labeling of activated microglial cells and macrophages in the cortex

Microscopic analysis of brain tissue

- Hypoplasia of the brain stem and the spinal cord
- Severe Wallerian degeneration of the lateral corticospinal tract

Immunoflurescence staining

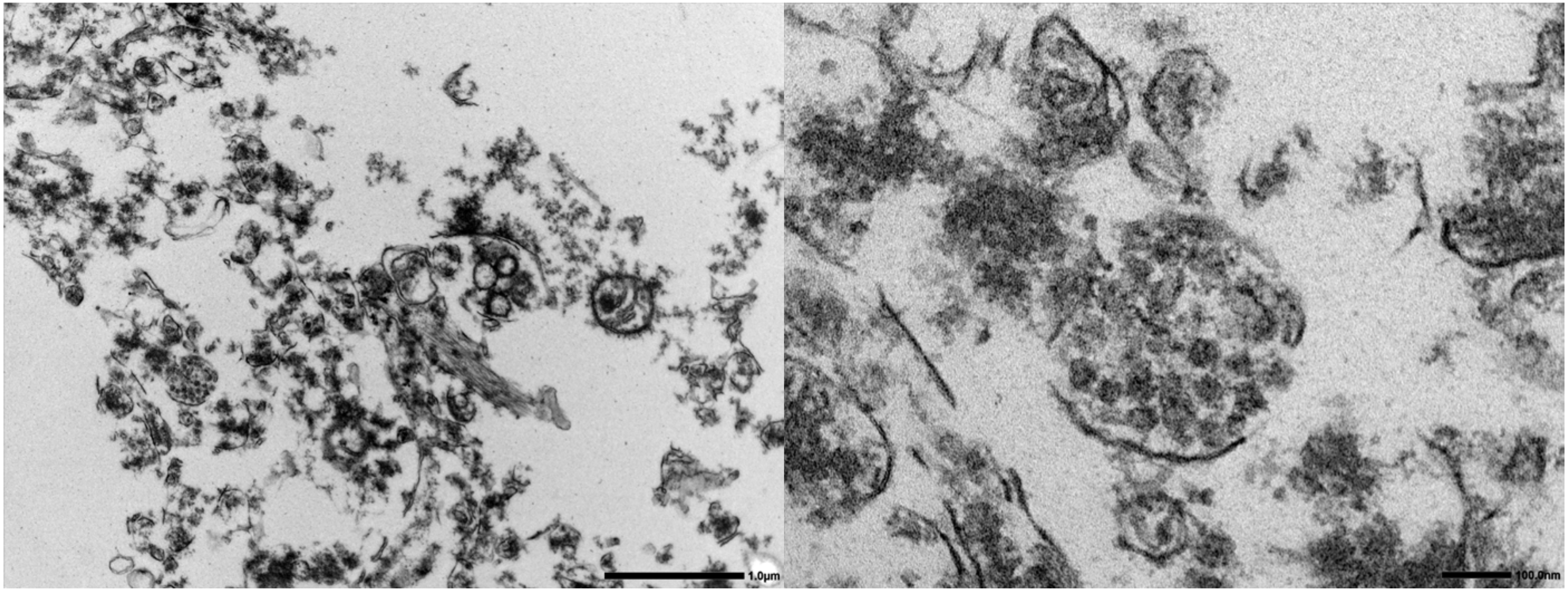


C. IIF with mother serum – reaction in destroyed neurons (lipofuscin granules)
D. IIF with healthy blood donor
E. A negative control
F. IIF on the brain tissue of an autopsied adult male (lipofuscin granules in cpl of neurons)

Histologic examination

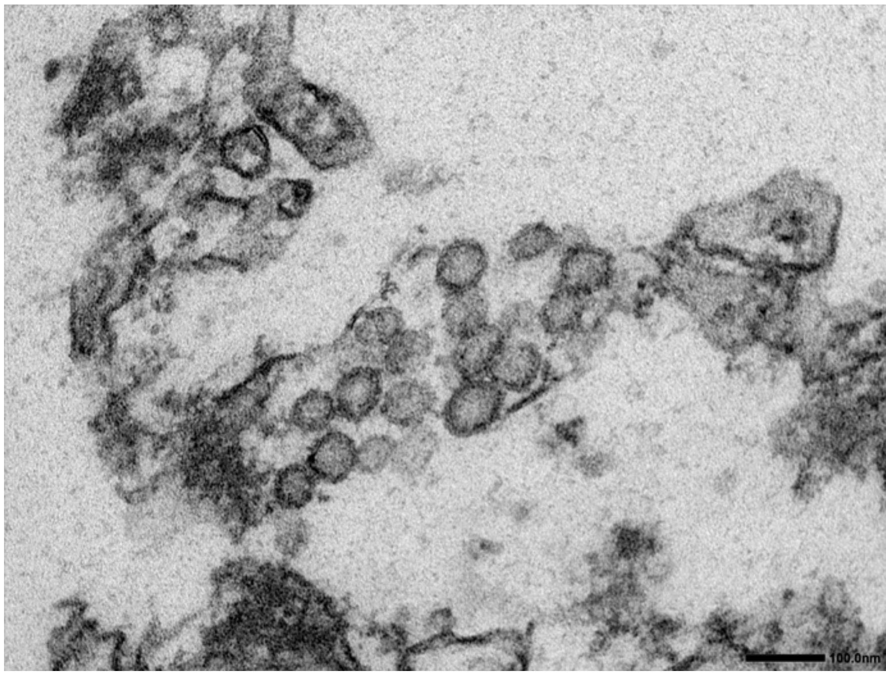
- Placenta: local calcifications in villi and decidua; no inflamation
- No relevant pathological changes in othe fetal organs
- No pathological changes in the umbilical cord
- No pathological changes in fetal membranes
- Fetal karyotyping showed normal male profile

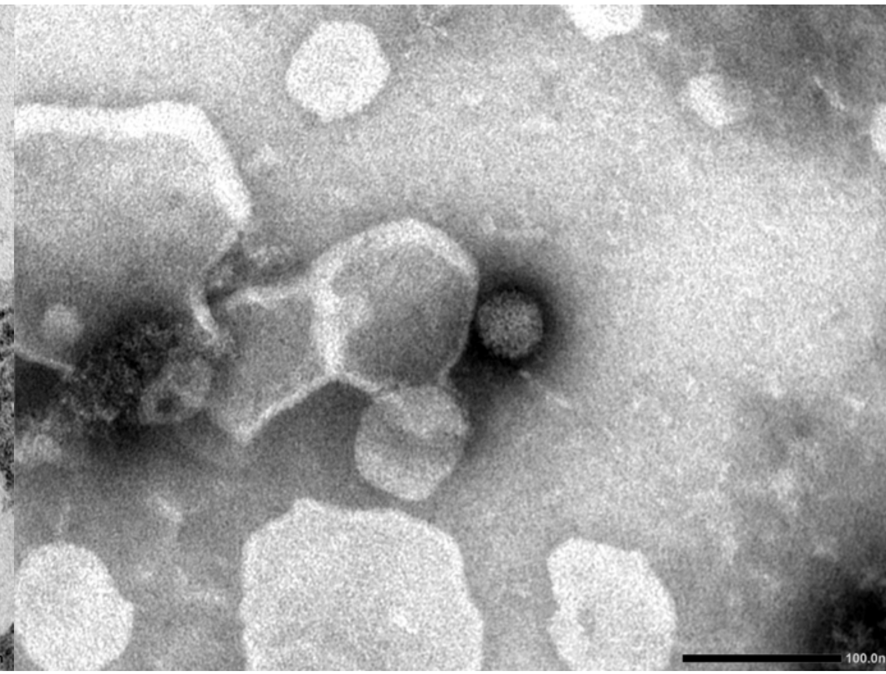
Ultrathin sections of fetal brain - TEM



Demaged brain cell with a cluster of virions located in the disruped ER

Staining of flavivirus-like particles- TEM





Group of enveloped structure with a bright interior – viral replication?

Viral particles from fetal brain – morphologic characteristic consistent with flaviviruses.

Microbiologic investigation

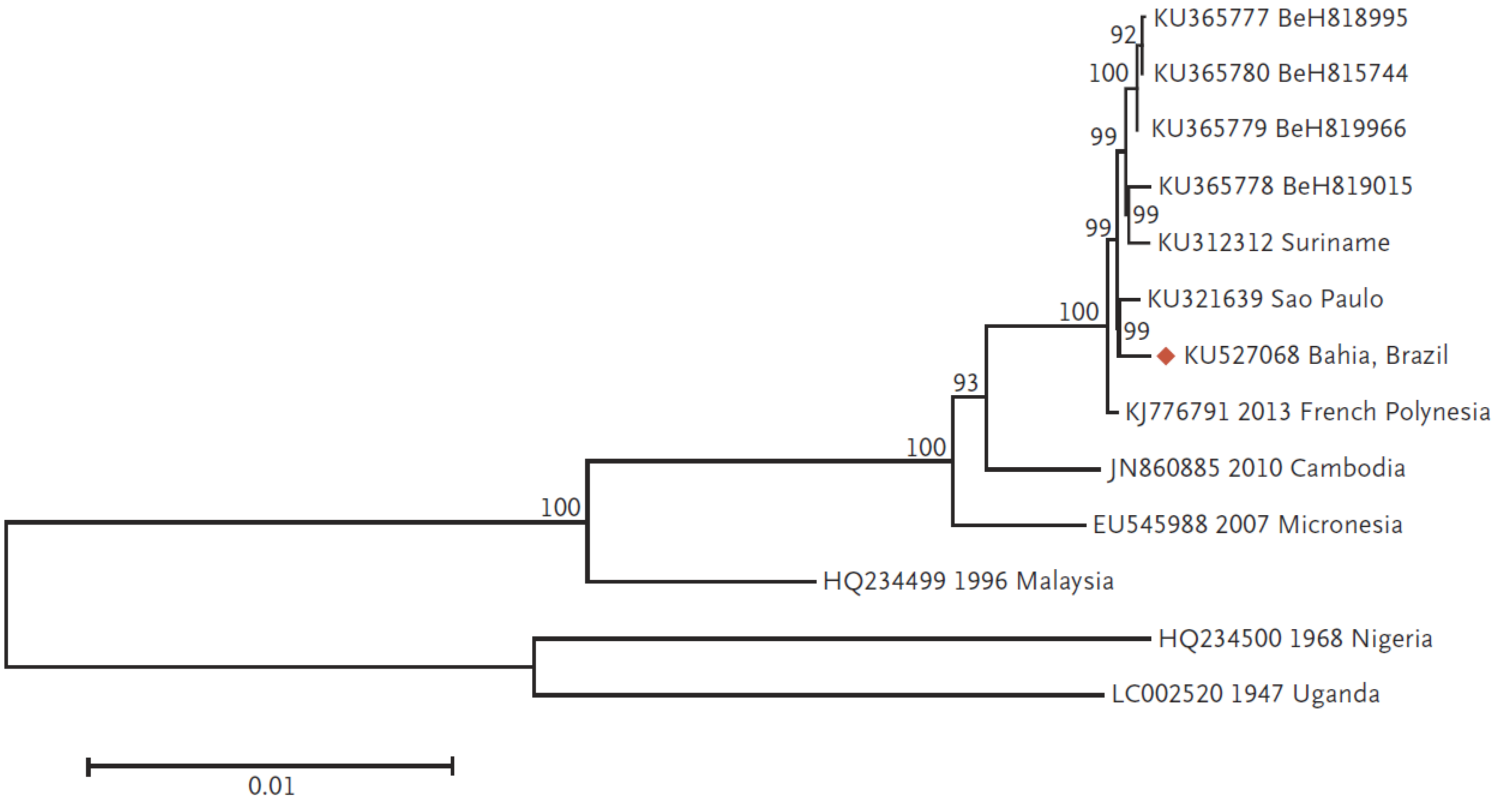
- RNA extracted from 10 mg of: placenta, lungs, heart, skin, spleen, thymus, liver, kidneys and cerebral cortex.
- ZIKV real-time RT-PCR (NS5) and one-step RT-PCR (E-protein) positive ONLY on brain tissue!
- Seqencing of amplicons confirmed ZIKV
- $6.5x10^7$ viral copies/mg of tissue!
- Complete ZIKV genome sequence recovered from the brain tissue.

Differential molecular testing of fetal autopsy tissue samples

Table S2. Differential molecular testing of fetal autopsy tissue samples

RT-PCR ZIKV	**Positive**	Faye O et al. Virol J 2013. 10:311
RT-PCR DENV	Negative	Simplexa™ Dengue Kit (Focus Diagnostics, Cypress, CA)
RT-PCR CHIK	Negative	RealStar® Chikungunya RT-PCR Kit (Altona Diagnostics, Hamburg, Germany)
RT-PCR YFV	Negative	Drosten C et al. J Clin Microbiol. 2002: 40: 2323-2330
RT-PCR WNV	Negative	Linke S et al. J Virol Methods. 2007:355-8
RT-PCR TBE	Negative	Schwaiger J et al. Clin Virol. 2003: 27:136-45
RT-PCR LCMV	Negative	Cordey S. J Virol Methods. 2011: 177:118-22
RT-PCR CMV	Negative	Argene® real-time PCR assay for detection of CMV (Biomerieux, France)
RT-PCR VZV	Negative	Argene® real-time PCR assay for detection of VZV (Biomerieux, France)
RT-PCR HSV 1	Negative	Argene® real-time PCR assay for detection of HSV-1 and HSV-2 (Biomerieux, France)
RT-PCR HSV 2	Negative	Argene® real-time PCR assay for detection of HSV-1 and HSV-2 (Biomerieux, France)
RT-PCR ENTV	Negative	Nijhuis et al. J Clin Microbiol. 2002: 40:3666
RT-PCR Parvo B19	Negative	*artus*® Parvo B19 TM PCR kit, v.1 (Qiagen, Hilden, Germany)
RT-PCR RUBV	Negative	Zhu Z et al. J Clin Microbiol. 2007: 45:2847-52
RT-PCR TOXO	Negative	Edvinsson B et al. Clin Microbiol Infect. 2006: 12:131-6

ZIKV sequence analysis



A complete ZIKV genome sequence (10,808 nt) was recovered from brain tissue and phylogenetic analysis showed that it emerged from ZIKV Asian lineage.
AA changes found in NS1 and NS4B regions.

Invetigation of maternal serum (convalescent phase)

Table S1. Investigation of maternal serum obtained at TOP	
Anti-ZIKV IgM*	Negative
Anti-ZIKV IgG*	**1:1024**
ZIKV NT**	**1:640**
Anti-DENV IgM[$]	Negative
Anti-DENV IgG (type 1-4)[$]	**1:100, 1:1000, 1:1000, 1:100**
Dengue NS1[$$]	Negative
DENV NT (type 2 and 3)	**1:40, 1:80**
Anti-CHIKV IgM[$]	Negative
Anti-CHIKV IgG[$]	Negative
Anti-YFV IgM[$]	Negative
Anti-YFV IgG[$]	**1:320**
YFV NT**	Negative
Anti-WNV IgM$	Negative
Anti-WNV IgG[$]	**1:100**
WNV NT**	Negative
Anti-TBEV IgM[#]	Negative
Anti-TBEV IgG[#]	**12.8 U/ml**
TBEV NT**	Negative
Anti-LCMV IgM*	Negative
Anti-LCMV IgG*	Negative
Anti-CMV IgM[&]	Negative
Anti-CMV IgG[&]	Negative
Anti-Parvo B19 IgM[&]	Negative
Anti-Parvo B19 IgG[&]	**8.0**
Anti-HSV IgM[#]	Negative
Anti-HSV IgG[#]	Negative
Anti-HSV2 IgG[&]	Negative
Anti-RUBV IgM[&]	Negative
Anti-RUBV IgG[&]	**194 IU/ml**
Anti-Toxo IgM[&]	Negative
Anti-Toxo IgG[&]	**>400 IU/ml**

Conclussions:

- Our results prooved strong evidence between ZIKV and microcephaly.
- Severe fetal injury associated with ZIKV vertical transmission
- Severly afected CNS and gross intrauterine retardation
- Demage of the placenta by the virus
- Neurotropism of the virus
- Possible location of the virus in the neurons
- Arrested development of the cortex at the embrionic age app. at 20 weeks
- Flavivirus-like particles seen in damaged ER – persistance of the virus in the the fetal brain

ZIKV situation – WHO 6 October, 2016

- 73 countries and territories report continuing mosquito-borne ZIKV transmission (56 from 2015 on).
- 12 countries have reported evidence of person-to-person transmission of Zika virus, probably via a sexual route.
- Microcephaly, and other fetal malformations potentially associated with Zika virus infection or suggestive of congenital infection, have been reported in 22 countries or territories. Thailand is the latest territory to report a case of microcephaly associated with Zika virus.
- 19 countries worldwide have reported an increased incidence of Guillain-Barré syndrome (GBS) and/or laboratory confirmation of a Zika virus infection among GBS cases.
- Based on research to date, there is scientific consensus that Zika virus is a cause of microcephaly and other CNS malformations and GBS.

Figure 1. Cumulative number of countries and territories by WHO region[2] reporting mosquito-borne Zika virus transmission for the first time in years (2007–2014), and monthly from 1 January 2015 to 5 October 2016

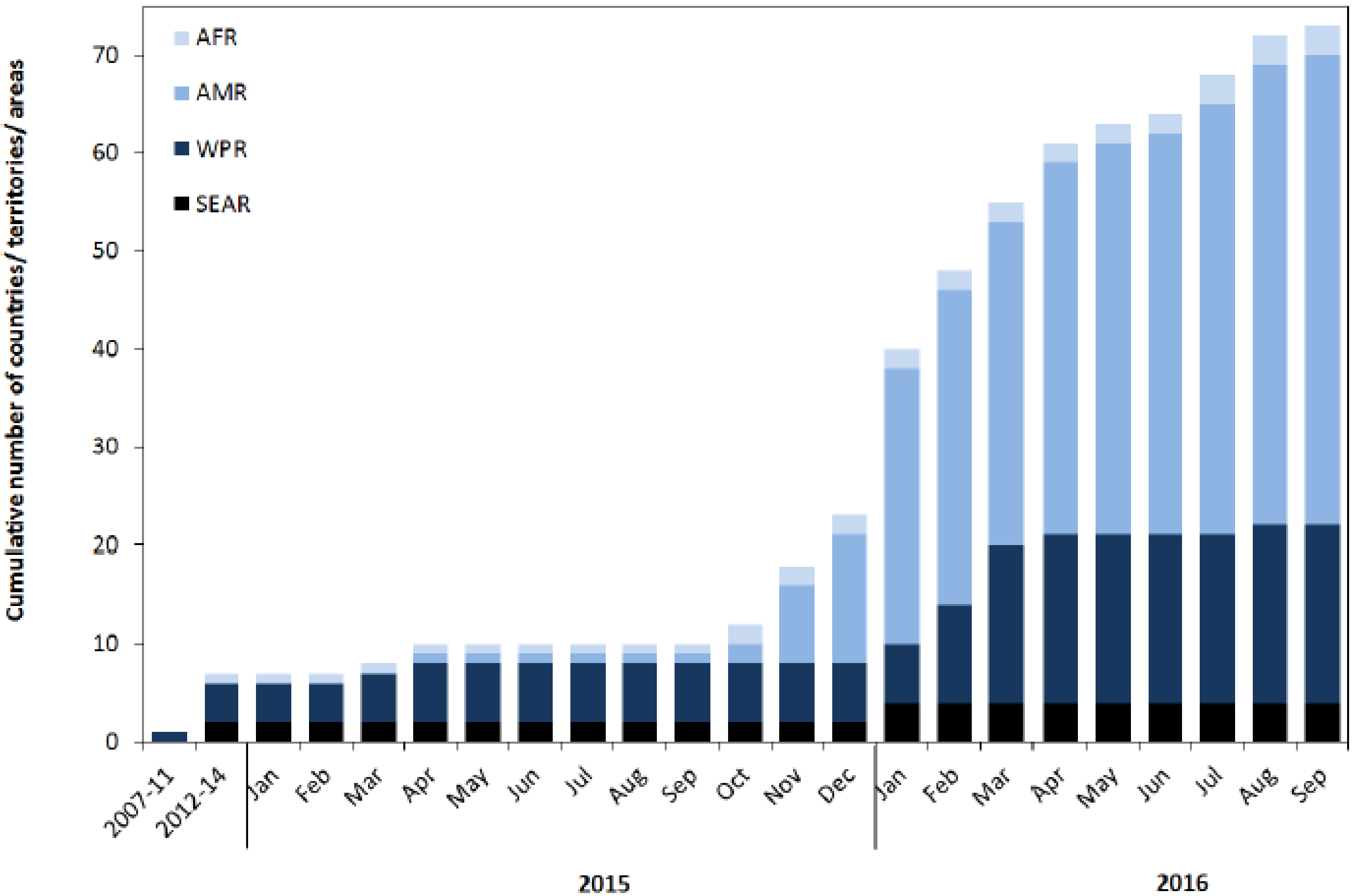


Table 2. Countries reporting non mosquito-borne Zika virus transmission since February 2016

Classification	WHO Regional Office	Country / territory	Total
Countries with evidence of person-to-person transmission of Zika virus, other than mosquito-borne transmission	AMRO/PAHO	Argentina, Canada, Chile, Peru, United States of America	5
	EURO	France, Germany, Italy, Netherlands, Portugal, Spain	6
	WPRO	New Zealand	1
Total			**12**

Table 3. Countries and territories reporting microcephaly and/or CNS malformation cases potentially associated with Zika virus infection

Reporting country or territory	Number of microcephaly and/or CNS malformation cases suggestive of congenital Zika infections or potentially associated with a Zika virus infection	Probable location of infection
Brazil	1949[3]	Brazil
Cabo Verde	9	Cabo Verde
Canada	1	Undetermined
Costa Rica	1	Costa Rica
Colombia	42[4]	Colombia
Dominican Republic	10[5]	Dominican Republic
El Salvador	4	El Salvador
French Guiana	3[6]	French Guiana
French Polynesia	8	French Polynesia
Guatemala	17[7]	Guatemala
Haiti	1	Haiti
Honduras	1	Honduras
Marshall Islands	1	Marshall Islands
Martinique	12[6]	Martinique
Panama	5	Panama
Paraguay	2[8]	Paraguay
Puerto Rico	1	Puerto Rico
Slovenia	1[9]	Brazil
Spain	2	Colombia, Venezuela (Bolivarian Republic of)
Suriname	1	Suriname
Thailand	2	Thailand
United States of America	26[10]	Undetermined*

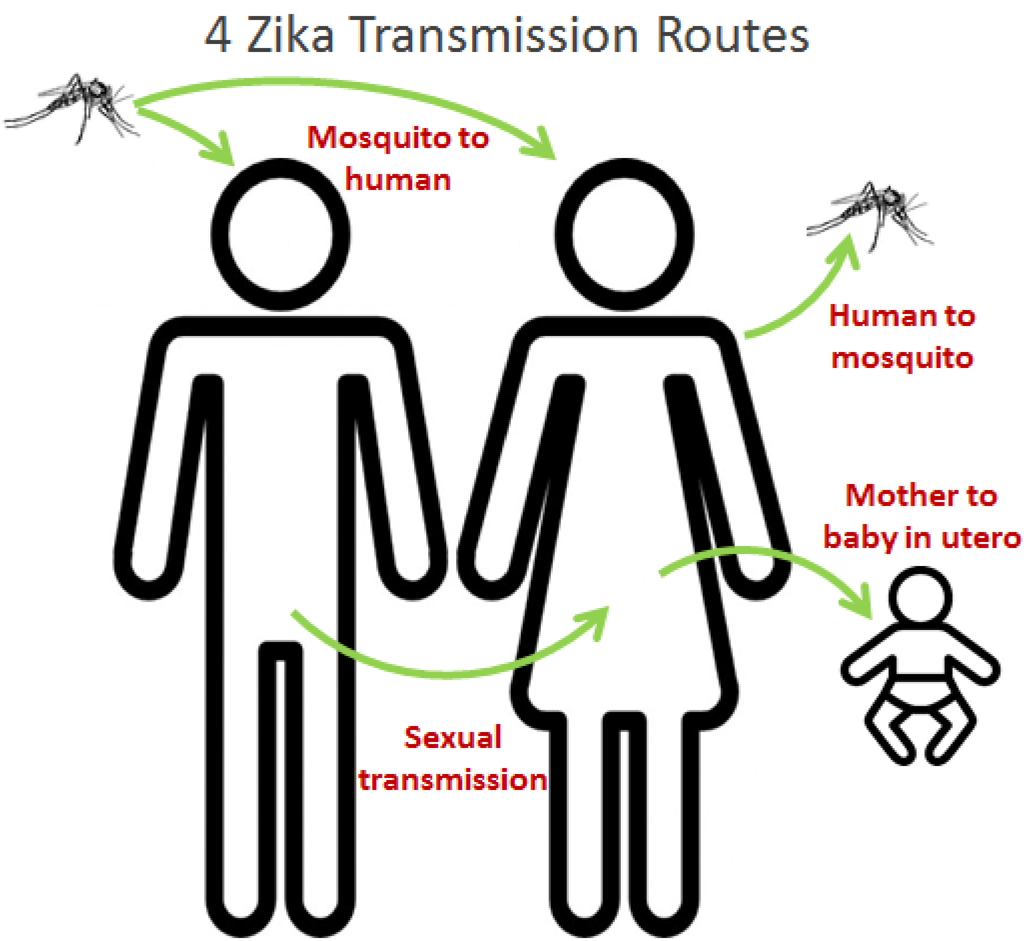
**The probable locations of three of the infections were Brazil (1 case), Haiti (1 case) and Mexico, Belize or Guatemala (1 case).*

Table 4. Countries and territories reporting Guillain-Barré syndrome (GBS) potentially associated with Zika virus infection

Classification	Country / territory
Reported increase in incidence of GBS cases, with at least one GBS case with confirmed Zika virus infection	Brazil, Colombia, Dominican Republic, El Salvador*, French Guiana, French Polynesia, Guadeloupe[11], Honduras, Jamaica, Martinique, Suriname**, Venezuela (Bolivarian Republic of)
No increase in GBS incidence reported, but at least one GBS case with confirmed Zika virus infection	Costa Rica, Grenada[12], Guatemala, Haiti, Mexico, Panama, Puerto Rico

**GBS cases with previous history of Zika virus infection were reported by the International Health Regulations (2005) National Focal Point in the United States of America.*

***One case living in continental Netherlands was diagnosed in mid-January 2016 and reported by the Netherlands.*


4 Zika Transmission Routes
Mosquito to human
Human to mosquito
Mother to baby in utero
Sexual transmission

Collaborators:

<u>Institute of Microbiology and Immunology</u>

Miša Korva

Mateja Poljšak Prijatelj

Marko Kolec

Miroslav Petrovec

<u>Institute of Pathology</u>

Mara Popović

Jernej Mlakar

Alenka Vizjak

Jerica Mraz

Jože Pižem

<u>UKC – Dept. of Perinatology</u>

Nataša Tul

Vesna Fabjan Vodušek

<u>UKC – Institute of Radiology</u>

Tina Vesnaver Vipotnik

Sofia


ZIKA
T.