The Dengue Fever vaccine:

How it can help against Zika

Sean Diehl, Ph.D.

Assistant Professor

Kristen Pierce, M.D.

Associate Professor

Medicine-Infectious Disease

The Robert Larner, M.D. College of Medicine
THE UNIVERSITY OF VERMONT

THE University of Vermont MEDICAL CENTER

The University of Vermont

Objectives

- Epidemiology of Dengue and Zika
- Clinical disease manifestations of Dengue and Zika
- Vaccine Development for Dengue
- Vaccine Development for Zika

WORLD'S DEADLIEST ANIMALS

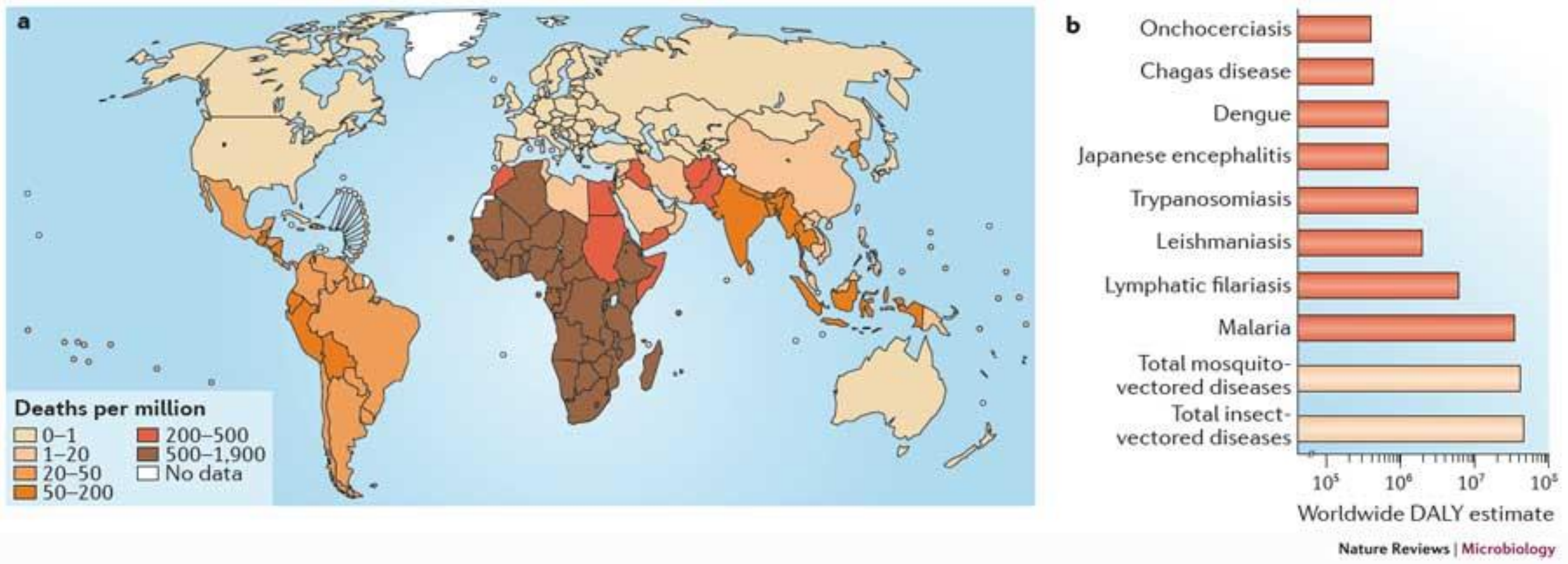
DEATHS PER YEAR

Mosquito	725,000
Freshwater snail	110,000
Ascaris roundworm	60,000
Venomous snake	50,000
Rabid dog	40,000
Assassin bug	12,000
Tsetse fly	9,000
Tapeworm	2,000
Crocodile	1,000
Hippo	500
Elephant	100
Lion	100
Wolf	10
Shark	10

SOURCE: DATA VIZEO

TND

Deaths and lost productivity Worldwide due to Infectious Disease



Nature Reviews | Microbiology

Nature Reviews

Transmission

- Factors
  - Initiation and maintenance of epidemic

Photo courtesy of Paul Zborowski ©2004

1) Strain of virus
   - Strains vary in virulence, duration of viremia

2) Density, behavior and competence of the vector
   - Rainy season
   - Increased transmission due to prolonged vector survival, shortens extrinsic incubation period

3) Susceptibility of human populations
   - Host factors

4) Introduction of virus into susceptible community

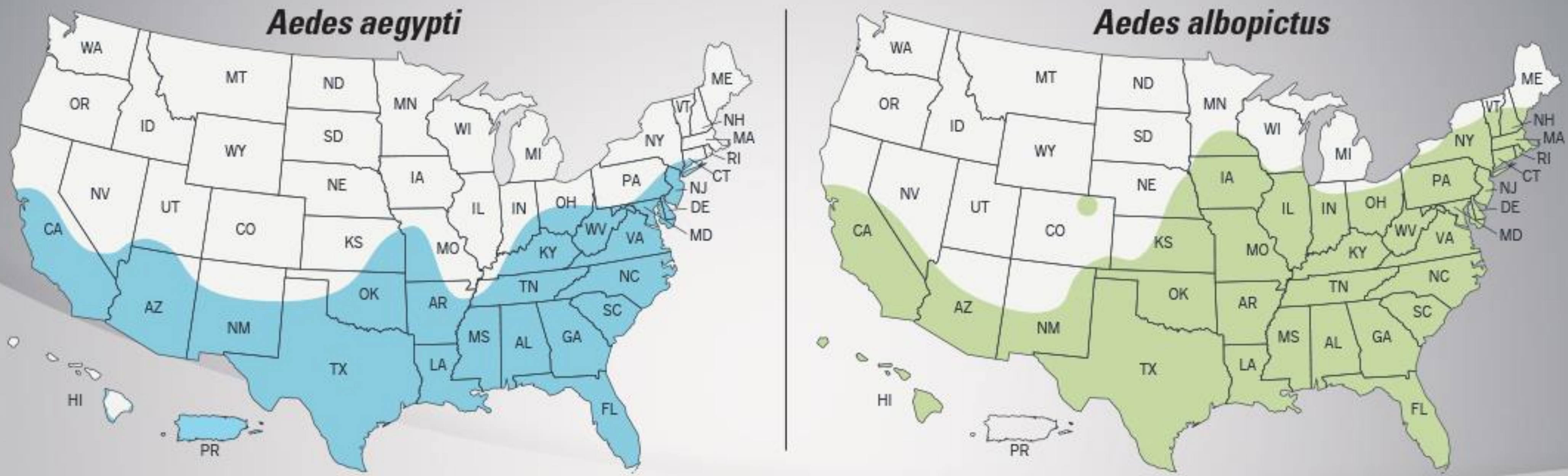
Dengue

- *Aedes aegypti, Aedes albopictus*

Zika

- *Aedes aegypti, Aedes albopictus*

Estimated range of *Aedes aegypti* and *Aedes albopictus* in the United States, 2016*



Aedes aegypti **mosquitoes are more likely to spread viruses like Zika, dengue, chikungunya than other types of mosquitoes such as *Aedes albopictus* mosquitoes.**

Emerging Infections

Emergence as a 2–step process

- 1) Introduction of an agent into a new host population
  - New infection
  - Variant of existing infection
- 2) establishment and dissemination within a new host (“adoption”)
  - Variety of factors associated with “spread”

Morse. Perspect. Biol. Med:34:387

Vector borne diseases

- Spread supported/facilitated by:
  - Global trade
  - Ineffectiveness of vector control
    - Biochemical
      - Resistance issues
    - Removal of breeding grounds
    - Biologic targeting of mosquitos
  - Urban crowding/living conditions
  - Poorly designed irrigation and water storage
  - Poor waste disposal
  - Increasing in global travel
  - Deforestation and habitat destruction
  - global warming?

Dengue and Zika

- Flavivirus

  West Nile

  Dengue

  Yellow Fever

  Tick-borne Encephalitis Virus (TBE)

  Saint Louis Encephalitis (SLE)

  Japanese Encephalitis Virus (JEV)

Dengue

Risk and Incidence

2 billion persons live in tropics/subtropics

- 40% of world’s population at risk

Most rapidly spreading mosquito-borne virus in the world

- 1950s annual case reports to WHO totaled 900
  - By 2005 annual case reports in 60 countries

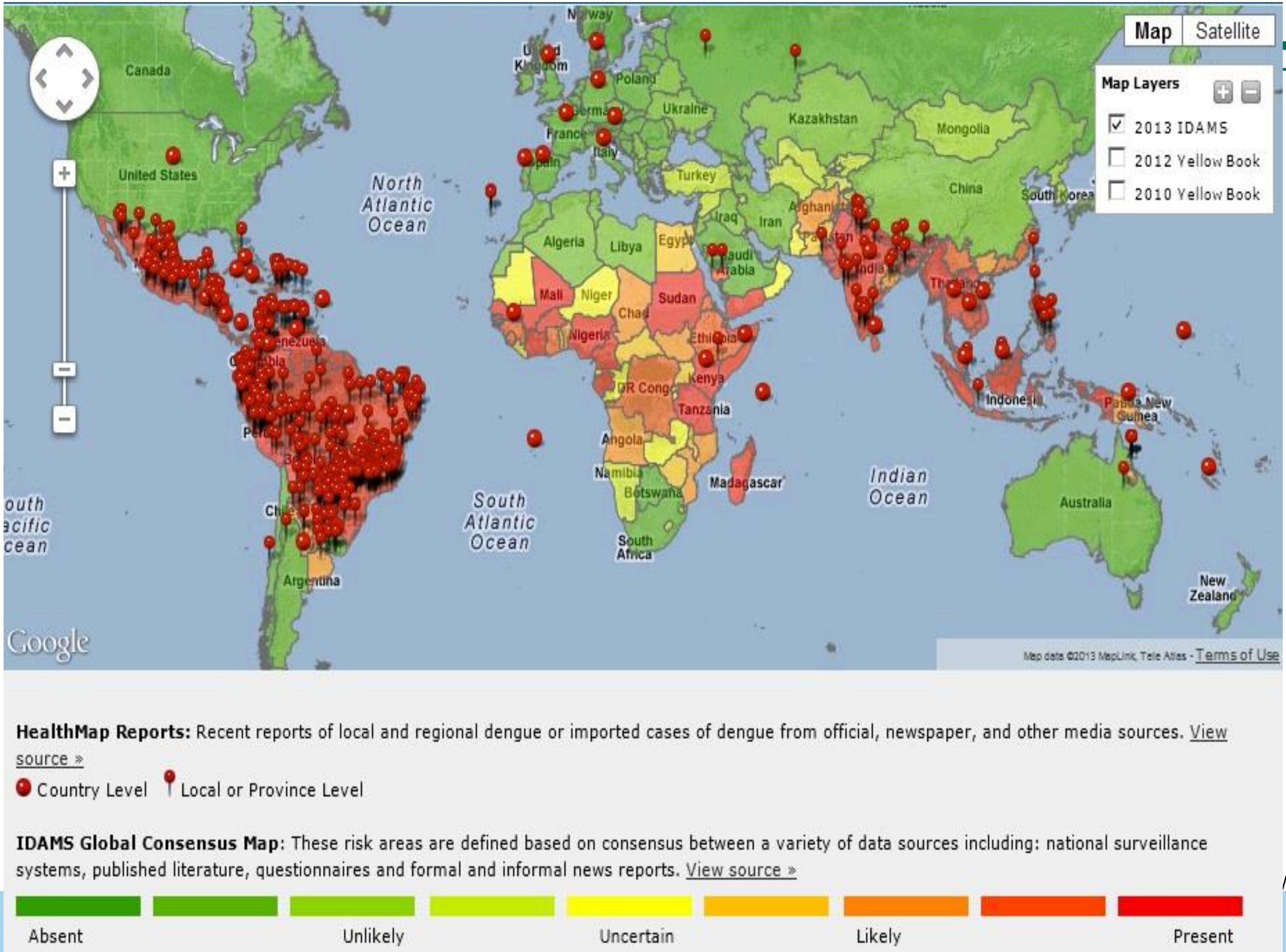
Annually:

- 120 million travel annually to these areas.
- 50-100 million cases dengue fever annually
- 250-500,000 cases Dengue Hemorrhagic Fever
- Approximately 20,000 deaths, but limited knowledge from many corners of the globe.

Epidemiology

Epidemic Dengue Hemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS)

- Emerged over 50 years ago in Southeast Asia
- Emerged in 1981 in the Americas
- Emerged in 1989 in Southern Asia
- Since post-WWII
  - Incidence of DHF/DSS has increased 500 fold
  - One of the leading causes of pediatric morbidity and mortality in Southeast Asia



HealthMap Reports: Recent reports of local and regional dengue or imported cases of dengue from official, newspaper, and other media sources. View source »

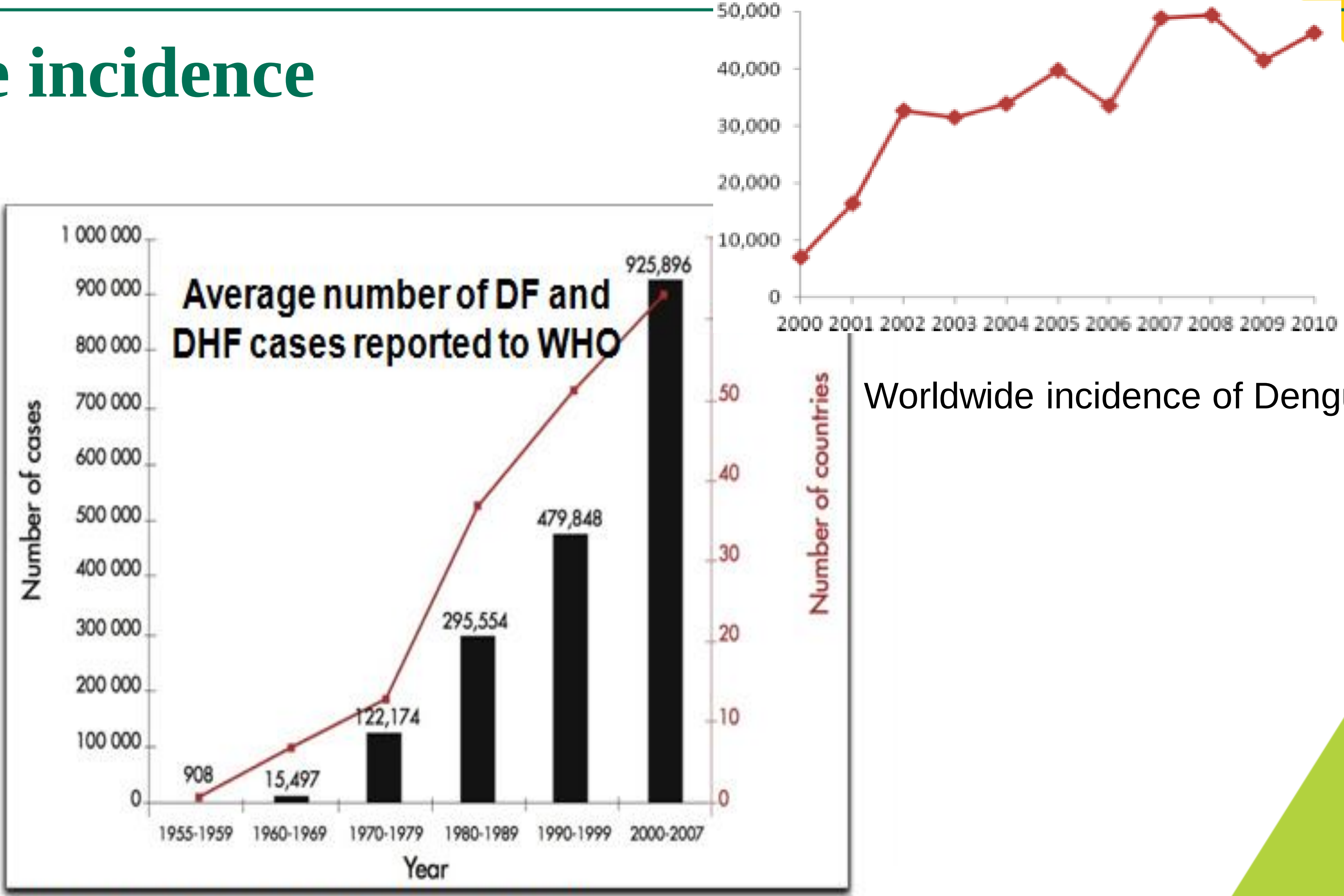
● Country Level 📍 Local or Province Level

IDAMS Global Consensus Map: These risk areas are defined based on consensus between a variety of data sources including: national surveillance systems, published literature, questionnaires and formal and informal news reports. View source »

/dengue/index.php

CDC.gov

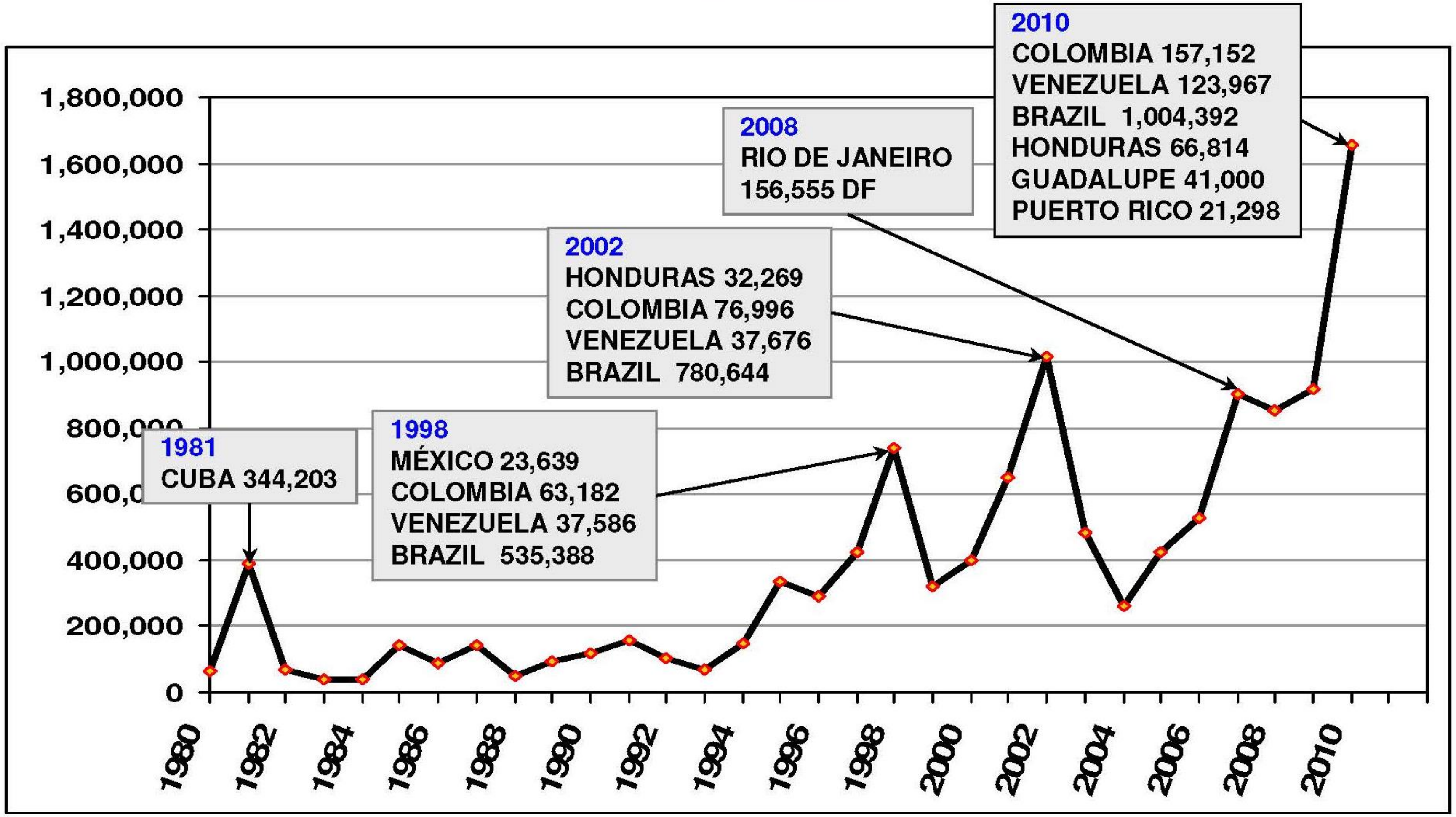
Dengue incidence



Worldwide incidence of Dengue

WHO. Dengue. Guidelines for Diagnosis, Treatment, Prevention and Control. Available at: http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf Last updated: 2009. Accessed July 21, 2011.

Dengue as an emerging disease in the Americas



1981
CUBA 344,203

1998
MÉXICO 23,639
COLOMBIA 63,182
VENEZUELA 37,586
BRAZIL 535,388

2002
HONDURAS 32,269
COLOMBIA 76,996
VENEZUELA 37,676
BRAZIL 780,644

2008
RIO DE JANEIRO
156,555 DF

2010
COLOMBIA 157,152
VENEZUELA 123,967
BRAZIL 1,004,392
HONDURAS 66,814
GUADALUPE 41,000
PUERTO RICO 21,298

Pan American Health Organization
Available at: http://new.paho.org/hq/index.php?option=com_content&task=view&id=264&Itemid=363&lang=en

Trends in Incidence in Dengue Fever among Hospitalized Patients in U.S.

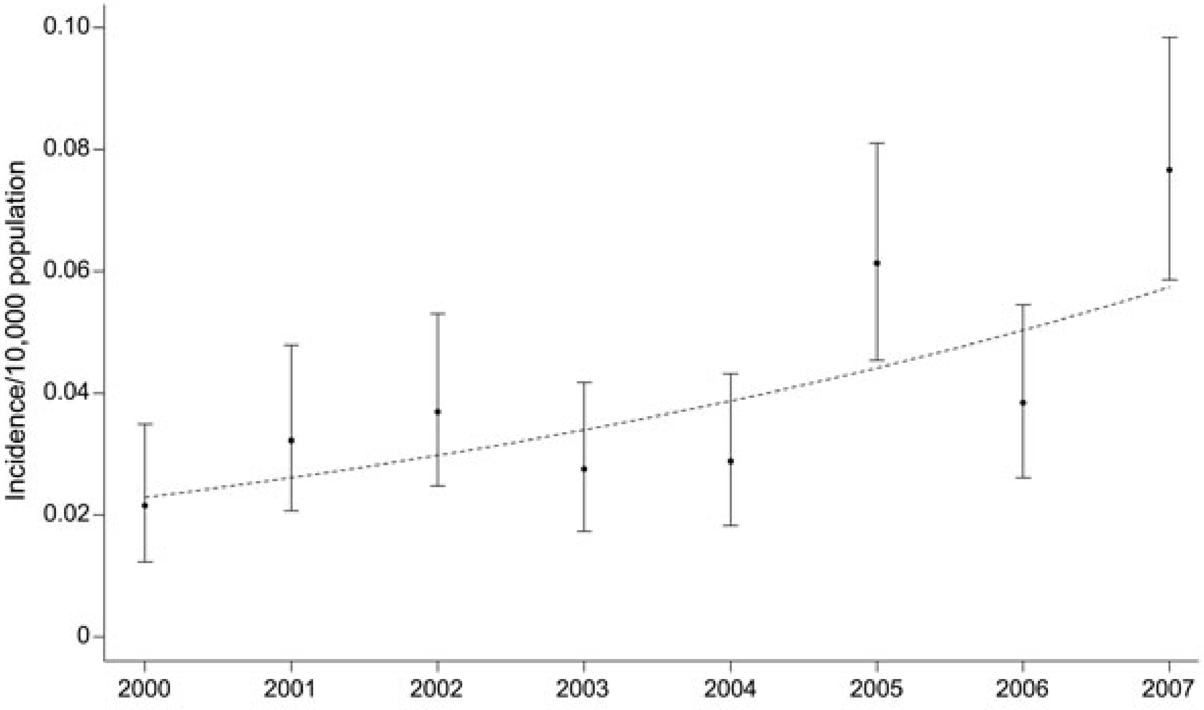
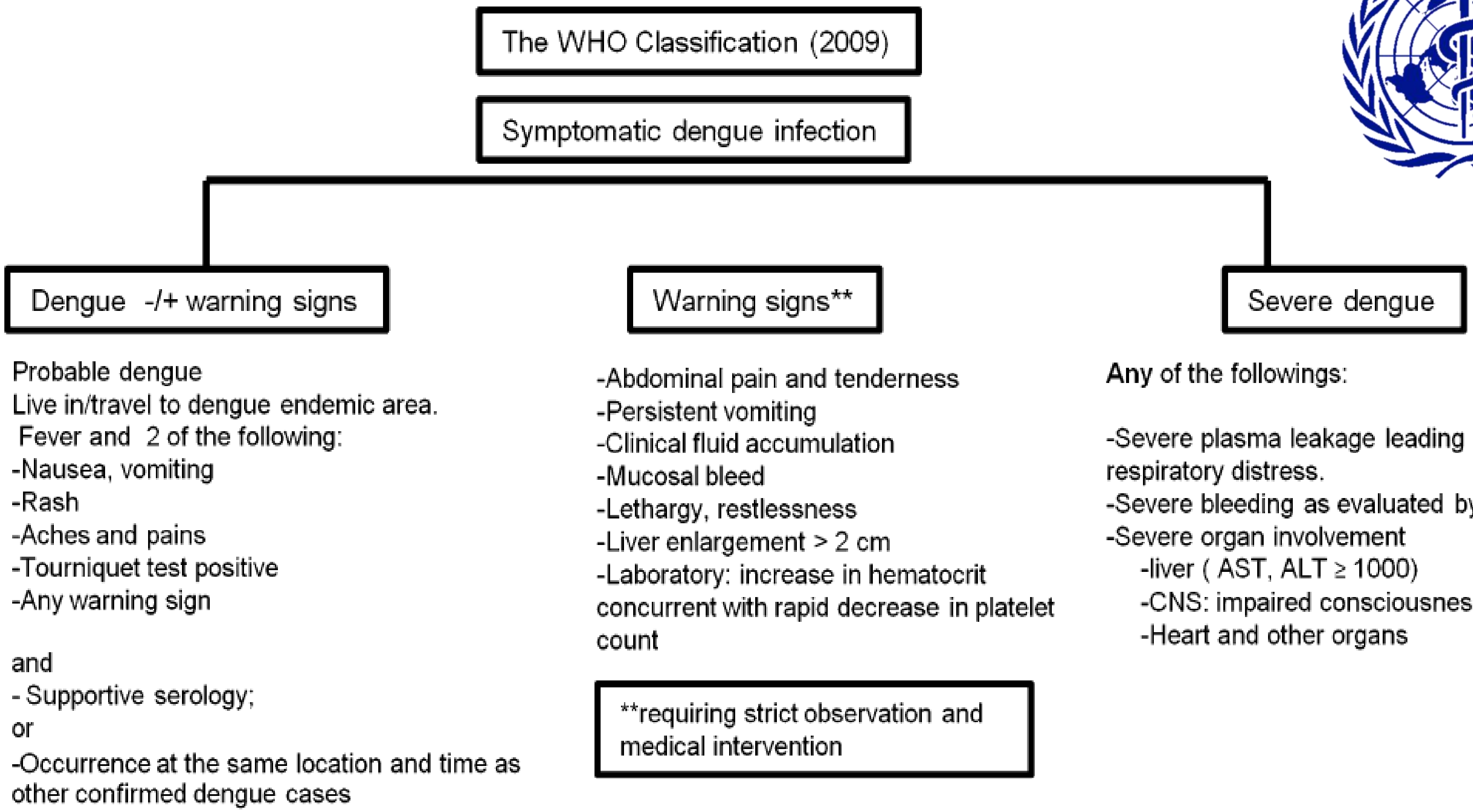


Figure. National estimates of dengue yearly incidence rates and 95% exact binomial confidence intervals (error bars), calculated by using data from the National Inpatient Sample, United States, 2000–2007. The trend (dotted line) is based on a logistic regression model fit by using generalized estimating equations. Note that the trend is curvilinear in the incidence rate, yet linear in the log odds of the incidence

Streit et al. *Emerg Infect Dis*. 2011; 17

“New” Clinical Dengue Classifications



The WHO Classification (2009)

Symptomatic dengue infection

Dengue -/+ warning signs

Probable dengue
Live in/travel to dengue endemic area.
Fever and 2 of the following:
- -Nausea, vomiting
- -Rash
- -Aches and pains
- -Tourniquet test positive
- -Any warning sign

and
- Supportive serology;
or
-Occurrence at the same location and time as other confirmed dengue cases

Warning signs**

- -Abdominal pain and tenderness
- -Persistent vomiting
- -Clinical fluid accumulation
- -Mucosal bleed
- -Lethargy, restlessness
- -Liver enlargement > 2 cm
- -Laboratory: increase in hematocrit concurrent with rapid decrease in platelet count

**requiring strict observation and medical intervention

Severe dengue

Any of the followings:

- -Severe plasma leakage leading to shock or respiratory distress.
- -Severe bleeding as evaluated by clinicians.
- -Severe organ involvement
  - -liver (AST, ALT ≥ 1000)
  - -CNS: impaired consciousness
  - -Heart and other organs

• CID 2011:53 (15 September) • Srikiatkhachorn et al

Clinical Disease Caused by Dengue Viruses

Dengue fever (DF):

- Fever

  and
- Headache
- Retro-orbital pain
- Myalgia
- Arthralgia
- Hemorrhage
- Rash

- Leukopenia
- Neutropenia
- Elevated ALT / AST
- Viremia
- Serum antibodies

Severe dengue:

Hemorrhagic Fever (DHF)

- Fever (2 - 7 days)

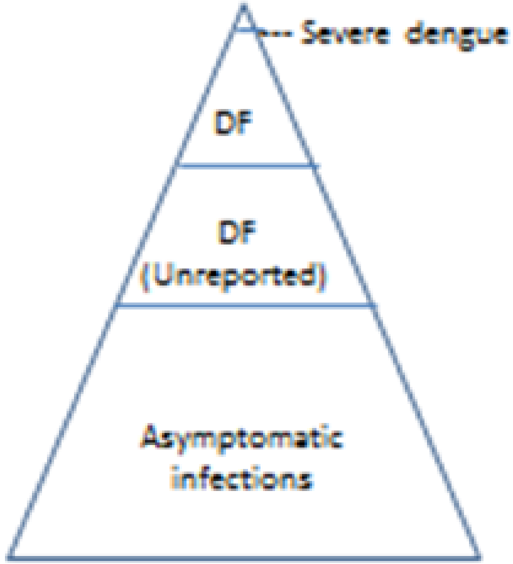
  and
- Thrombocytopenia

  and
- Petechial rash
- Bruising
- Bleeding
- Coagulopathy

  and
- Vascular leakage
- Pleural effusion
- Ascites
- Hemoconcentration

Shock Syndrome (DSS):

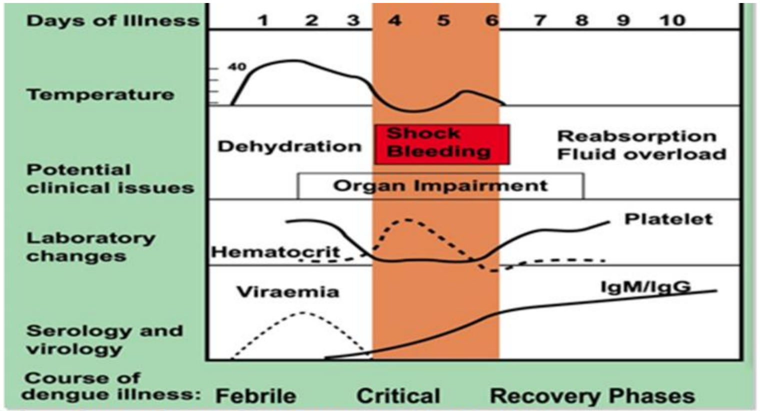
- Hypotension
- Shock



Based on WHO 1975, 1997

Clinical Disease

- Classic disease
  - Incubation period 3-14 days (average 4-7)
  - May have asymptomatic disease or mild febrile illness
- 3 phases
  - 1) Febrile Phase
  - 2) Critical Phase
  - 3) Recovery Phase



Center for Disease Control and Prevention. Clinician's case management card. Available at: http://www.cdc.gov/Dengue/resources/Dengue%20Case%20Management_card_125085_12x6_Zcard_Dengue.pdf Accessed July 21, 2011.

Clinical

- Risk factors for severe disease

  AB blood group

  Race

  Young age

  Viral strain

  Female sex

  High BMI

  Genetic variants of Human Leukocyte Antigen (HLA)

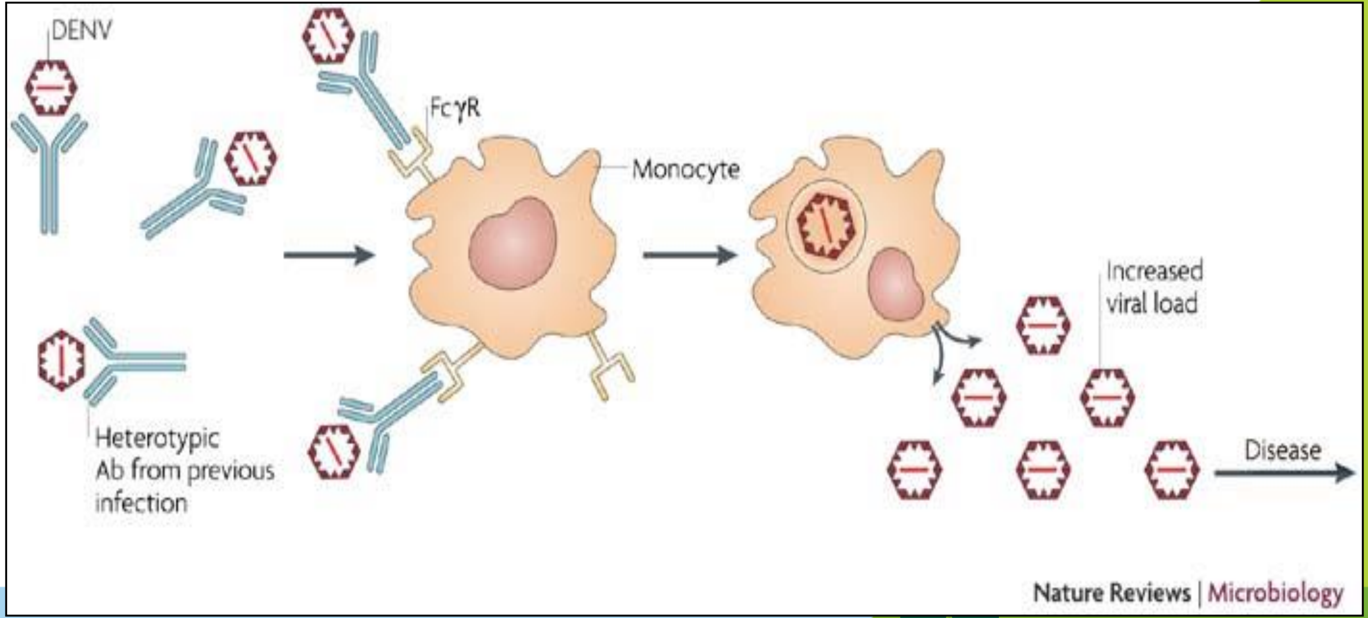
  Possibly chronic disease: Sickle cell disease, diabetes, asthma

- Factors that Decrease risk of severe disease
  - Race
  - Malnutrition
  - Polymorphisms in Fcγ and Vitamin D receptor

Immunopathogenesis of Severe Disease

Antibody dependent Enhancement (ADE)

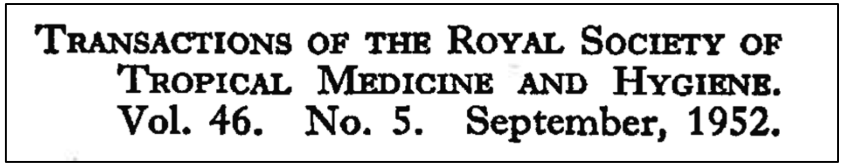
- After initial infection antibodies remain cross reactive with other serotypes
- Non-neutralizing antibodies could then mediate an increased uptake of virus into monocyte/macrophage
- Leading to increased viral replication, immune activation and cytokine release



Whitehead et al. Nature Rev Micro 2007

Zika

The Beginning


TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. 46. No. 5. September, 1952.

Dr. George Dick

Countries and territories showing historical time-line of Zika virus spread (1947 - 2016)

World Health Organization

1947 - 1952
- Uganda
- United Republic of Tanzania

1954
- Nigeria

1960 - 1983
- Central African Republic
- Senegal
- Pakistan
- Burkina Faso
- Cote D'ivoire
- Cameroon
- Sierra Leone
- Gabon
- Indonesia
- Malaysia
- Nigeria
- Costa Rica
- Cambodia

2007 - 2009
- YAP (Micronesia (Federal states of))
- Gabon

2012 - 2014
- French Polynesia
- EASTER ISLAND (Chile)
- Cook Islands
- New Caledonia
- Malaysia
- Philippines
- Cambodia
- Indonesia
- Thailand

Jan - Oct 2015
- Brazil
- Vanuatu
- Fiji
- Colombia
- Cabo Verde
- Samoa
- Solomon Islands

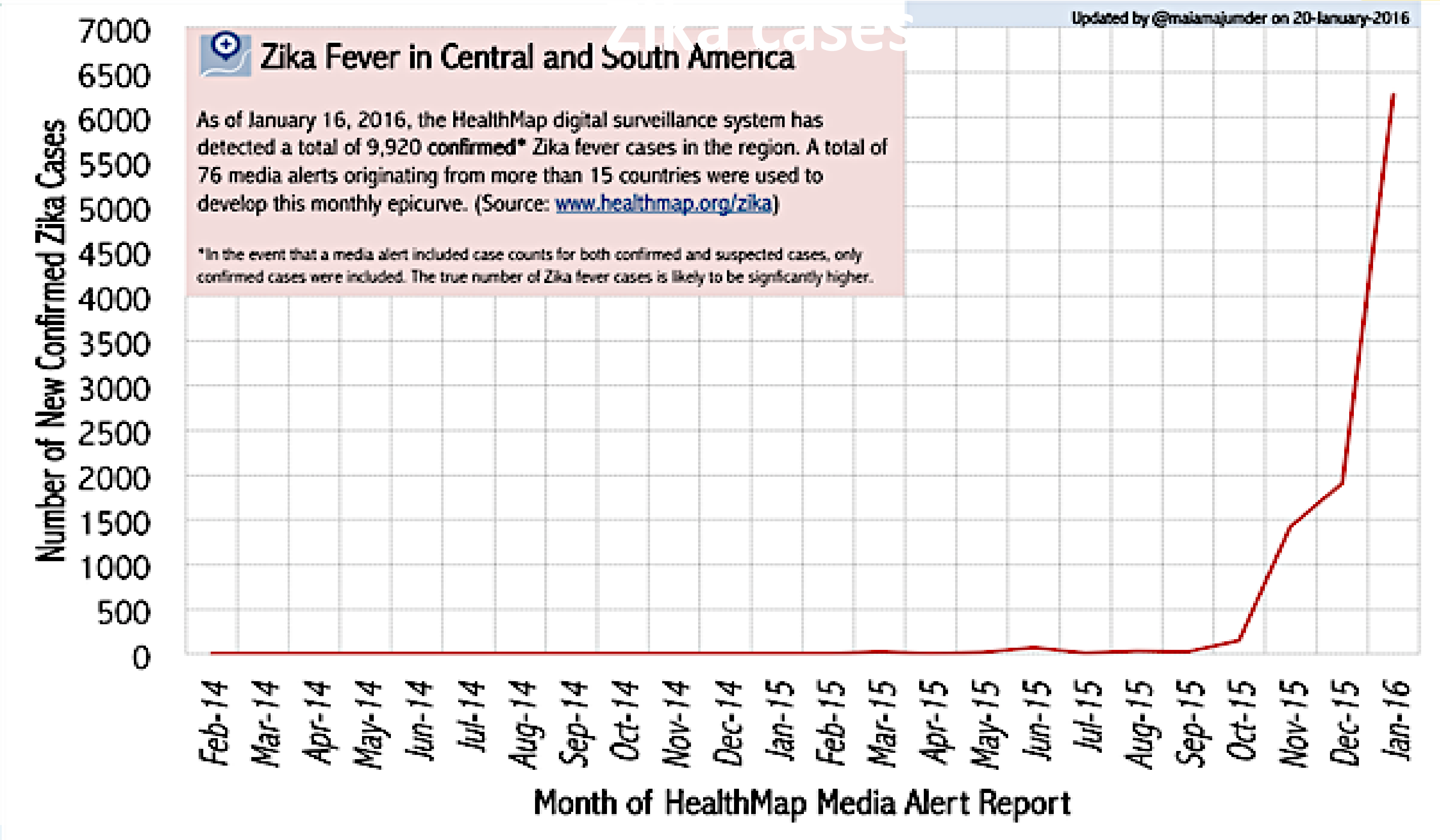
Nov - 2015
- El Salvador
- Guatemala
- Mexico
- Paraguay
- Suriname
- Venezuela (Bolivarian Republic of)

Dec - 2015
- French Guiana
- Honduras
- Martinique
- Panama
- Puerto Rico

Jan - 2016
- Bolivia (Plurinational State of)
- United States Virgin Islands
- Dominican Republic
- Costa Rica
- Guadeloupe
- Saint Martin
- Nicaragua
- Barbados
- Maldives
- Ecuador
- Guyana
- Jamaica
- Curaçao
- Samoa
- Haiti

Feb - 2016
- Tonga

Zika cases



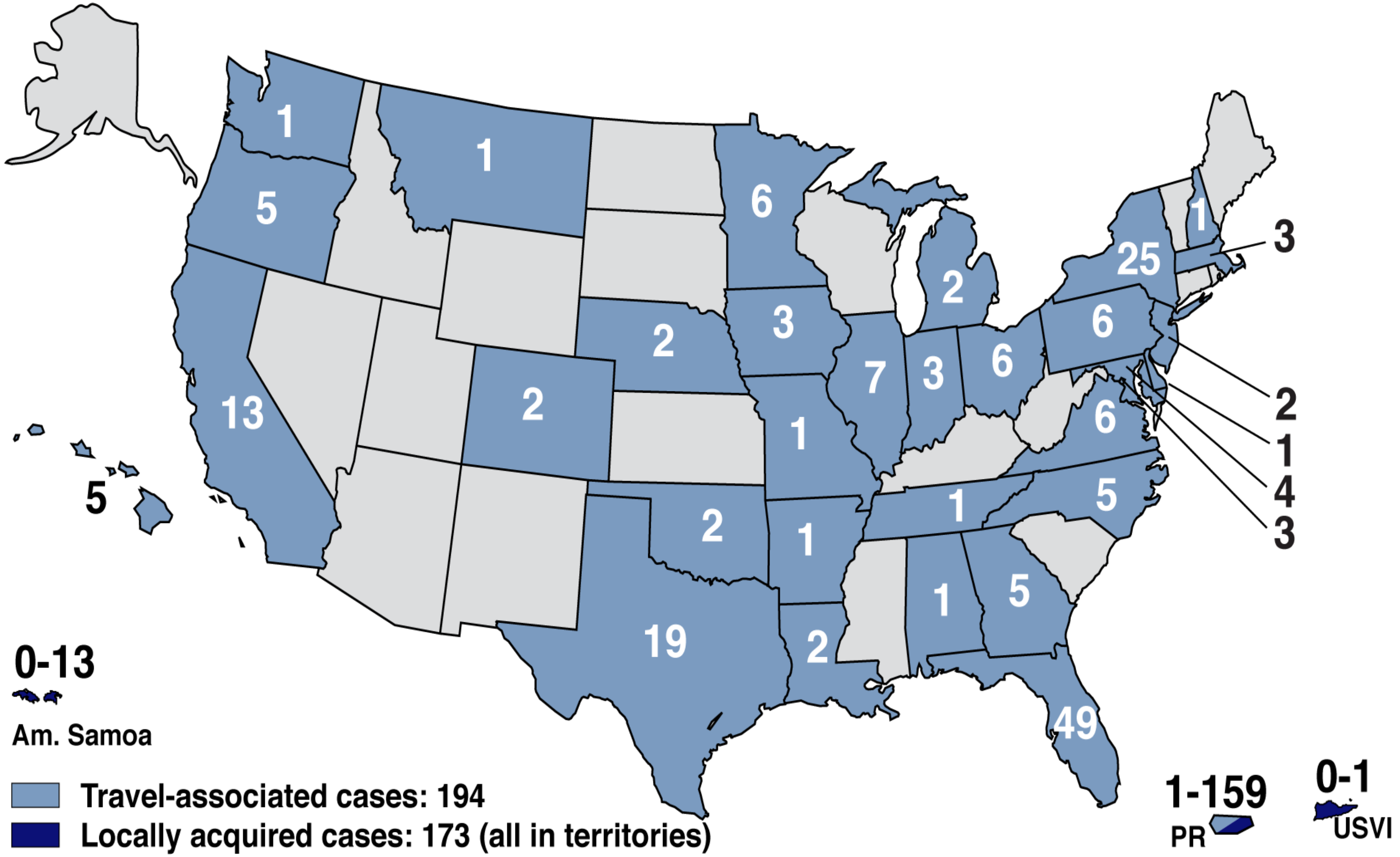
Updated by @maiamajumder on 20-January-2016

Zika Fever in Central and South America

As of January 16, 2016, the HealthMap digital surveillance system has detected a total of 9,920 **confirmed*** Zika fever cases in the region. A total of 76 media alerts originating from more than 15 countries were used to develop this monthly epicurve. (Source: www.healthmap.org/zika)

*In the event that a media alert included case counts for both confirmed and suspected cases, only confirmed cases were included. The true number of Zika fever cases is likely to be signficantly higher.

Zika Virus Cases in the United States and U.S. Territories, 2015–2016

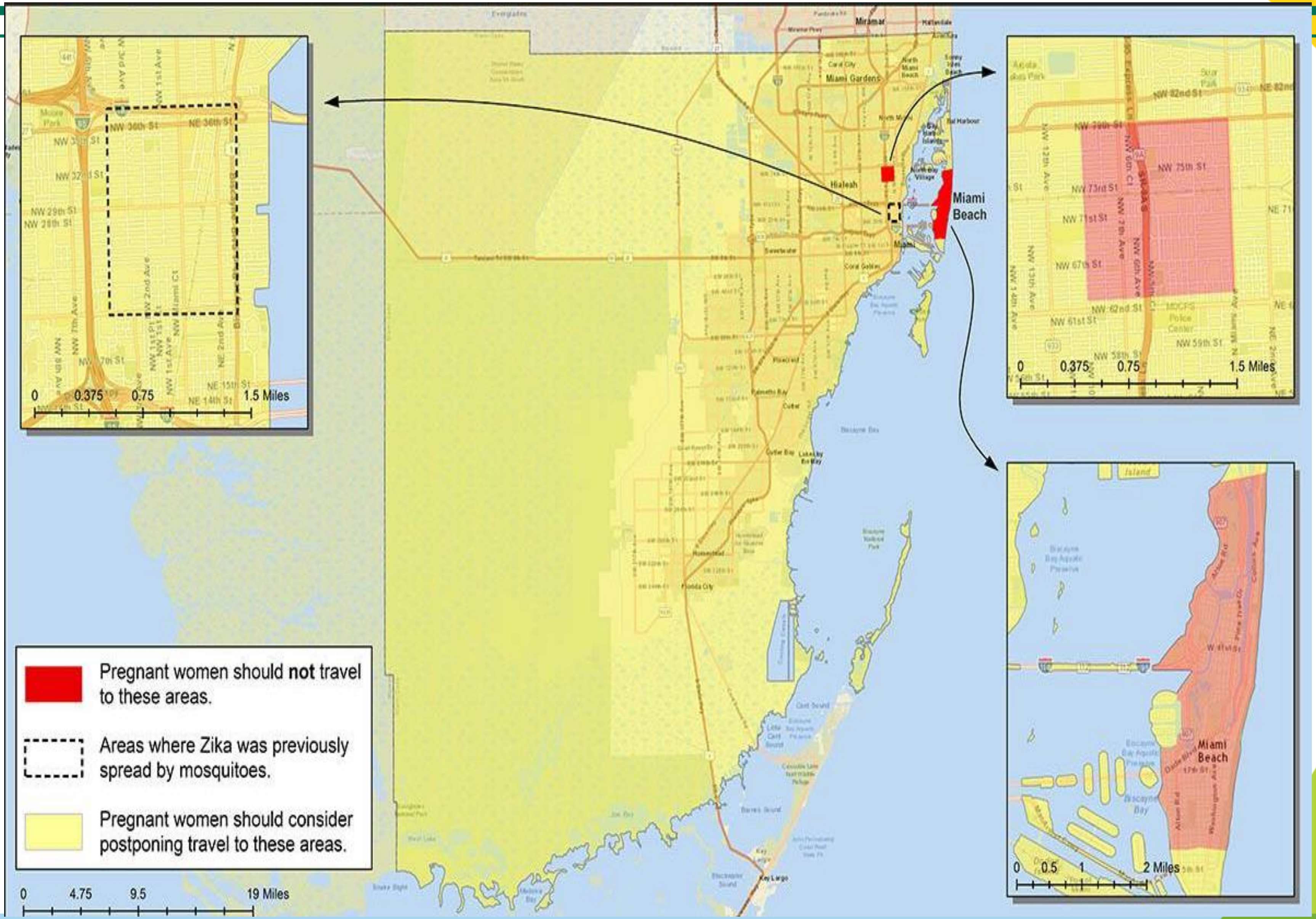


▨ Travel-associated cases: 194

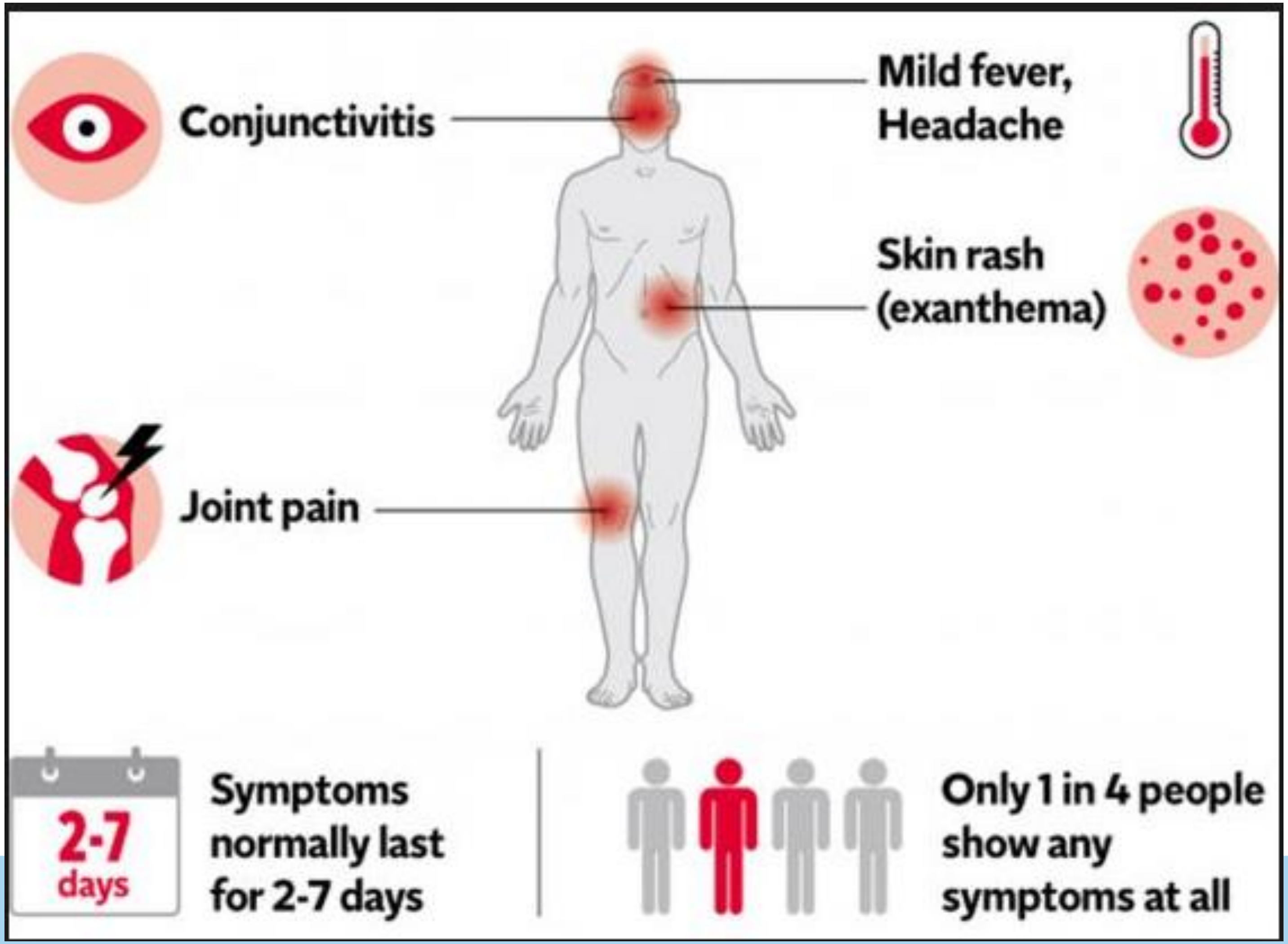
■ Locally acquired cases: 173 (all in territories)

AS Fauci/NIAID

Source: CDC. Data as of 3/09/16.


Miami Beach
🟥 Pregnant women should **not** travel to these areas.
Areas where Zika was previously spread by mosquitoes.
🟨 Pregnant women should consider postponing travel to these areas.
0 4.75 9.5 19 Miles
0 0.375 0.75 1.5 Miles
0 0.375 0.75 1.5 Miles
0 0.5 1 2 Miles
Miami Beach
NW 36th St
NE 36th St
NW 29th St
NW 28th St
NW 79th St
NW 75th St
NW 73rd St
NW 71st St
NW 67th St
NW 62nd St
NW 61st St
NW 82nd St
Hialeah
Miami
Miami Gardens
Homestead
Florida City
Key Largo
Biscayne Bay

Symptoms of Zika



Conjunctivitis

Mild fever,
Headache

Skin rash
(exanthema)

Joint pain

Symptoms
normally last
for 2-7 days

Only 1 in 4 people
show any
symptoms at all

Zika clinical signs

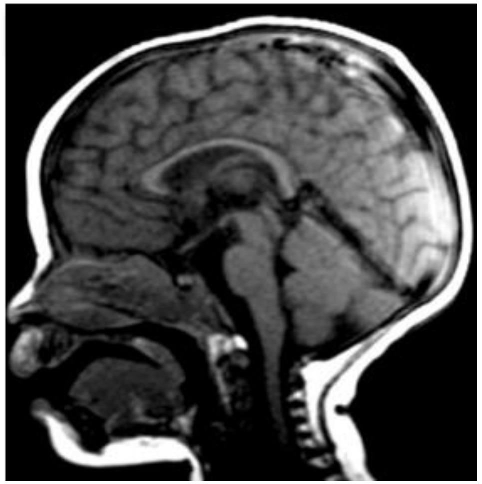
Lab Findings

- Low White blood cell counts
- Low Platelet counts
- Elevated Liver Enzymes

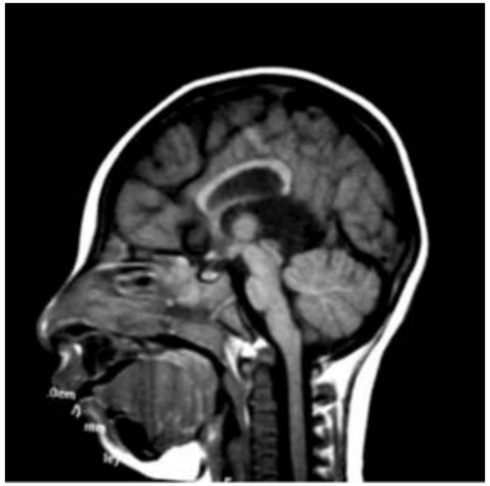
Nenad Macesic et al. Clin Infect Dis. 2015;61:1485-1486


What is ZIKA?

What is microcephaly



Normocephalic

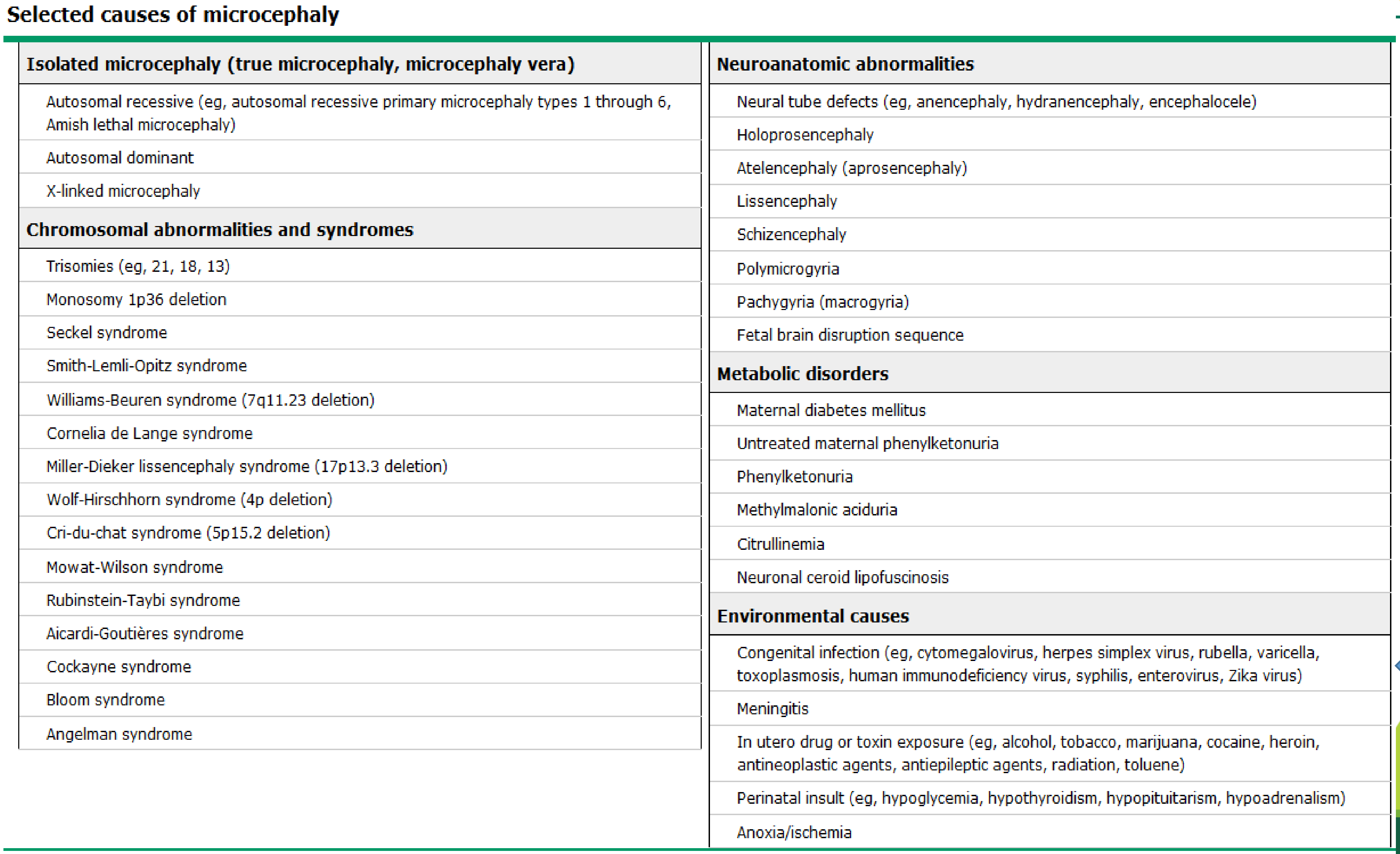


Microcephalic

Infant's head is smaller than the heads of other infants of same age and sex

Can occur as a result of congenital insult or post-natal insult

Microcephaly



Selected causes of microcephaly

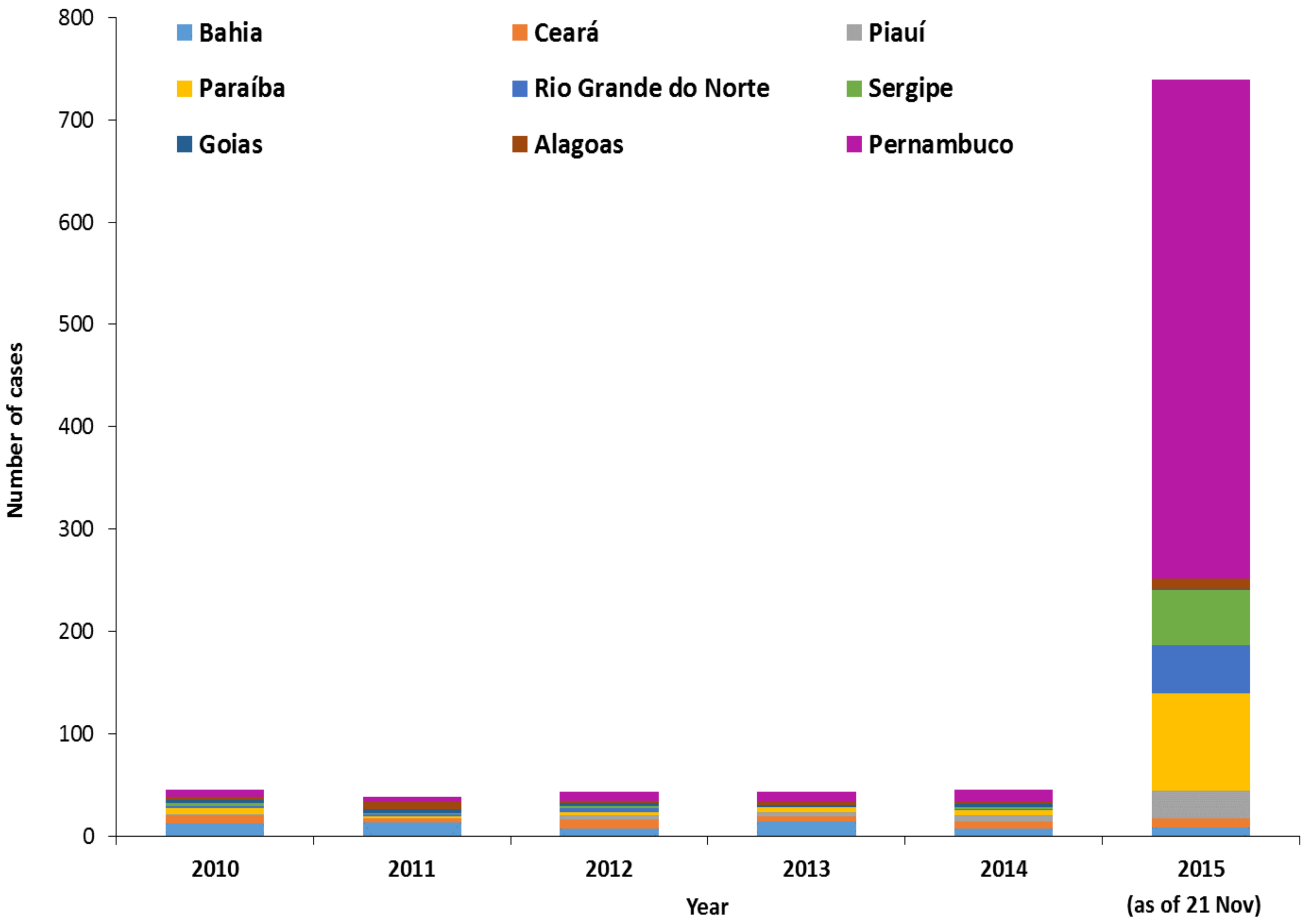
Isolated microcephaly (true microcephaly, microcephaly vera)
Autosomal recessive (eg, autosomal recessive primary microcephaly types 1 through 6, Amish lethal microcephaly)
Autosomal dominant
X-linked microcephaly

Chromosomal abnormalities and syndromes
Trisomies (eg, 21, 18, 13)
Monosomy 1p36 deletion
Seckel syndrome
Smith-Lemli-Opitz syndrome
Williams-Beuren syndrome (7q11.23 deletion)
Cornelia de Lange syndrome
Miller-Dieker lissencephaly syndrome (17p13.3 deletion)
Wolf-Hirschhorn syndrome (4p deletion)
Cri-du-chat syndrome (5p15.2 deletion)
Mowat-Wilson syndrome
Rubinstein-Taybi syndrome
Aicardi-Goutières syndrome
Cockayne syndrome
Bloom syndrome
Angelman syndrome

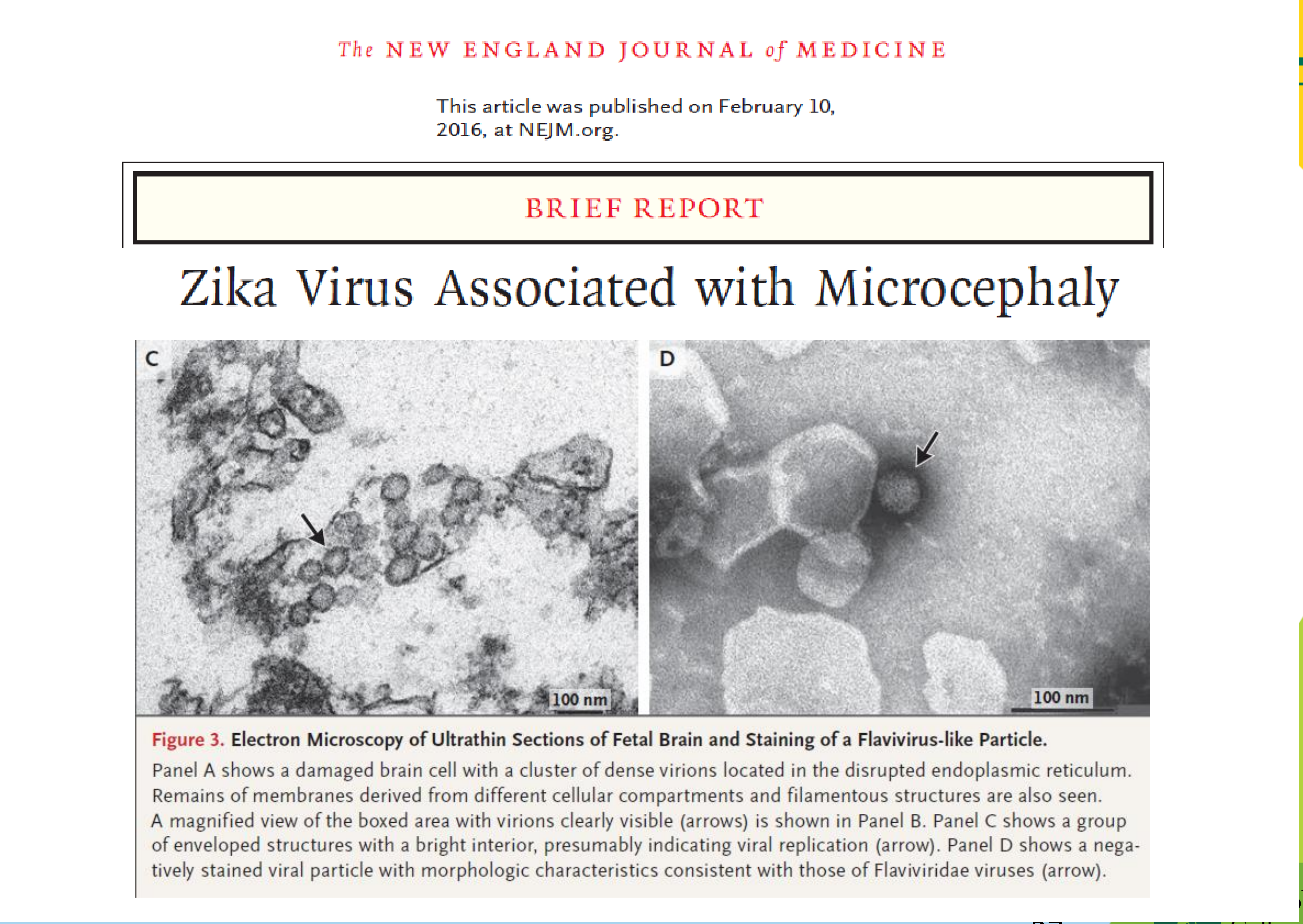
Neuroanatomic abnormalities
Neural tube defects (eg, anencephaly, hydranencephaly, encephalocele)
Holoprosencephaly
Atelencephaly (aprosencephaly)
Lissencephaly
Schizencephaly
Polymicrogyria
Pachygyria (macrogyria)
Fetal brain disruption sequence

Metabolic disorders
Maternal diabetes mellitus
Untreated maternal phenylketonuria
Phenylketonuria
Methylmalonic aciduria
Citrullinemia
Neuronal ceroid lipofuscinosis

Environmental causes
Congenital infection (eg, cytomegalovirus, herpes simplex virus, rubella, varicella, toxoplasmosis, human immunodeficiency virus, syphilis, enterovirus, Zika virus)
Meningitis
In utero drug or toxin exposure (eg, alcohol, tobacco, marijuana, cocaine, heroin, antineoplastic agents, antiepileptic agents, radiation, toluene)
Perinatal insult (eg, hypoglycemia, hypothyroidism, hypopituitarism, hypoadrenalism)
Anoxia/ischemia



Rates of suspected Microcephaly in Brazilian states by year Euro CDC



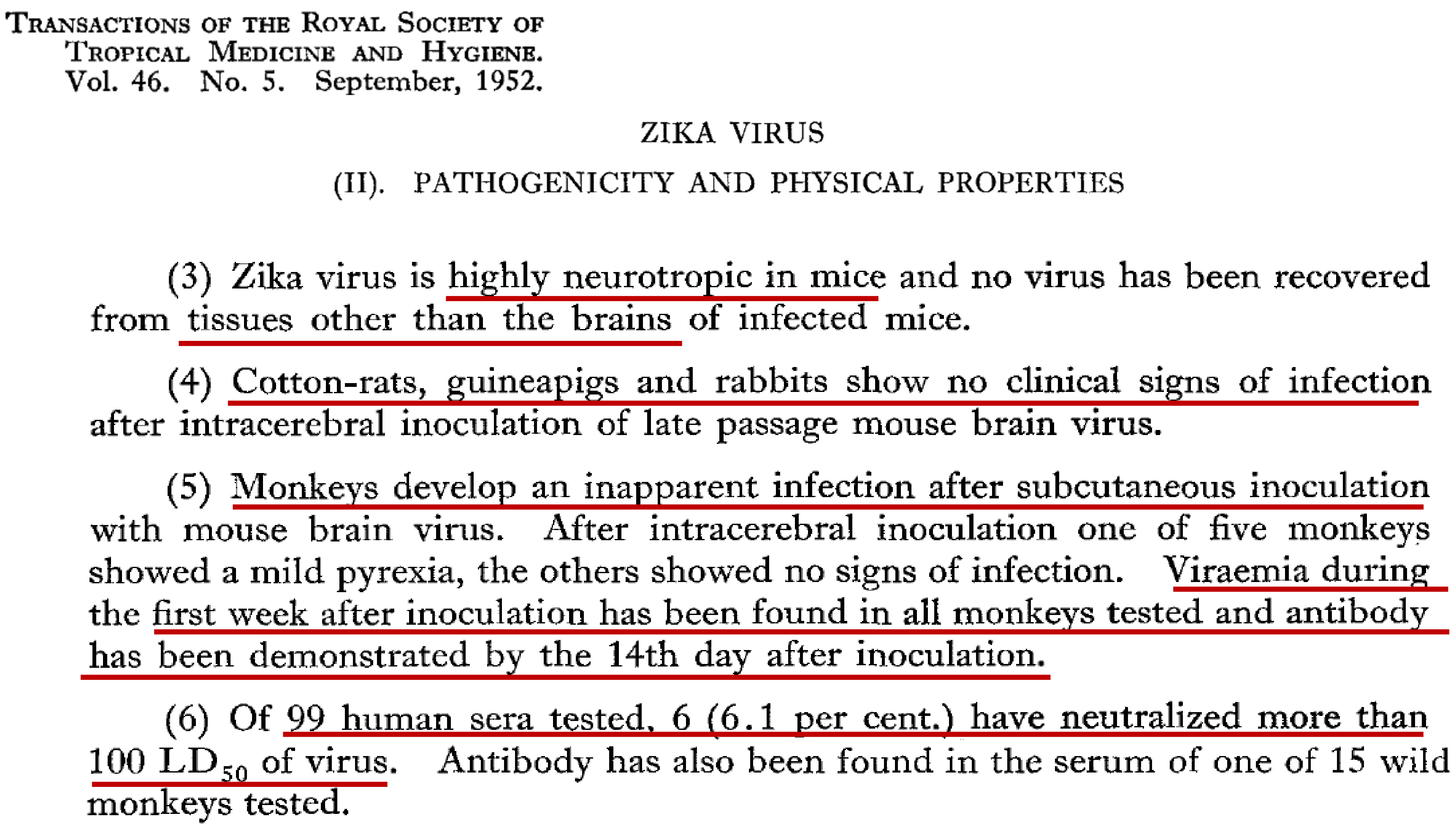
The NEW ENGLAND JOURNAL *of* MEDICINE

BRIEF REPORT

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).



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ZIKA VIRUS

(II). PATHOGENICITY AND PHYSICAL PROPERTIES

(3) Zika virus is highly neurotropic in mice and no virus has been recovered from tissues other than the brains of infected mice.

(4) Cotton-rats, guineapigs and rabbits show no clinical signs of infection after intracerebral inoculation of late passage mouse brain virus.

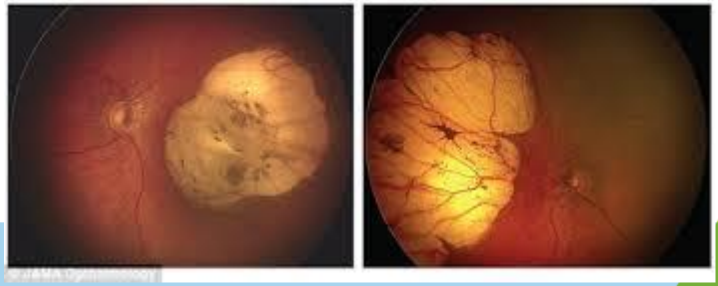
(5) Monkeys develop an inapparent infection after subcutaneous inoculation with mouse brain virus. After intracerebral inoculation one of five monkeys showed a mild pyrexia, the others showed no signs of infection. Viraemia during the first week after inoculation has been found in all monkeys tested and antibody has been demonstrated by the 14th day after inoculation.

(6) Of 99 human sera tested, 6 (6.1 per cent.) have neutralized more than 100 LD_{50} of virus. Antibody has also been found in the serum of one of 15 wild monkeys tested.

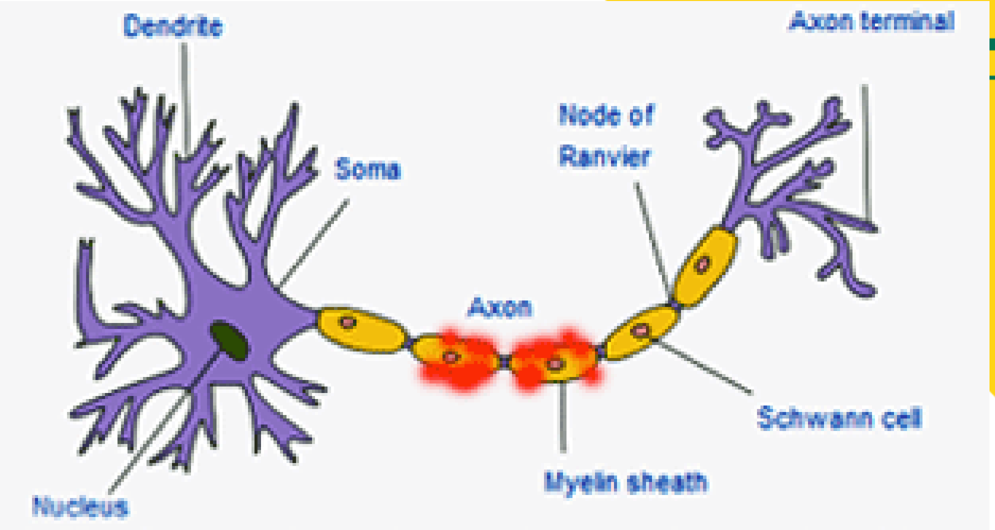
Congenital ZIKV Infection
….not just microcephaly

- Microcephaly
- Brain atrophy
- Ventricular enlargement
- Intracranial calcifications
- Ocular defects
- Joint contractures

- Absence of the corpus callosum
- Agenesis of the vermis
- Thalamus absent
- Cataracts
- Hydrops fetalis


© JAMA Ophthalmology

Guillain–Barré Syndrome (GBS)



Guillian-Barre syndrome destroys the protective covering of the peripheral nerves (myelin sheath), preventing nerves from transmitting signals to the brain.

Acute, immune mediated

May lead to paralysis

Roughly 25, 000 cases/yr in the US

Preceding infection in previous weeks

10-30% will require mechanical ventilation

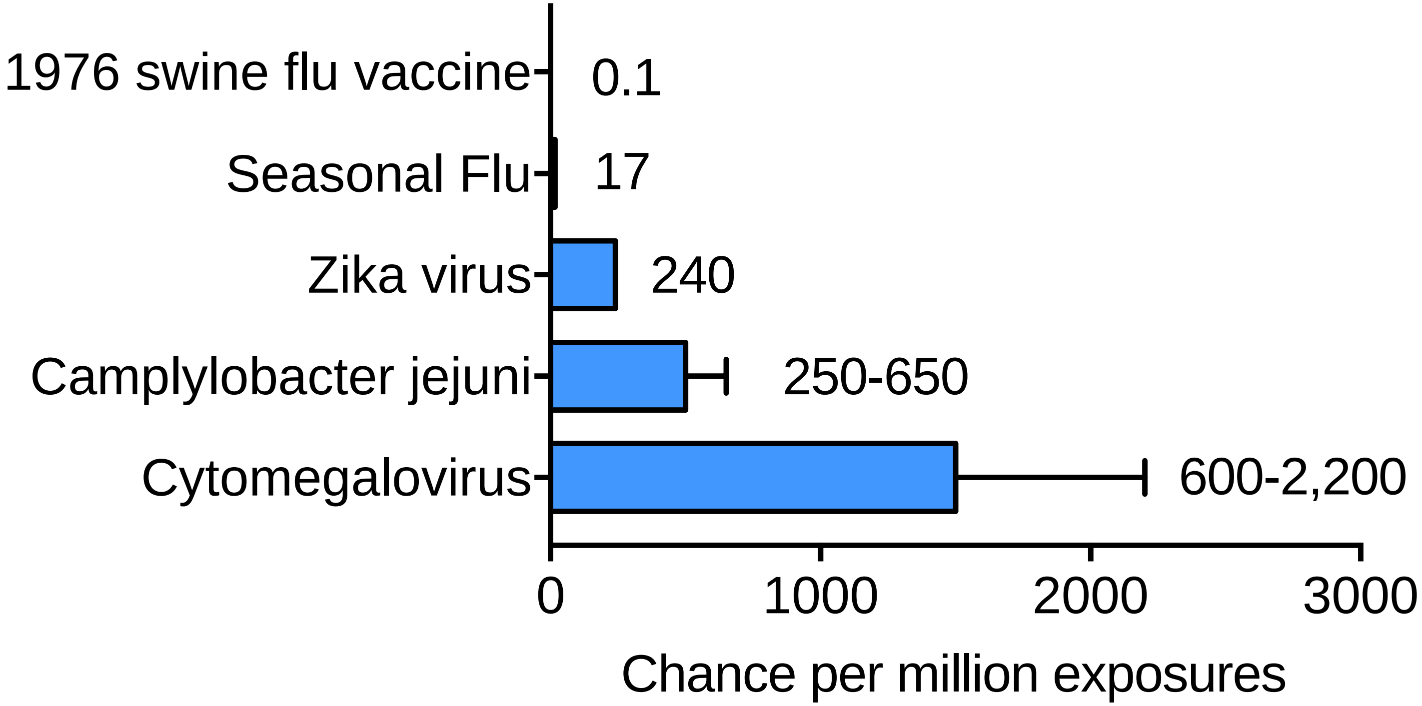
Most fully recover

Mortality rate 5%

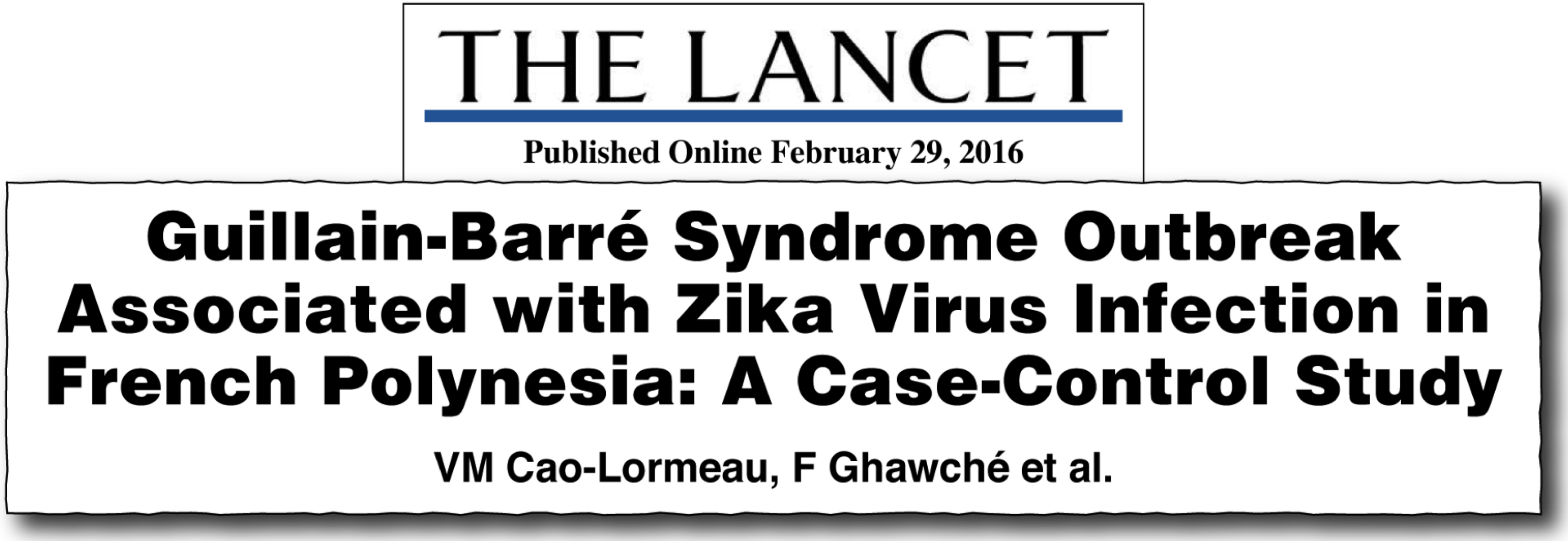
Risk of GBS and Infectious Disease

Guillain-Barré Syndrome

chance per million exposures



Source: CDC and Kwong JC et al. Risk of Guillain-Barre syndrome after seasonal influenza vaccination and influenza in health care encounters. *Lancet Infect. Dis.* (2013) 13(9):769-76



THE LANCET

Published Online February 29, 2016

Guillain-Barré Syndrome Outbreak Associated with Zika Virus Infection in French Polynesia: A Case-Control Study

VM Cao-Lormeau, F Ghawché et al.

Results:

- 98% of the 42 patients with GBS had anti-ZIKV IgM or IgG, and 100% had neutralizing antibody against ZIKV compared with 56% of 98 patients in control group ($p<0.0001$)
- 88% of 42 patients with GBS reported symptoms of ZIKV infection 6 days before onset of neurological symptoms.
- Estimated rate of GBS with ZIKV infection = 1/5000

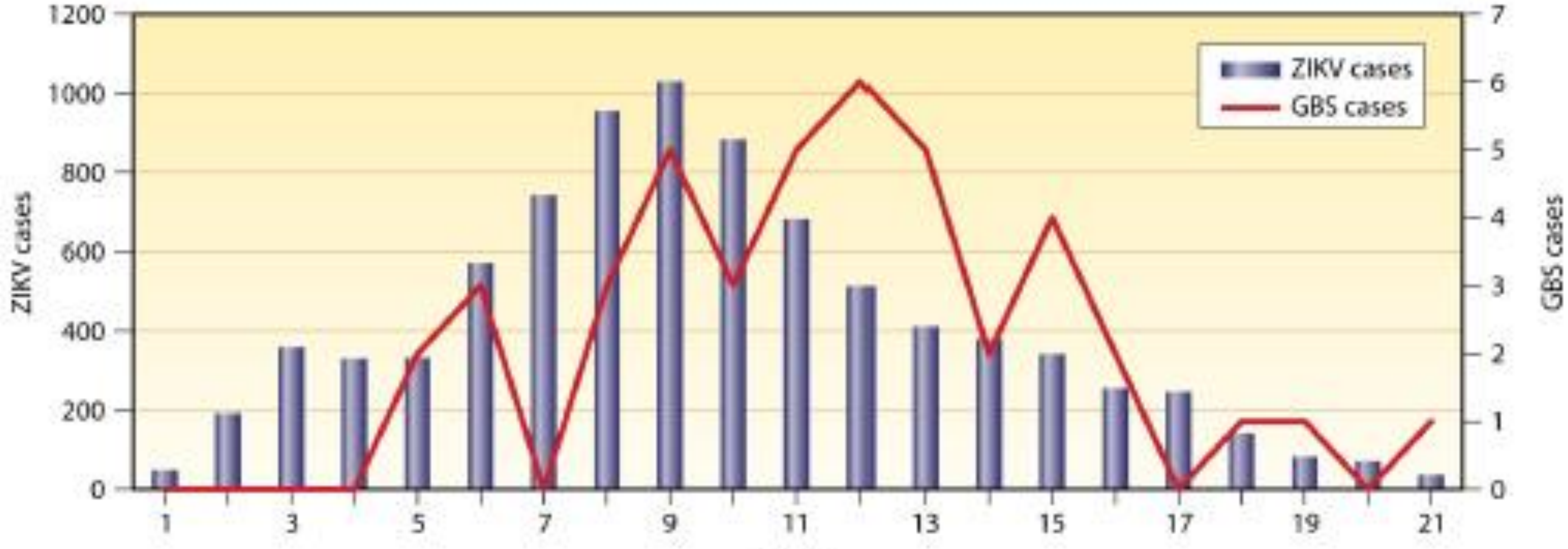
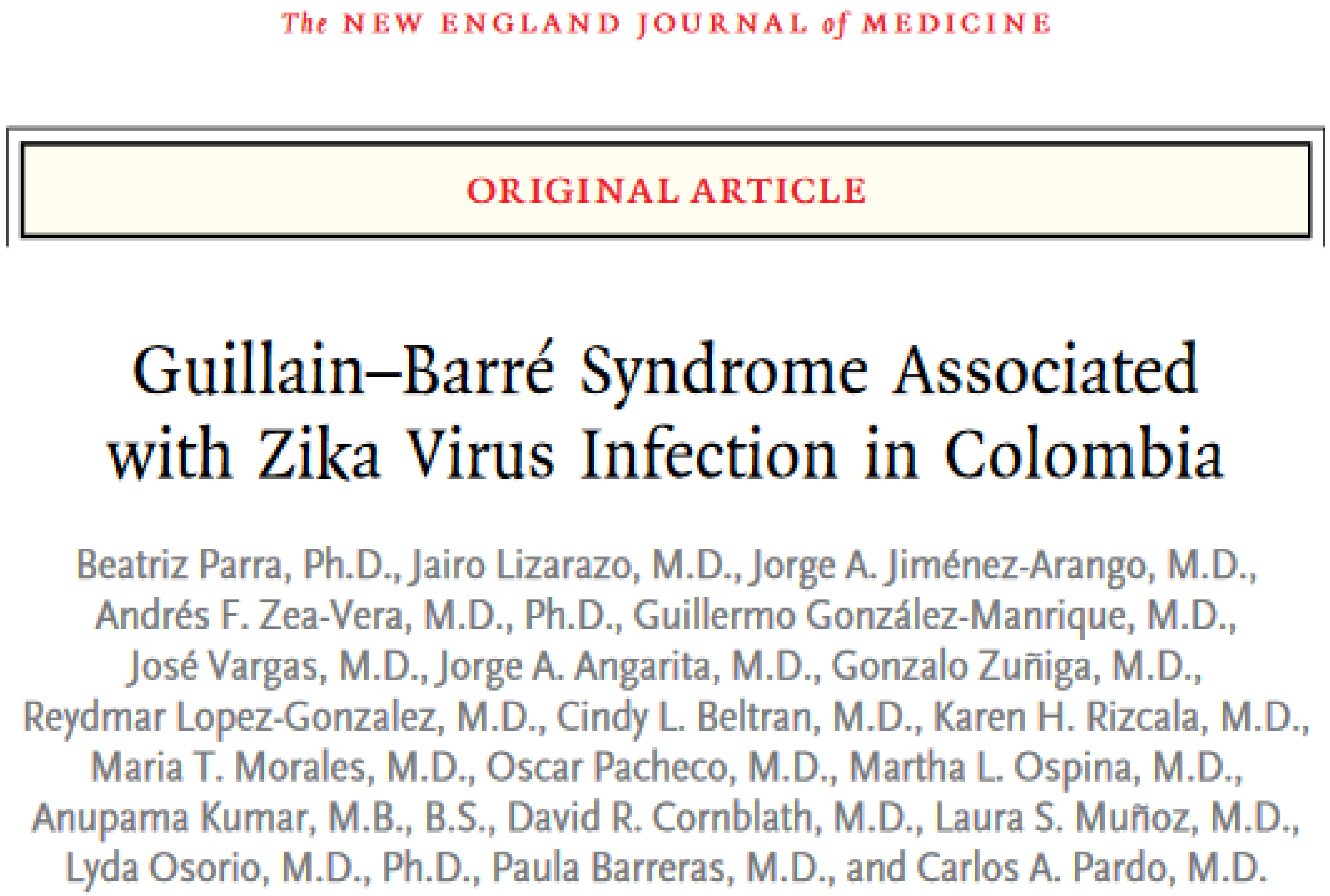


FIG 9 Temporal association between cases of Zika fever (blue columns) and GBS (red line) during the French Polynesian outbreak.

Clinical Microbiology Reviews. 2016



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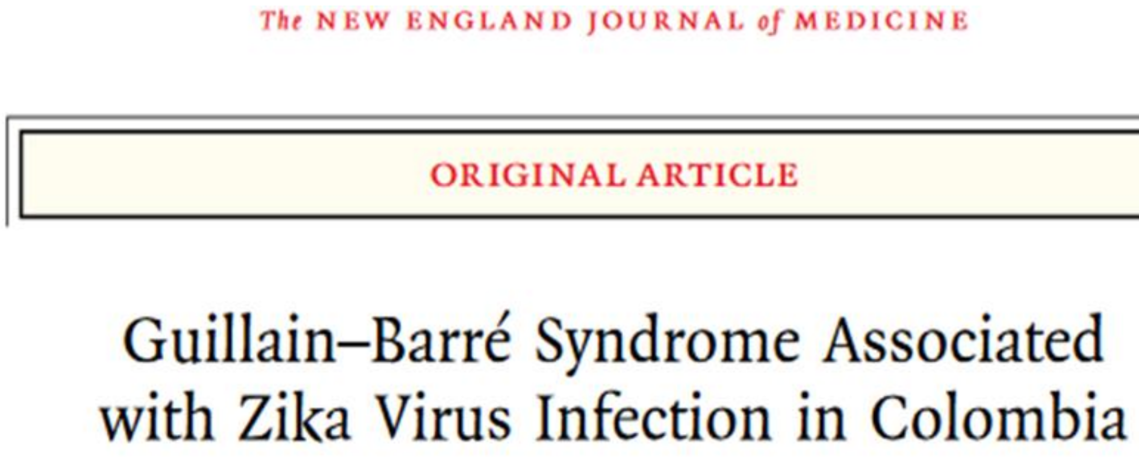
ORIGINAL ARTICLE

Guillain–Barré Syndrome Associated with Zika Virus Infection in Colombia

Beatriz Parra, Ph.D., Jairo Lizarazo, M.D., Jorge A. Jiménez-Arango, M.D., Andrés F. Zea-Vera, M.D., Ph.D., Guillermo González-Manrique, M.D., José Vargas, M.D., Jorge A. Angarita, M.D., Gonzalo Zuñiga, M.D., Reydmar Lopez-Gonzalez, M.D., Cindy L. Beltran, M.D., Karen H. Rizcala, M.D., Maria T. Morales, M.D., Oscar Pacheco, M.D., Martha L. Ospina, M.D., Anupama Kumar, M.B., B.S., David R. Cornblath, M.D., Laura S. Muñoz, M.D., Lyda Osorio, M.D., Ph.D., Paula Barreras, M.D., and Carlos A. Pardo, M.D.

NEJM. 2016



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Guillain–Barré Syndrome Associated with Zika Virus Infection in Colombia

68 patients with GBS

- 66 (97%) with previous symptoms c/w Zika
    - 42 patients with +PCR
        - Urine/CSF
- ?increased risk/link between Zika and GBS with previous Dengue infection

Detection of Zika Virus in Urine

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 21, No. 1, January 2015

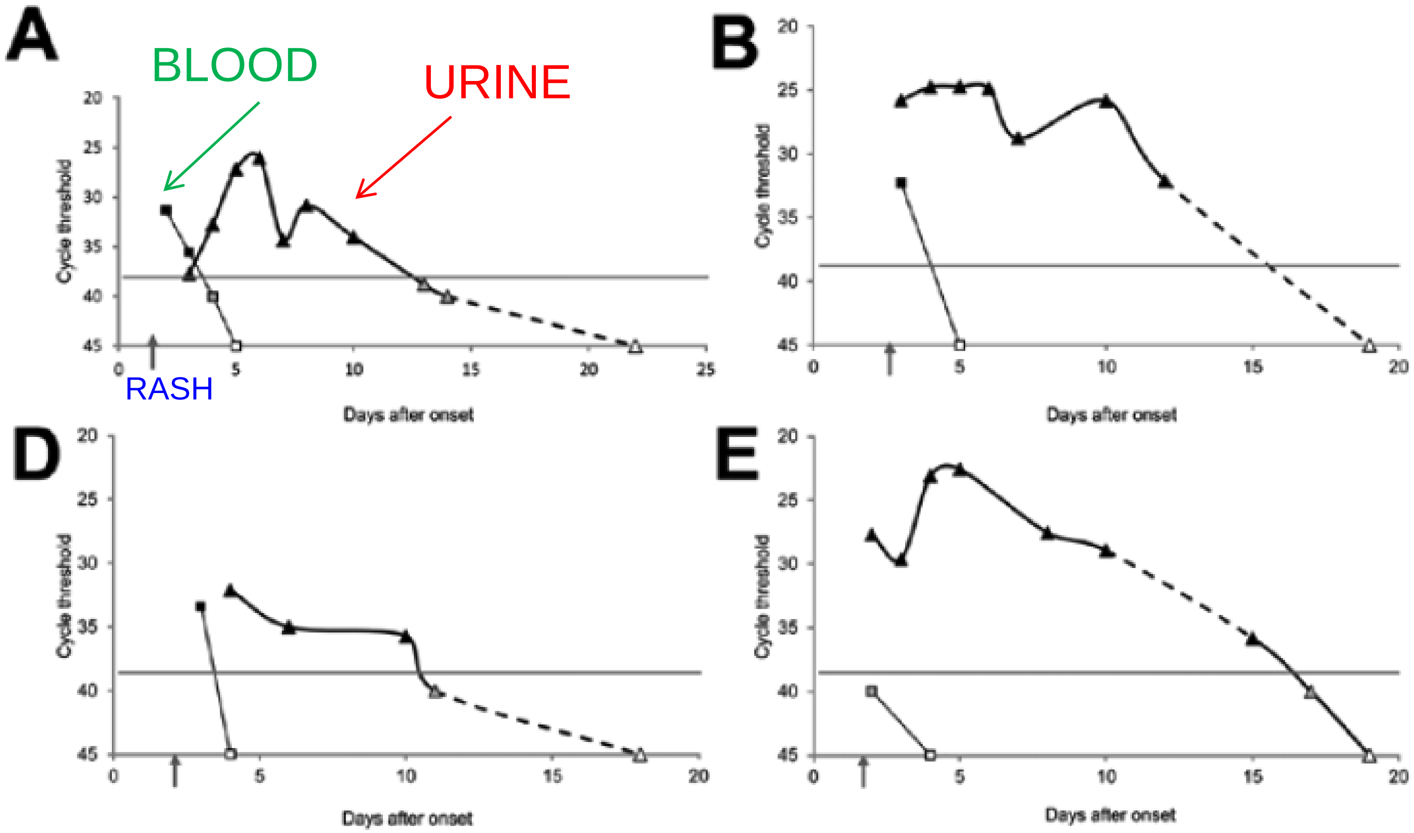
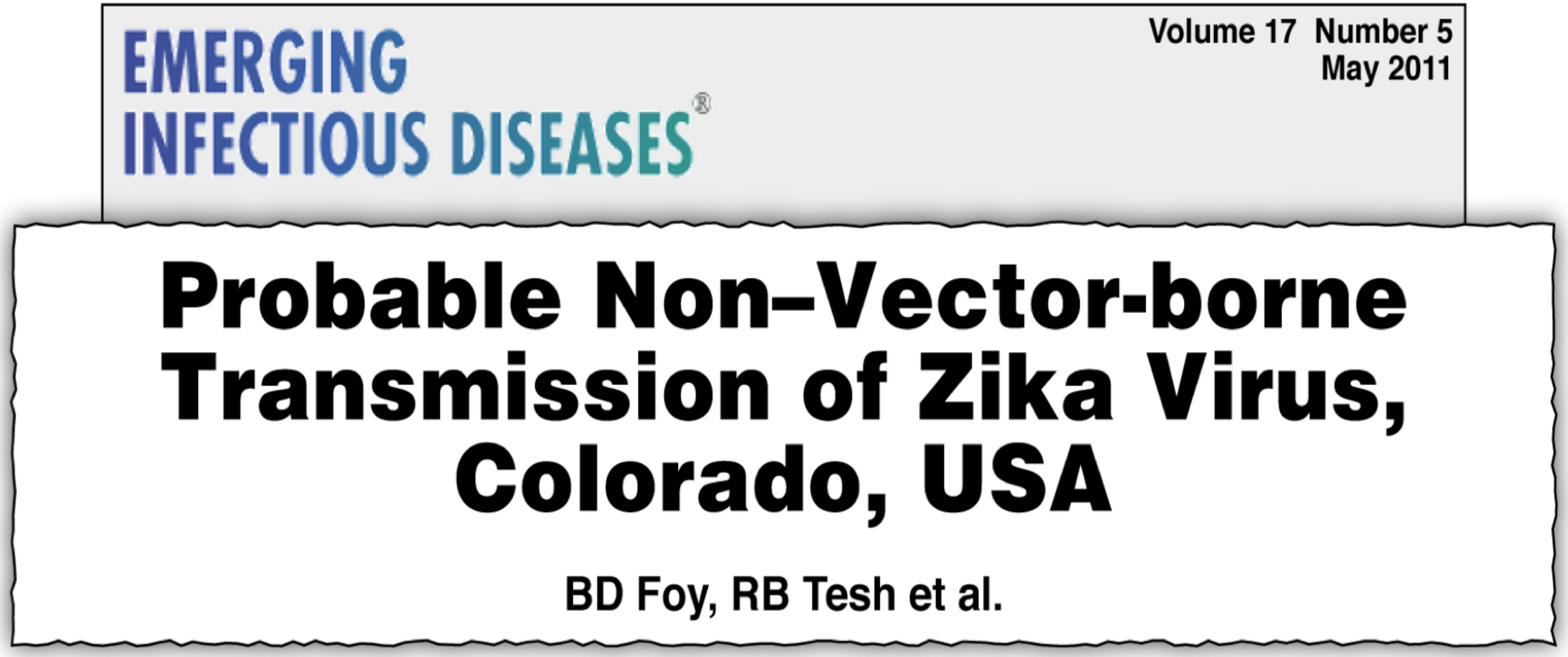


Figure. Detection of Zika virus in blood and urine specimens of 6 patients by using real-time reverse transcription PCR with primers/probe 1086/1162c/1107-Cy5 (*11*) New Caledonia, 2014. A) Patient 1; B) Patient 2; C) Patient 3; D) Patient 4; E) Patient 5; F) Patient 6. Triangles indicate urine samples and squares indicate serum samples. The cutoff cycle threshold (C_t) value is 38.5, as previously reported (*11*) and is indicated by horizontal lines. Black symbols indicate amplifications with C_t<38.5, gray symbols indicate amplifications with C_t ≥38.5, and white symbols indicate negative amplifications. Onset of disease (day 0) was defined retrospectively after questioning patients about initial symptoms. Dashed lines indicate a period >2 days without a sample being obtained. Arrows indicate onset of rash.

First Reported Case of Sexually Transmitted Zika Virus



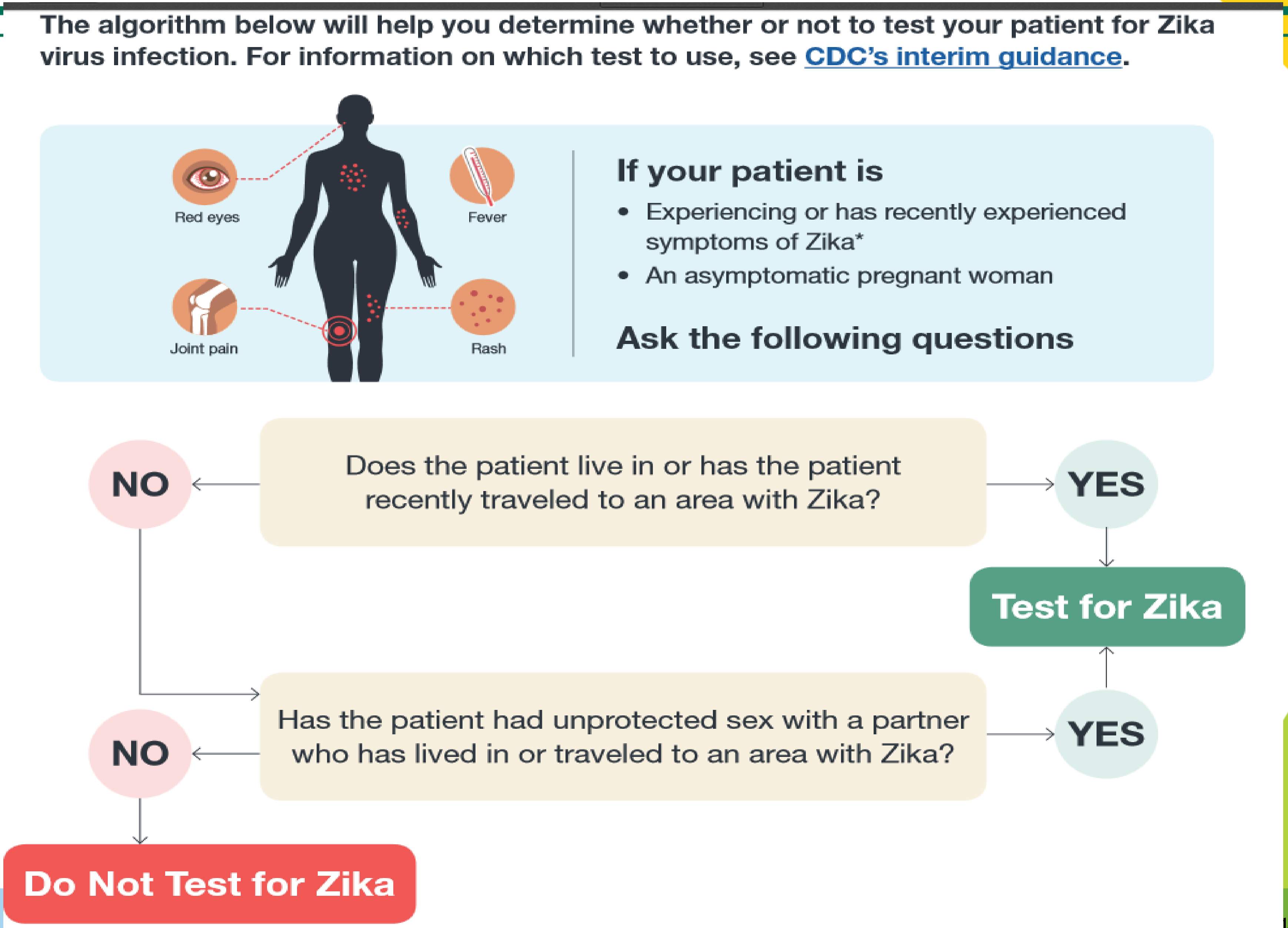
EMERGING INFECTIOUS DISEASES®

Volume 17 Number 5
May 2011

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

BD Foy, RB Tesh et al.

- American scientist contacted Zika virus infection in Senegal in 2008 and transmitted virus to his wife after his return home
- Sexual contact implicated as transmission route



The algorithm below will help you determine whether or not to test your patient for Zika virus infection. For information on which test to use, see CDC's interim guidance.

If your patient is

- Experiencing or has recently experienced symptoms of Zika*
- An asymptomatic pregnant woman

Ask the following questions

Does the patient live in or has the patient recently traveled to an area with Zika?

YES → **Test for Zika**

NO → Has the patient had unprotected sex with a partner who has lived in or traveled to an area with Zika?

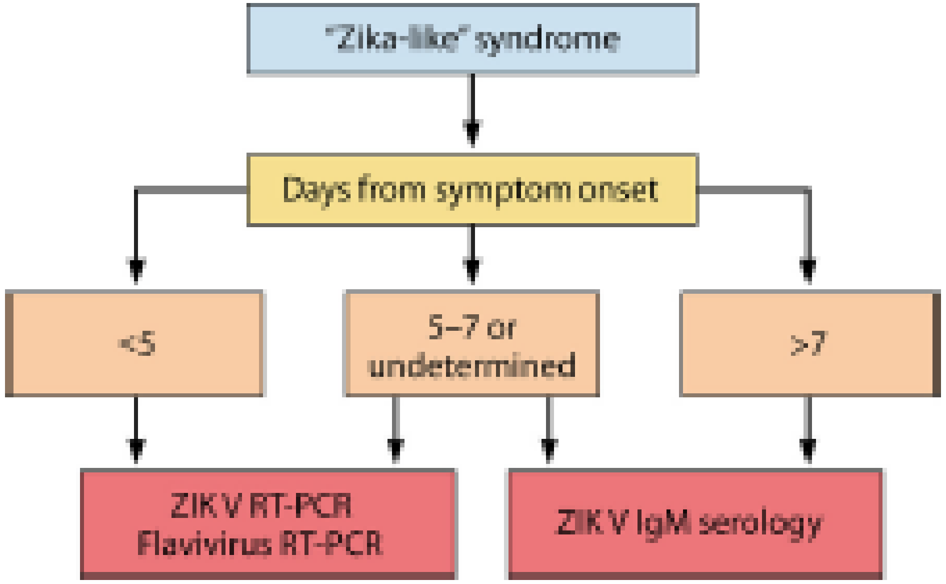
YES → **Test for Zika**

NO → **Do Not Test for Zika**

Asymptomatic Men, Children, Women who are not pregnant

Zika testing

- Serum
    - Antibody testing and PCR
        - IgM lasts up to 12 weeks (present by D4)
            - Cross-reactivity with other flaviviruses
- Urine
    - Submitted alongside serum samples
- CSF
- Amniotic fluid
- Tissue
- Saliva
    - Alternative if blood cannot be collected



"Zika-like" syndrome

Days from symptom onset

<5

5–7 or undetermined

>7

ZIKV RT-PCR
Flavivirus RT-PCR

ZIKV IgM serology

“Newer” recommendations

- Men
  - Wait 6 months from last possible exposure before trying to conceive with partner
- Women
  - Wait 8 weeks from last possible exposure before trying to conceive

- Pregnant women
  - PCR screening of blood and urine up to 14 days after last possible exposure
  - If evaluated 2-12 weeks after travel
    - IgM and PCR

CDC.gov. 2016

The Dengue Fever vaccine:
How it can help against Zika

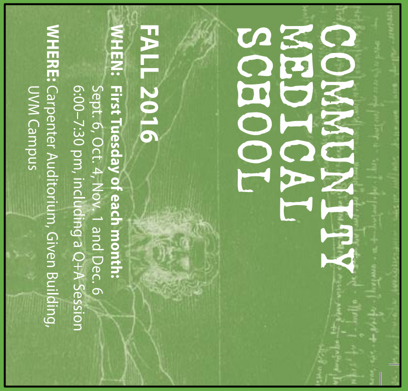
Sean Diehl, Ph.D.

Assistant Professor

Kristen Pierce, M.D.

Associate Professor

Medicine-Infectious Disease



COMMUNITY
MEDICAL
SCHOOL

FALL 2016

WHEN: First Tuesday of each month:
Sept. 6, Oct. 4, Nov. 1 and Dec. 6
6:00–7:30 pm, including a Q+A Session

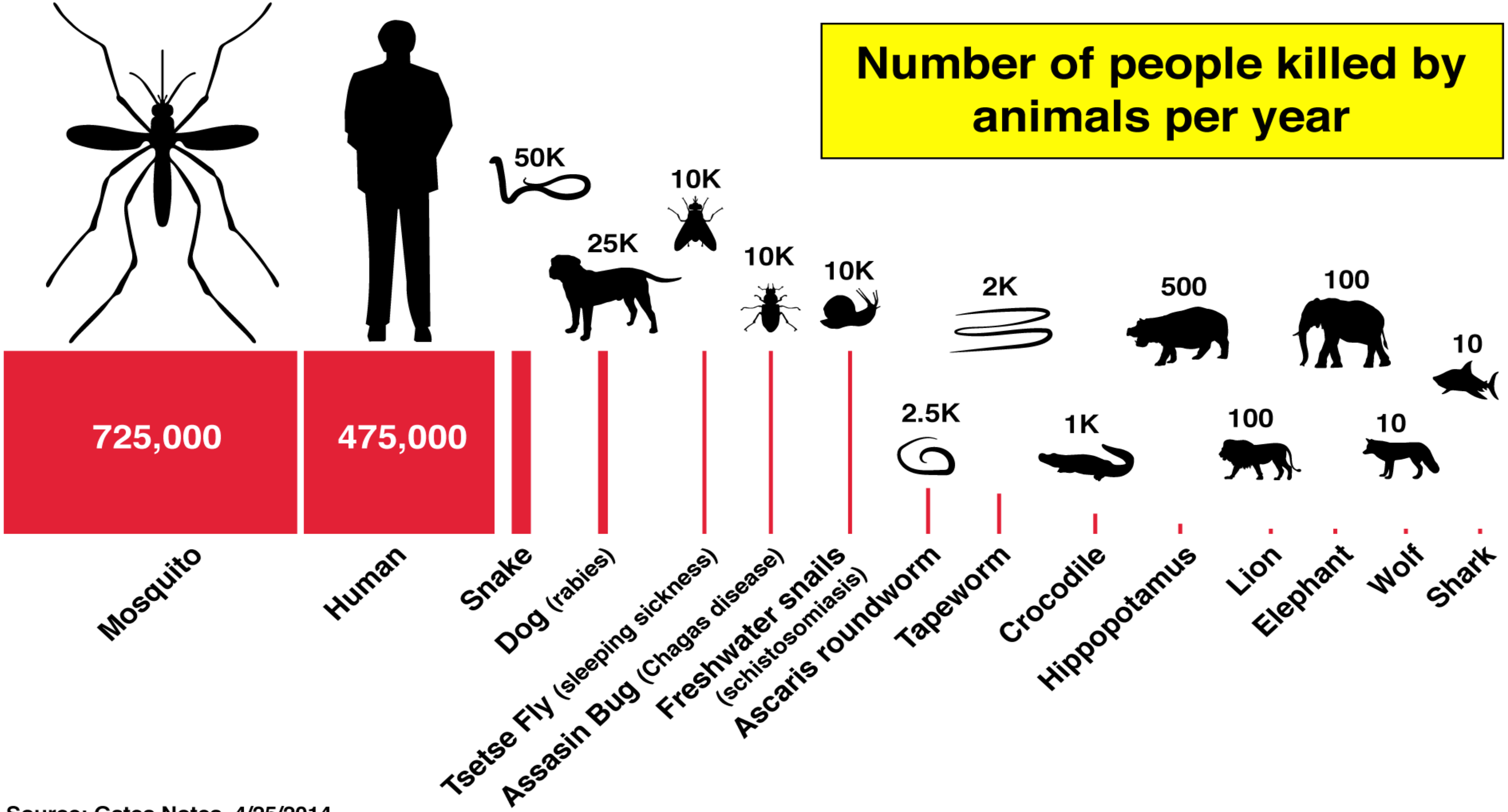
WHERE: Carpenter Auditorium, Given Building,
UVM Campus

The Robert Larner, M.D.
College of Medicine
THE UNIVERSITY OF VERMONT

THE
University of Vermont
MEDICAL CENTER

The University of Vermont

The world’s most dangerous animals



Source: Gates Notes, 4/25/2014

AS Fauci/NIAID

How Vaccines Work

General Rule: The more similar a vaccine is to the disease-causing form of the organism, the better the immune response to the vaccine

Introduce the immune system to a pathogen in a "controlled" environment

Cause the immune system to remember the pathogen and to respond to it

Enable the immune system to effectively clear the pathogen to prevent disease

Arms of the Immune system

Front line defense

Innate immune cells

Dispatchers/coordinators

Antigen-presenting cells

Decision makers/archivists

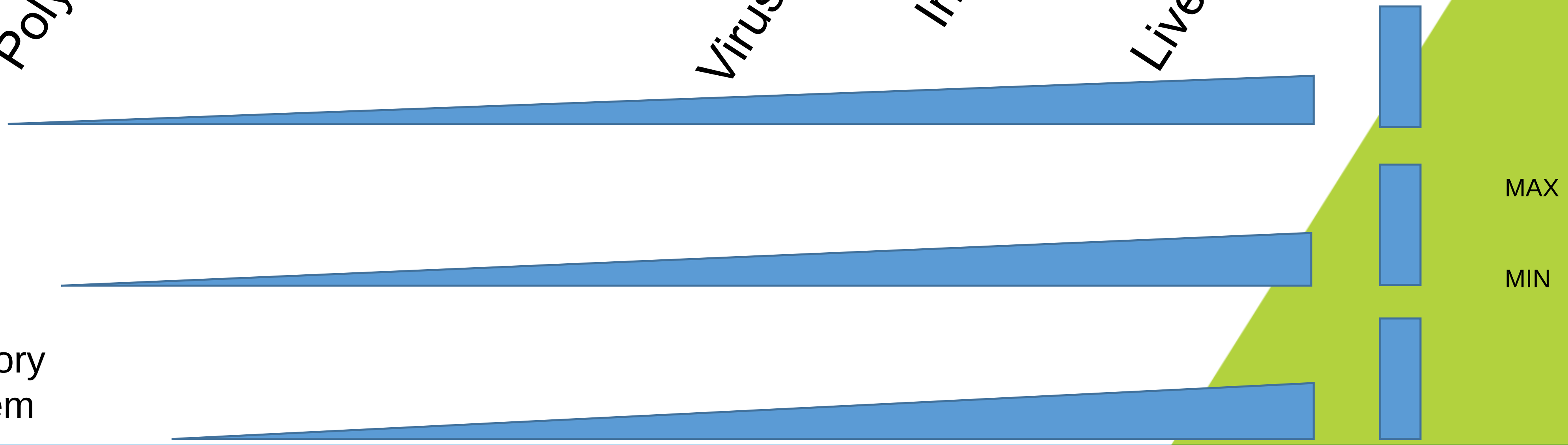
T cells – Generals / commanders / admirals

B cells – fighter pilots
- make antibodies = missles/bombs

Vaccines engage the immune system like an emergency preparedness drill to be ready for the real threat.

Vaccine Types

Example	Typhoid	Tetanus, HepB Pneumococcal	Ebola, Zika (experimental)	HPV	FLU shot	MMR, Flumist YFV, JEV, Dengue, (Zika)	
Type	Polysaccharide	Subunit	DNA	Virus-like particle	Inactivated	Live attenuated	Pathogen



Reactogenicity

Pathogen - likeness

Ability to induce memory
engage immune system

Why develop a live attenuated vaccine?

Live attenuated vaccines have been successful for other flaviviruses: yellow fever and Japanese encephalitis virus

Highly immunogenic, requiring only one dose

Expected to induce lifelong immunity

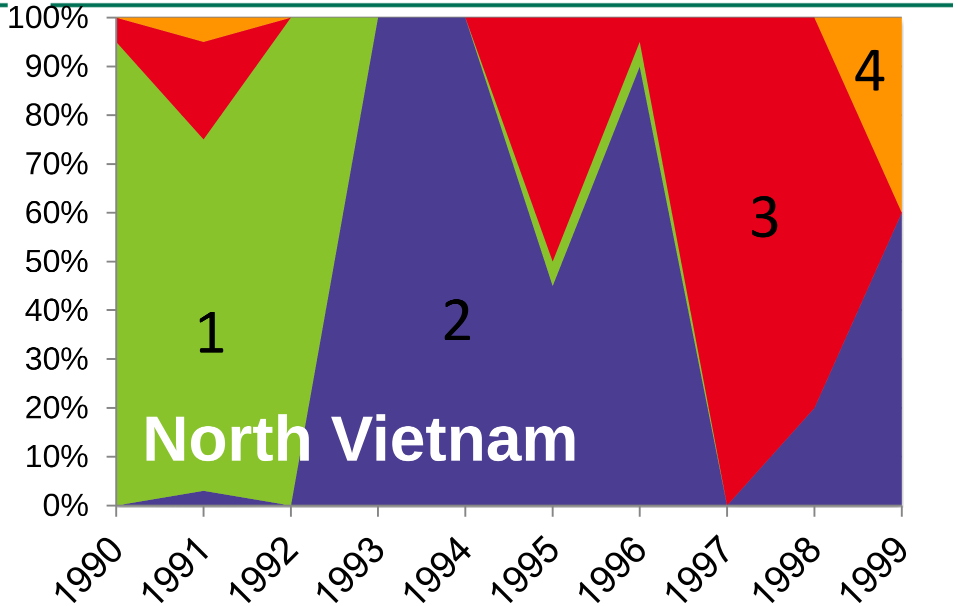
Can be very economical to produce and can be manufactured locally in endemic countries

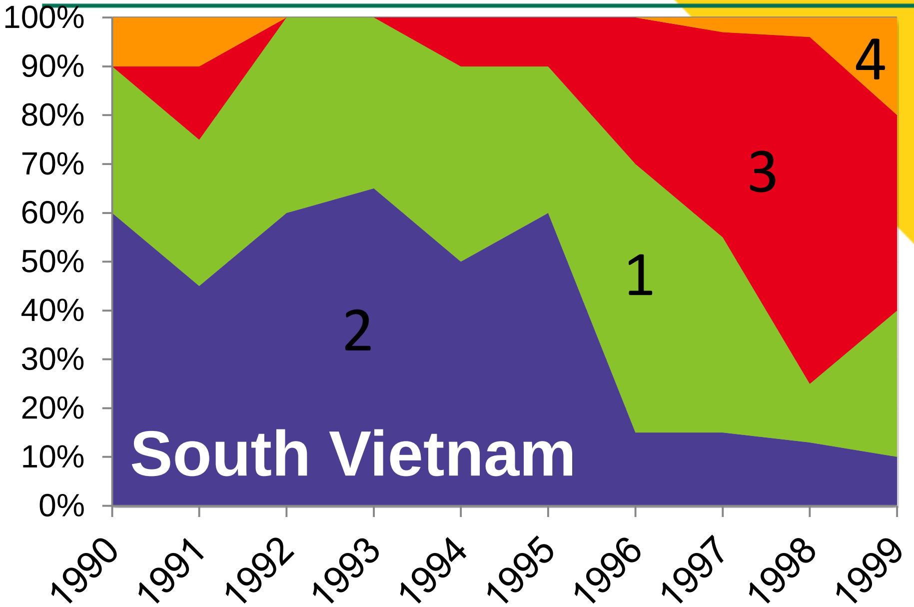
Induces both humoral and cellular immune responses

Challenges to dengue vaccine development

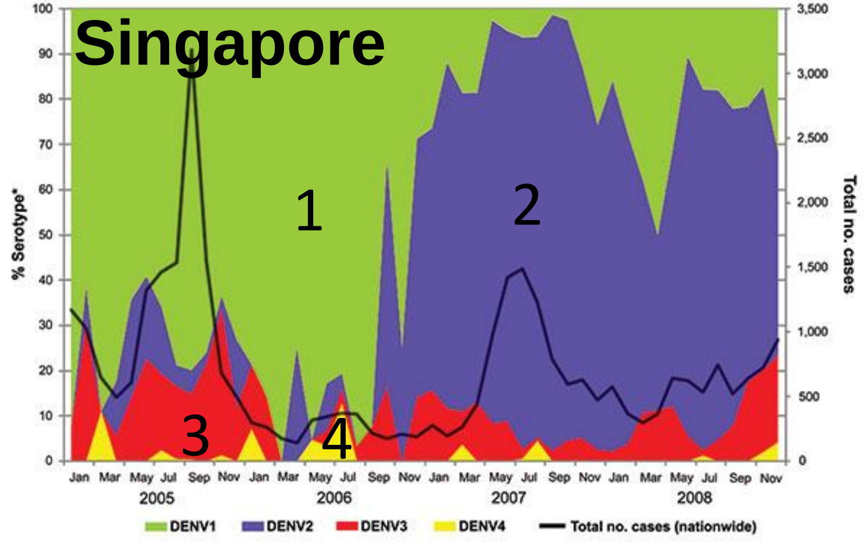
- Four serotypes cause disease
- Cannot predict circulation patterns
- Usually the 2nd DIFFERENT dengue serotype is the culprit
- Need to avoid interference between viruses in vaccine
- Attenuation in a specific way
- Engaging the whole immune system (not just antibodies)

Dengue circulation patterns are unpredictable



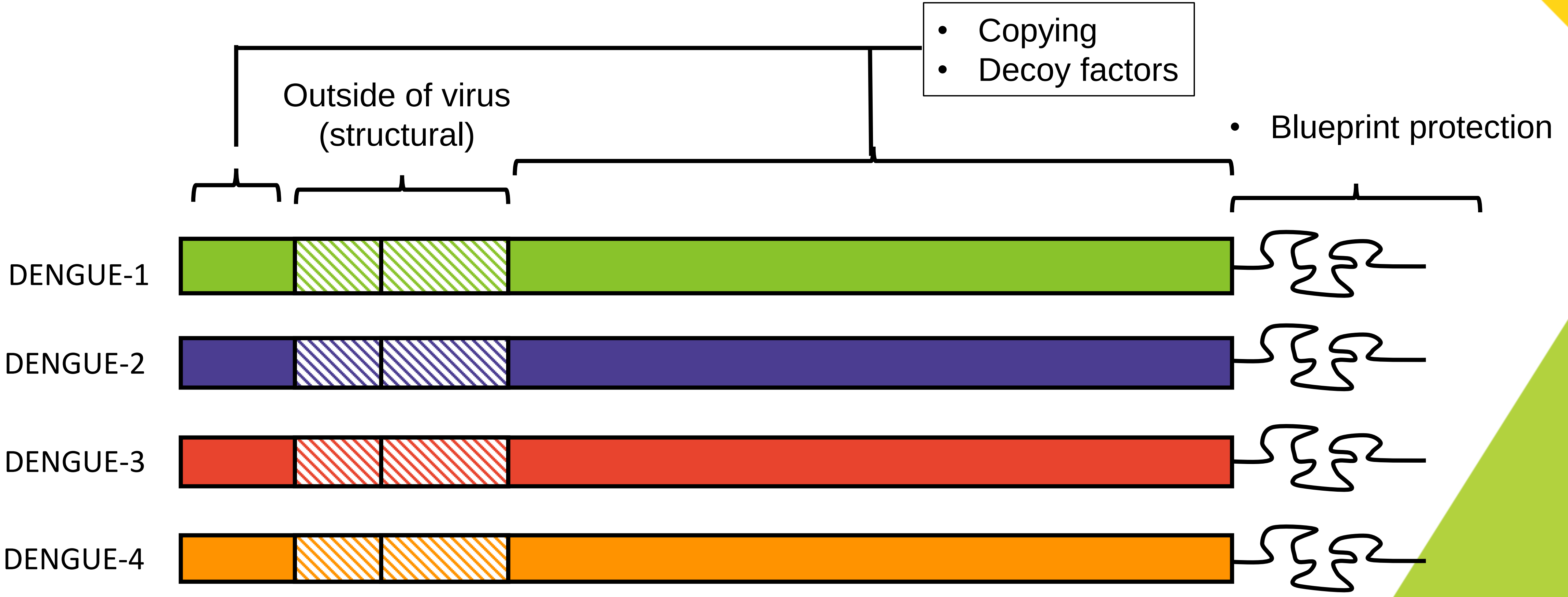


Nguyen Thi Kim Tien, etal., 2007 Dengue Bull 23: 34-39.



Lee K-S, Lai Y-L, et al. Emerg Infect Dis. 2010 May http://wwwnc.cdc.gov/eid/article/16/5/09-1006.htm

Dengue is caused by any of four distinct dengue viruses



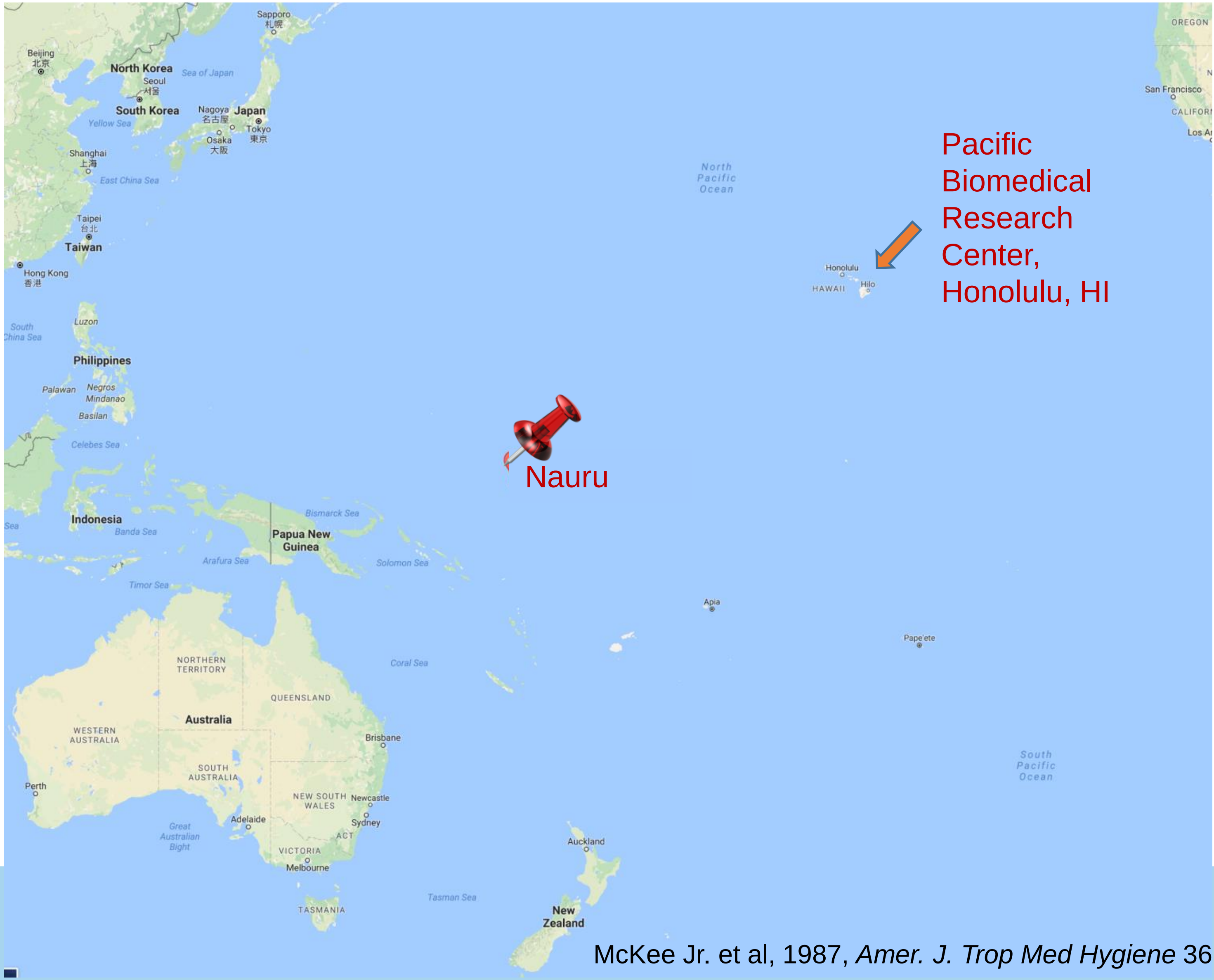
- Copying
- Decoy factors

Outside of virus (structural)

- Blueprint protection

Where do these vaccine candidates come from?

DENGUE 1



Dengue 1 Western Pacific.

1974

From the serum of a Chinese traveler to Nauru, reporting mild dengue at the Pacific Biomedical Research Center in Honolulu, Hawaii

McKee Jr. et al, 1987, *Amer. J. Trop Med Hygiene* 36(2): 435-442

DENGUE 2

New Guinea C[1]:

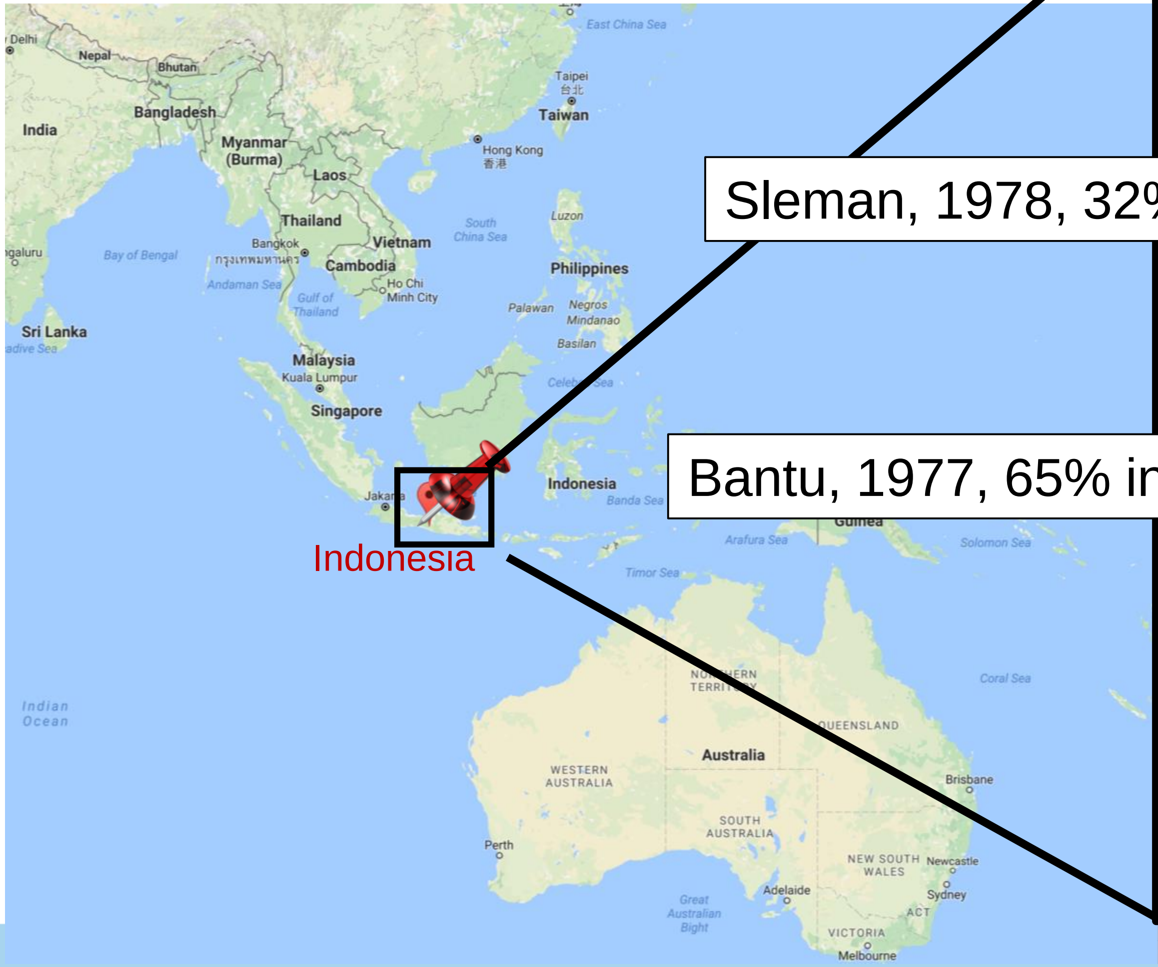
- 1944: Mild diseease

Tonga[2]:

- 1974: outbreak of mild disease, 17% infection rate.
- 1975: severe disease, high attack rate.

1. Sabin, A.B., (1950) *Bacteriol. Rev* 14: 225
2. Gubler DJ, et al. (1978) Am. J Trop Med Hyg 27(3) 581-589

DENGUE 3



Indonesia

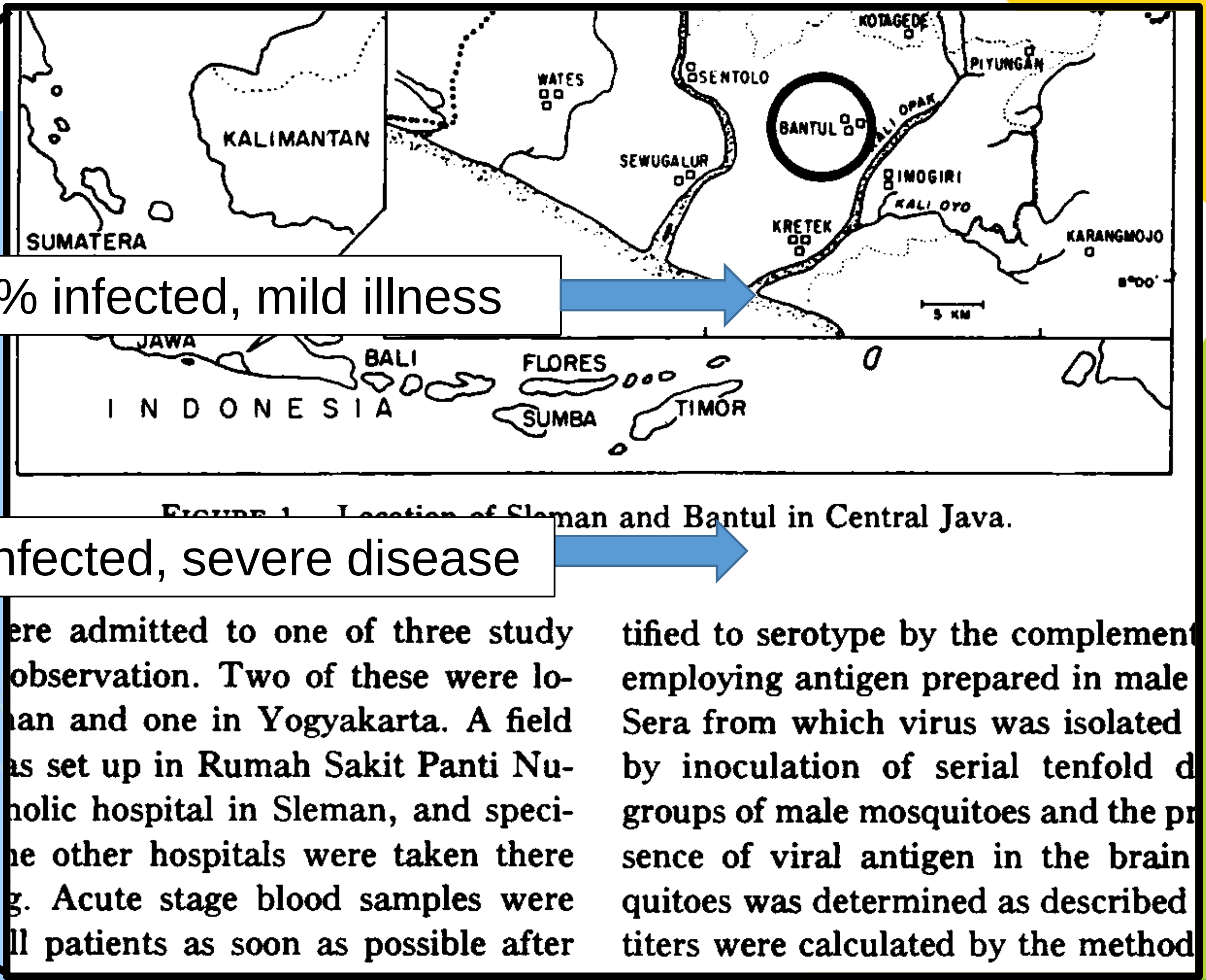


FIGURE 1. Location of Sleman and Bantul in Central Java.

ere admitted to one of three study observation. Two of these were lo- an and one in Yogyakarta. A field as set up in Rumah Sakit Panti Nu- holic hospital in Sleman, and speci- he other hospitals were taken there g. Acute stage blood samples were ll patients as soon as possible after

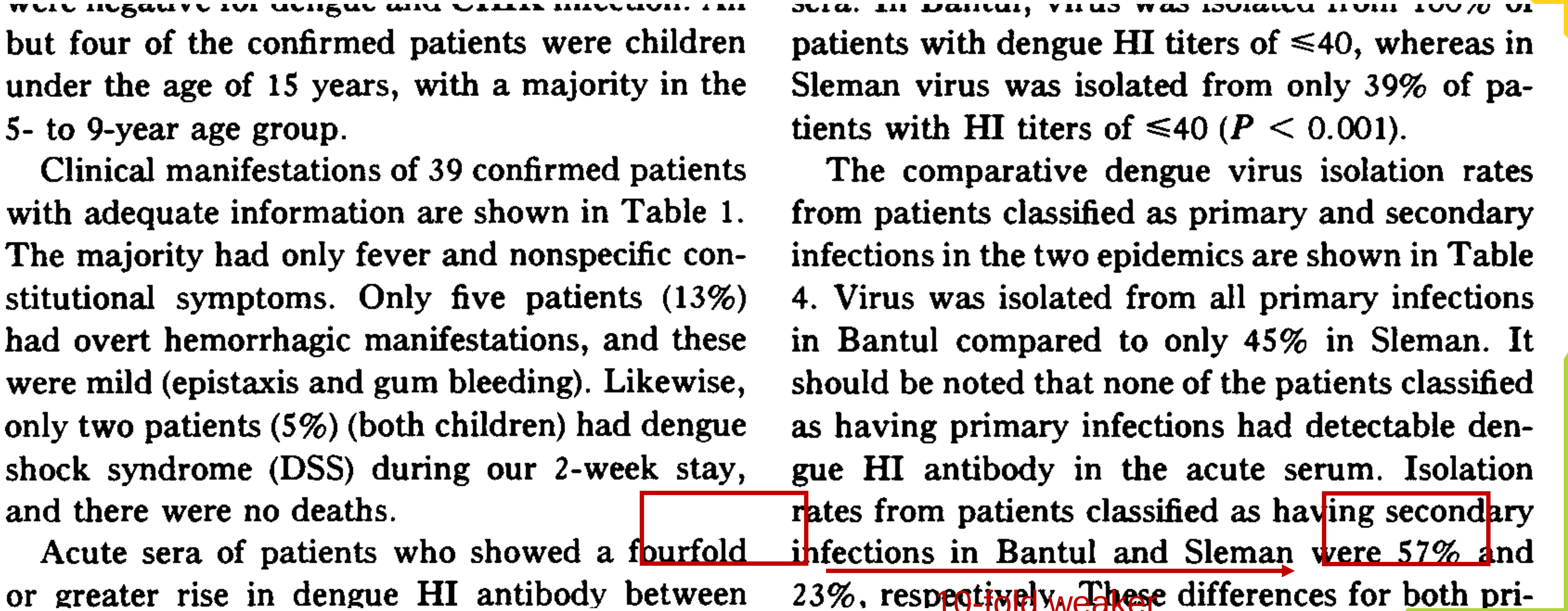
tified to serotype by the complement employing antigen prepared in male Sera from which virus was isolated by inoculation of serial tenfold d groups of male mosquitoes and the pr sence of viral antigen in the brain quitoes was determined as described titers were calculated by the method

Sleman, 1978, 32% infected, mild illness

Bantu, 1977, 65% infected, severe disease

1. Gubler DJ. et al, 1979, *Amer. J. Trop Med Hygiene* 28: 701- 710
2. Gubler DJ. et al, 1981, *Amer. J. Trop Med Hygiene* 30(5): 1094-1099

DENGUE 3



were negative for dengue and CHIK infection. All but four of the confirmed patients were children under the age of 15 years, with a majority in the 5- to 9-year age group.

Clinical manifestations of 39 confirmed patients with adequate information are shown in Table 1. The majority had only fever and nonspecific constitutional symptoms. Only five patients (13%) had overt hemorrhagic manifestations, and these were mild (epistaxis and gum bleeding). Likewise, only two patients (5%) (both children) had dengue shock syndrome (DSS) during our 2-week stay, and there were no deaths.

Acute sera of patients who showed a fourfold or greater rise in dengue HI antibody between

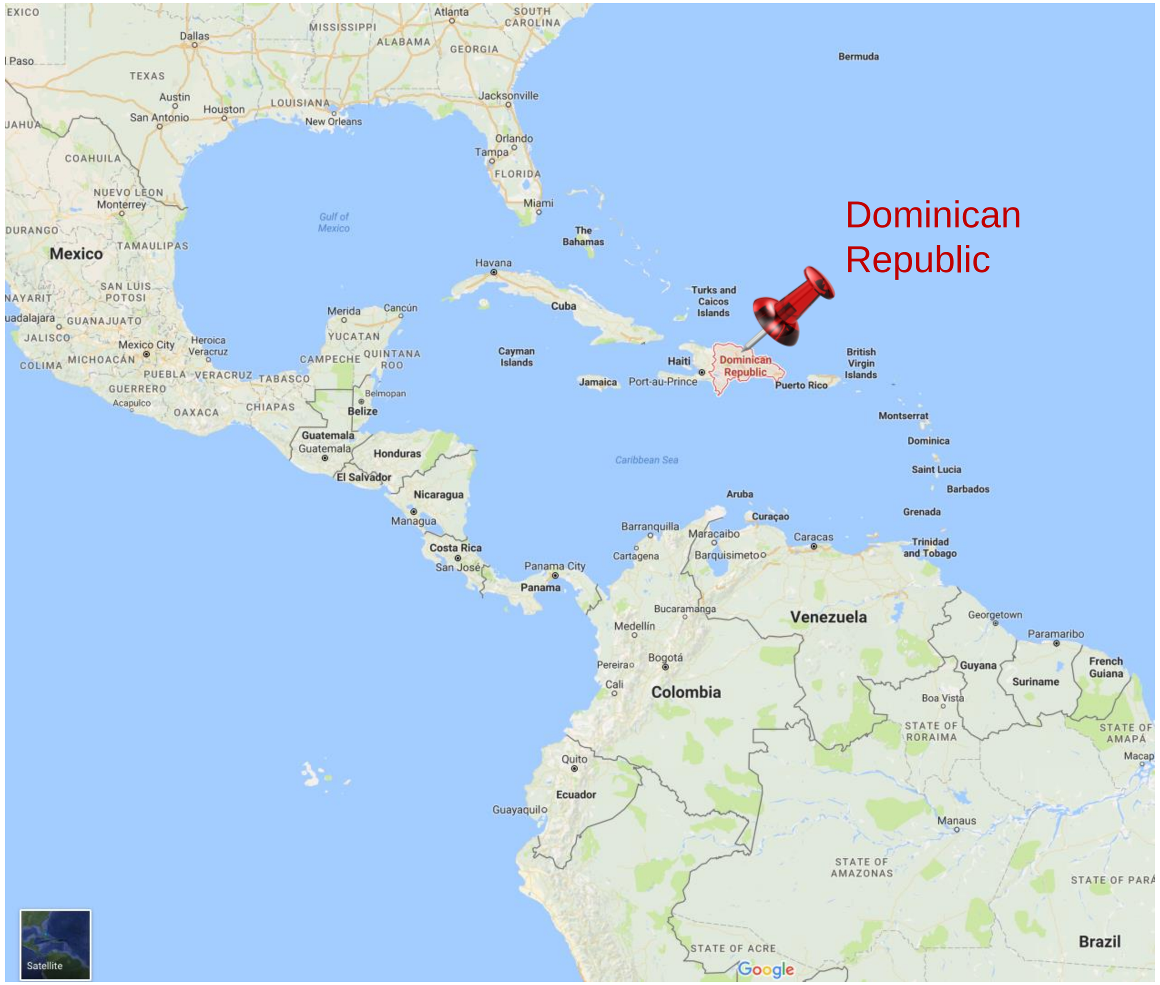
sera. In Bantul, virus was isolated from 100% of patients with dengue HI titers of ≤40, whereas in Sleman virus was isolated from only 39% of patients with HI titers of ≤40 ($P < 0.001$).

The comparative dengue virus isolation rates from patients classified as primary and secondary infections in the two epidemics are shown in Table 4. Virus was isolated from all primary infections in Bantul compared to only 45% in Sleman. It should be noted that none of the patients classified as having primary infections had detectable dengue HI antibody in the acute serum. Isolation rates from patients classified as having secondary infections in Bantul and Sleman were 57% and 23%, respectively. These differences for both pri-

10-fold weaker

Perfect illustration of how to pick a vaccine virus (Sleman, not Bantu)

DENGUE 4



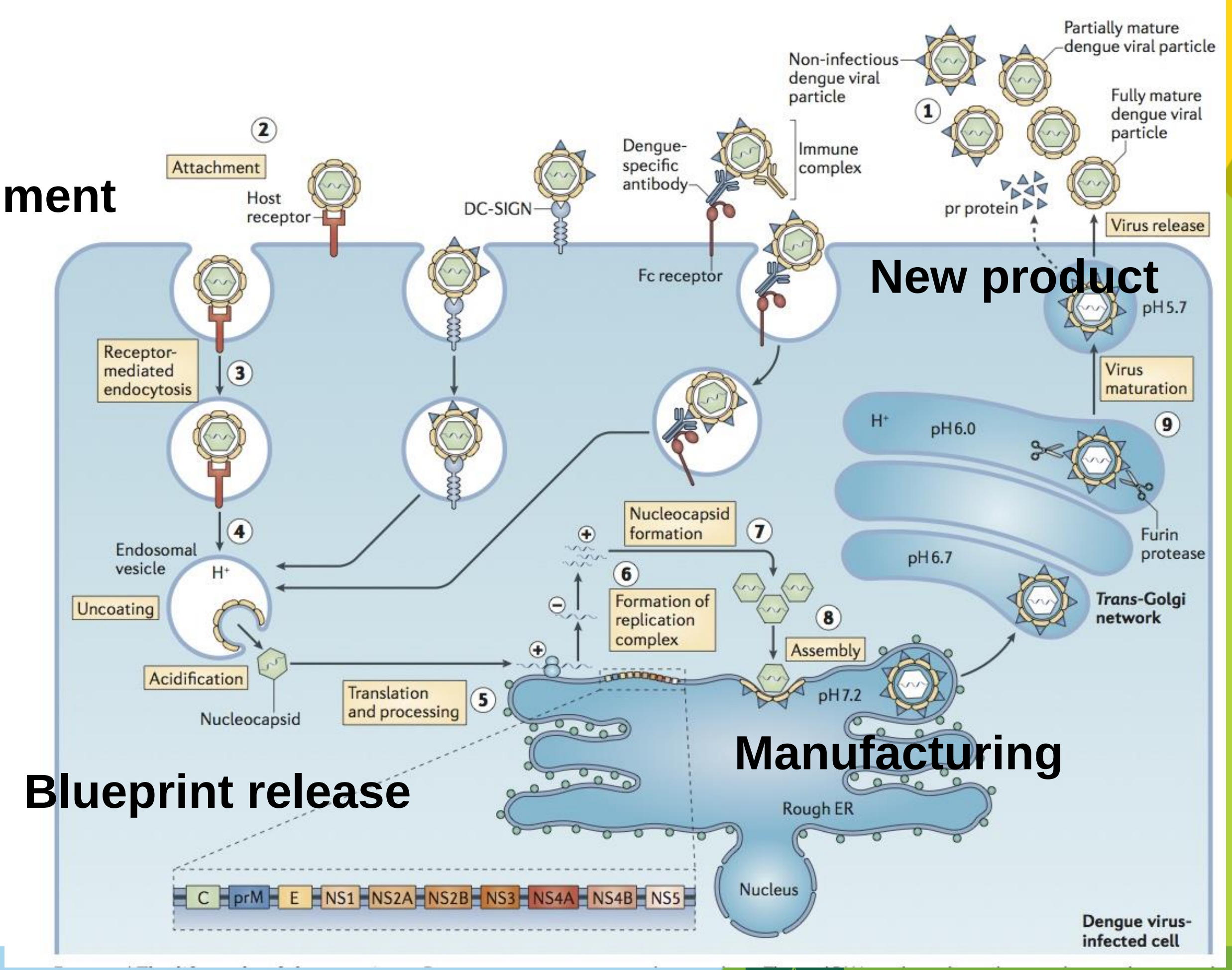
May 1981:
Generally mild disease.

Distinct from the concurrent 1981 Cuba outbreak, which led to severe disease – which was DENV2.

1. Henchal E.A. et al, (1986), *Amer. J. Trop Med Hygiene* 35(2): 393- 400

Dengue and Zika viruses carry their own blueprints and the host cell builds new viruses.

Attachment

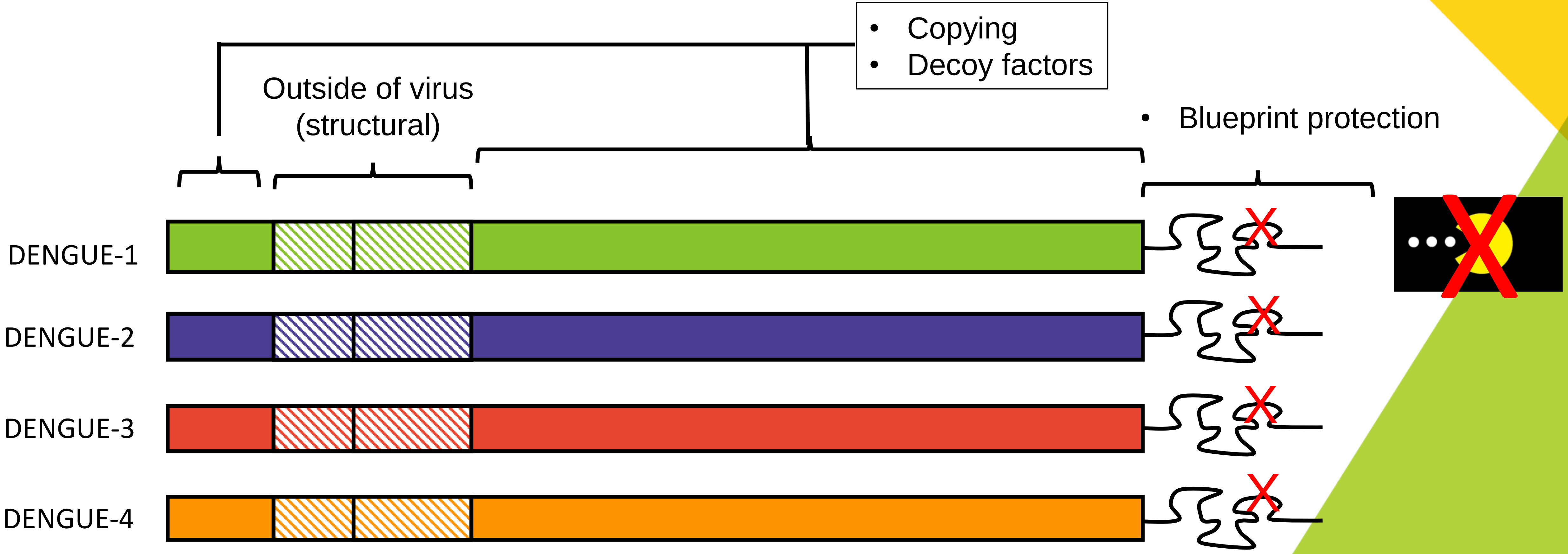


New product

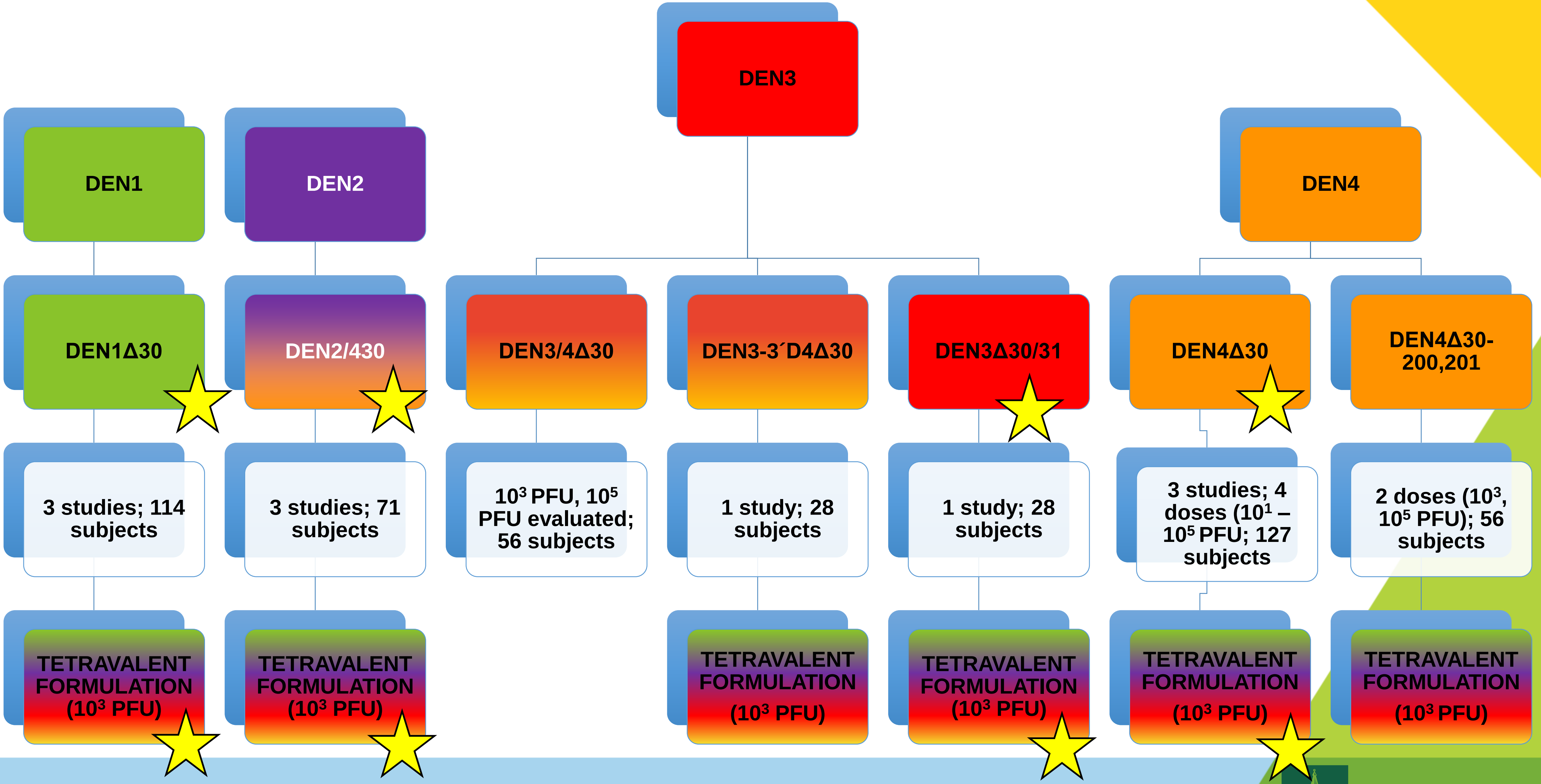
Manufacturing

Blueprint release

Live attenuation strategy: editing dengue genome



Testing individual dengue vaccine candidates to ensure a balanced immune response (antibody)



DEN3

DEN1

DEN2

DEN4

DEN1Δ30

DEN2/430

DEN3/4Δ30

DEN3-3´D4Δ30

DEN3Δ30/31

DEN4Δ30

DEN4Δ30-200,201

3 studies; 114 subjects

3 studies; 71 subjects

10^3 PFU, 10^5 PFU evaluated; 56 subjects

1 study; 28 subjects

1 study; 28 subjects

3 studies; 4 doses (10^1 – 10^5 PFU; 127 subjects

2 doses (10^3, 10^5 PFU); 56 subjects

TETRAVALENT FORMULATION (10^3 PFU)

TETRAVALENT FORMULATION (10^3 PFU)

TETRAVALENT FORMULATION (10^3 PFU)

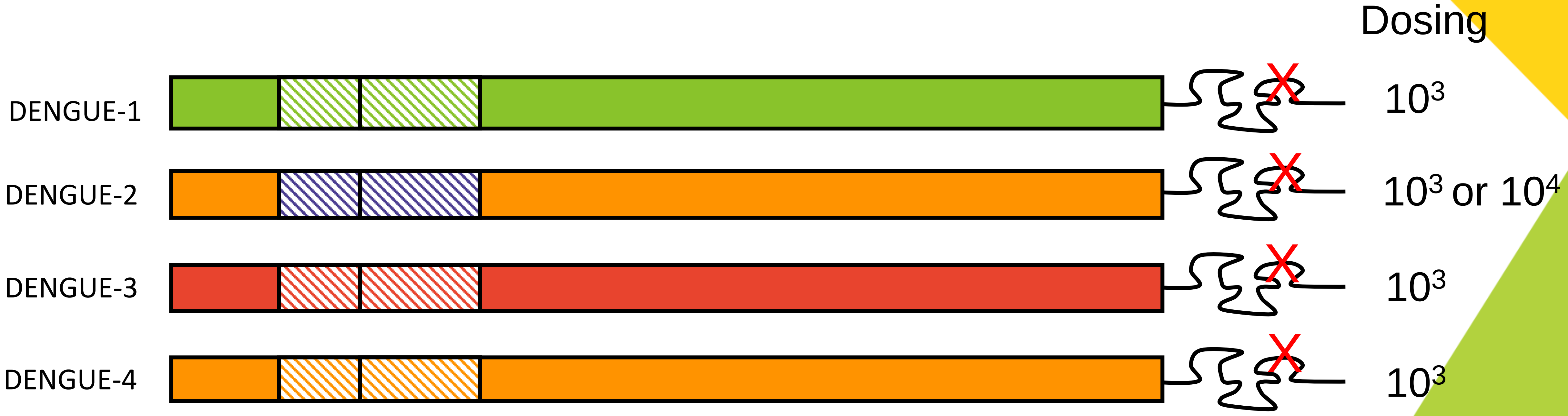
TETRAVALENT FORMULATION (10^3 PFU)

TETRAVALENT FORMULATION (10^3 PFU)

TETRAVALENT FORMULATION (10^3 PFU)

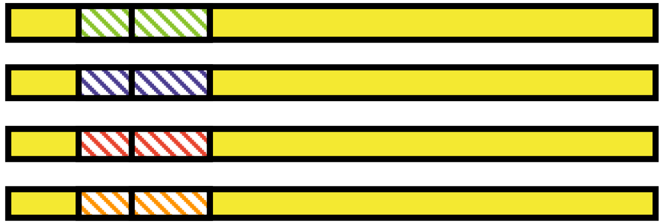
Over 400 subjects at UVM and Johns Hopkins participated in testing of safety of each component and as a mixture.

Final tetravalent vaccine formulation TV003



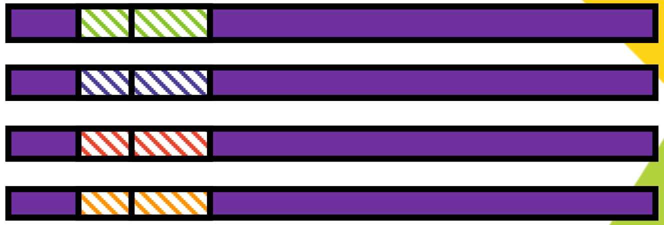
TV003 gives balanced immune response to all four DENV

Sanofi (CYD)



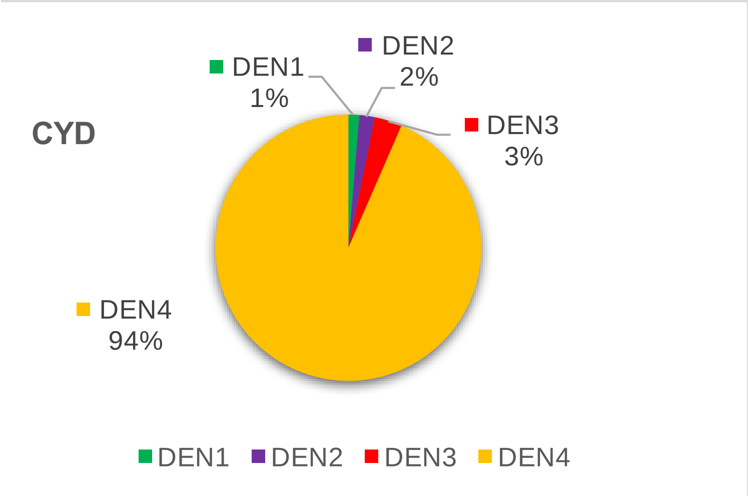
NIH (TV003)

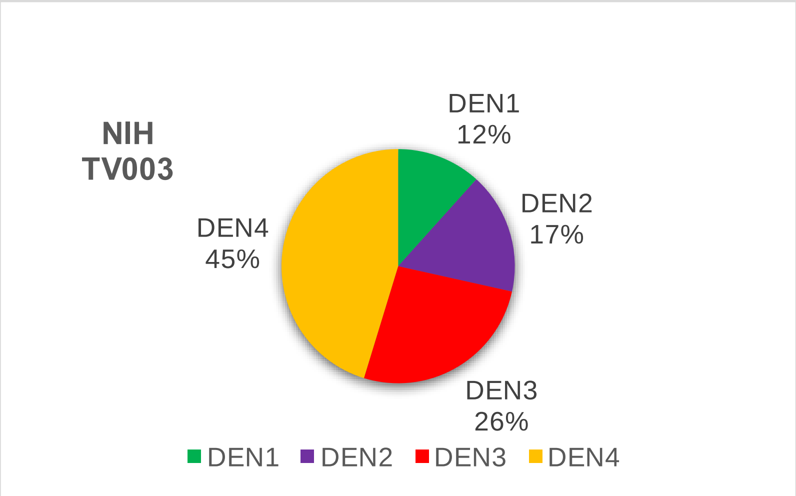
Takeda

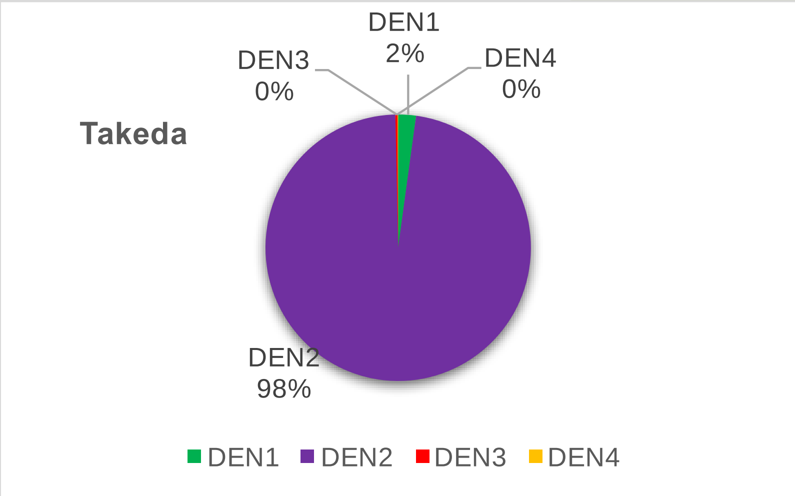


After 1 dose

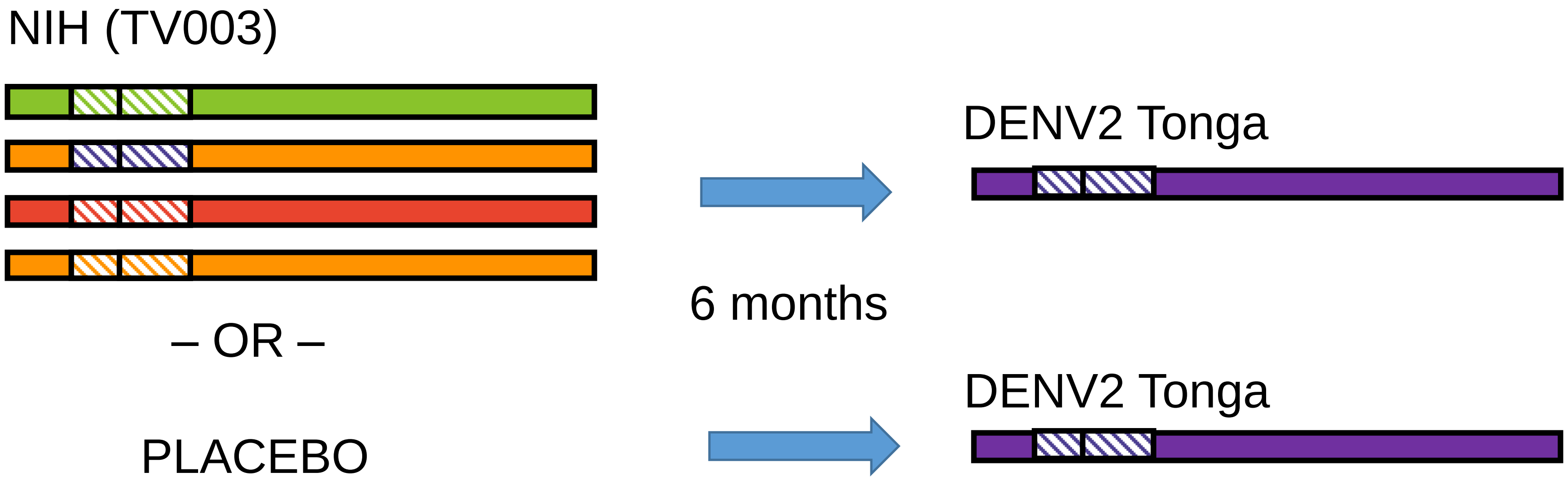
ANTIBODY responses







Will it work? Dengue human infection model



NIH (TV003)

– OR –

PLACEBO

6 months

DENV2 Tonga

DENV2 Tonga

16 MARCH 2016

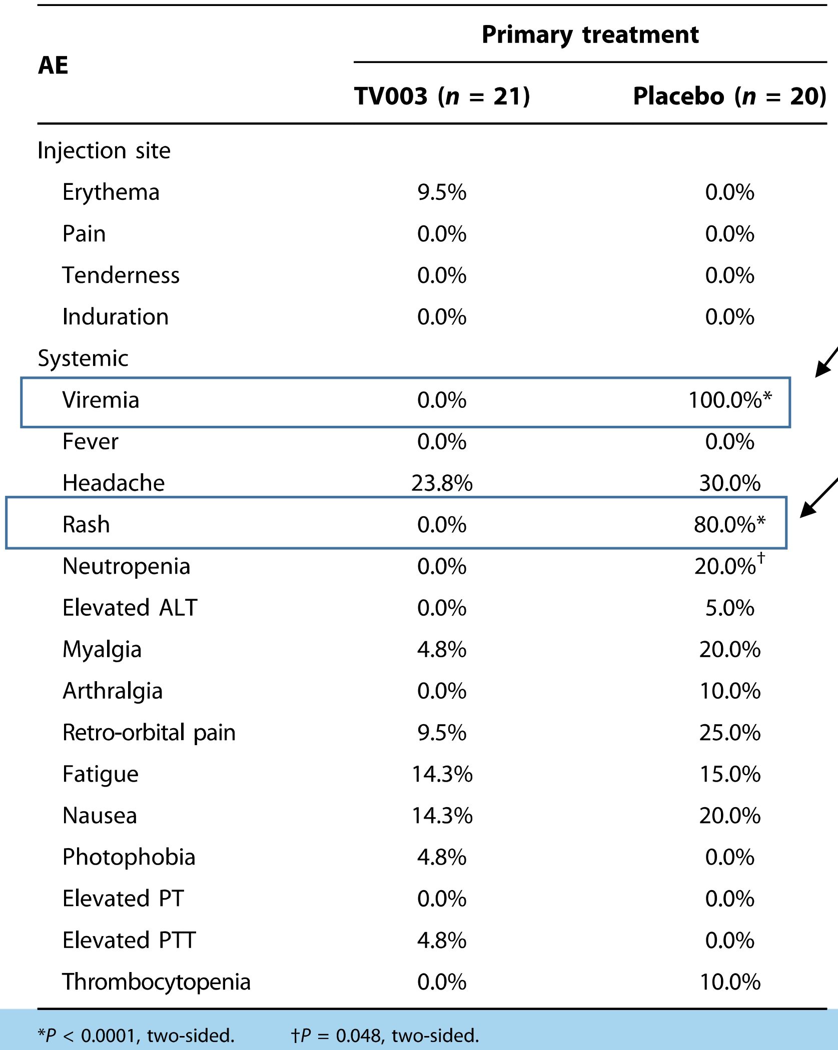
Science Translational Medicine

AAAS

Cover story March 2016

Kirkpatrick, Durbin, Whitehead, Pierce, Diehl et al. (2016) Science Translational Medicine

Will it work?



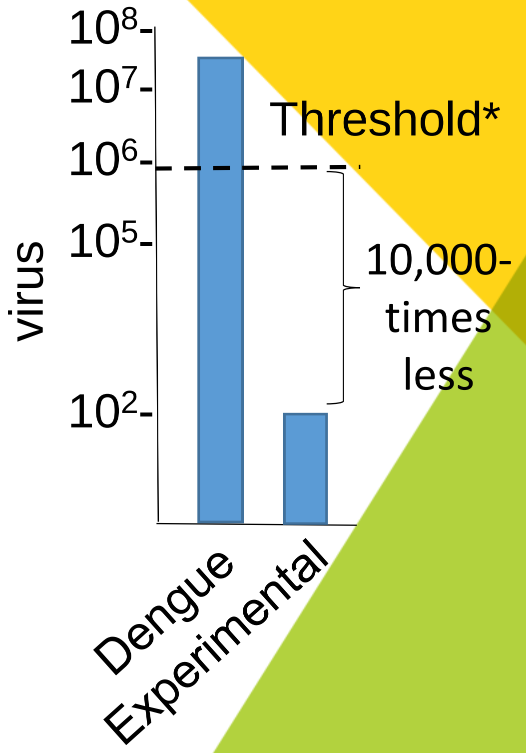
AE	Primary treatment	
	TV003 (*n* = 21)	Placebo (*n* = 20)
Injection site		
Erythema	9.5%	0.0%
Pain	0.0%	0.0%
Tenderness	0.0%	0.0%
Induration	0.0%	0.0%
Systemic		
Viremia	0.0%	100.0%*
Fever	0.0%	0.0%
Headache	23.8%	30.0%
Rash	0.0%	80.0%*
Neutropenia	0.0%	20.0%†
Elevated ALT	0.0%	5.0%
Myalgia	4.8%	20.0%
Arthralgia	0.0%	10.0%
Retro-orbital pain	9.5%	25.0%
Fatigue	14.3%	15.0%
Nausea	14.3%	20.0%
Photophobia	4.8%	0.0%
Elevated PT	0.0%	0.0%
Elevated PTT	4.8%	0.0%
Thrombocytopenia	0.0%	10.0%

*$P < 0.0001$, two-sided. †$P = 0.048$, two-sided.

- TV003 protects against DEN2 virus in blood

- DEN2 virus gives a rash
- TV003 protects against this

Rash:
- Mild (usually unnoticed)
- Lasts 1 day
- Indicates good response



*Vaughn et al. (2000). *J Infect. Dis.* (181(1):2-9

Kirkpatrick, Pierce, Diehl et al. (2016) Science Translational Medicine

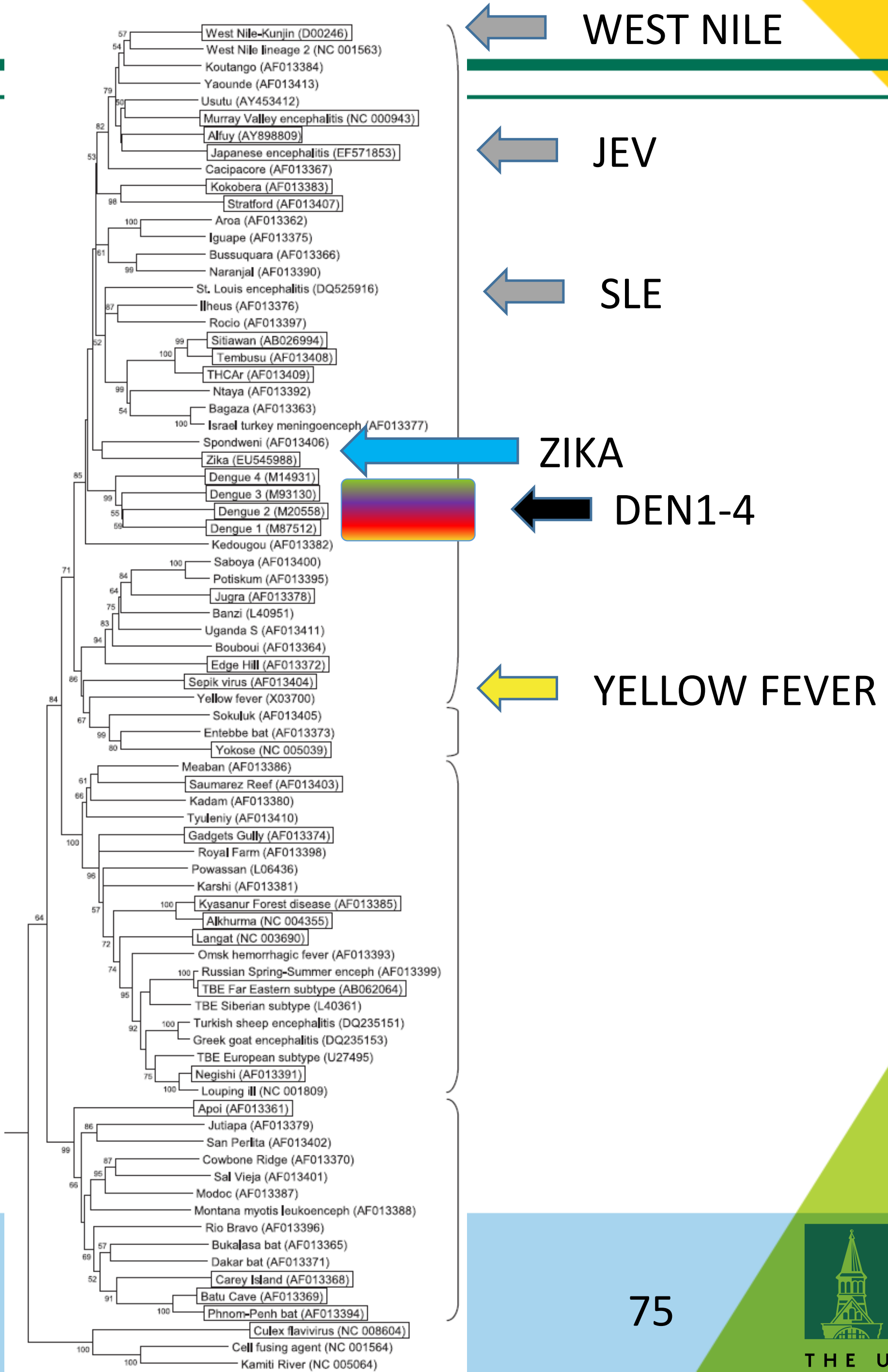
What's next for dengue vaccine

- How many serotypes needed to get protection? (2,3, all 4?)
- Protection against other serotypes (DENGUE-3)
- Safety and efficacy in field trials
    - Bangladesh
    - Thailand
    - Brazil (17,000 subjects) 3 years
- Which parts of immune system are necessary for protection?
- Combination with ZIKA virus?

Flaviviruses

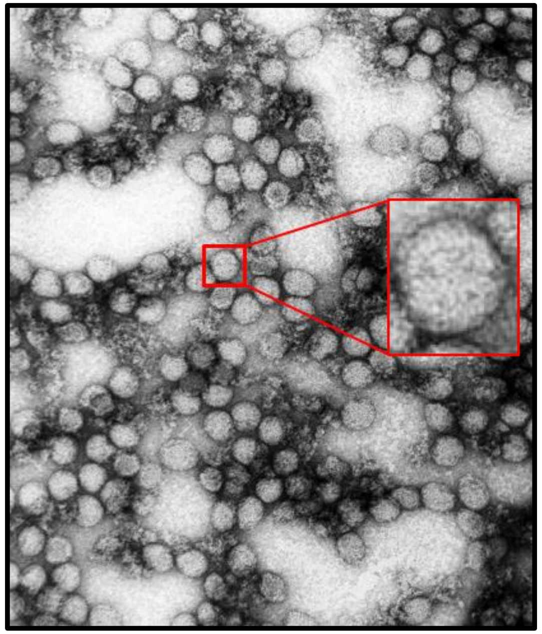
From Latin, flavi = yellow)

- Yellow Fever Virus
- Dengue (Dengue 1, 2, 3, and
- Japanese encephalitis (JEV)
- St Lous Encephalitis
- West Nile Virus
- **Zika virus**



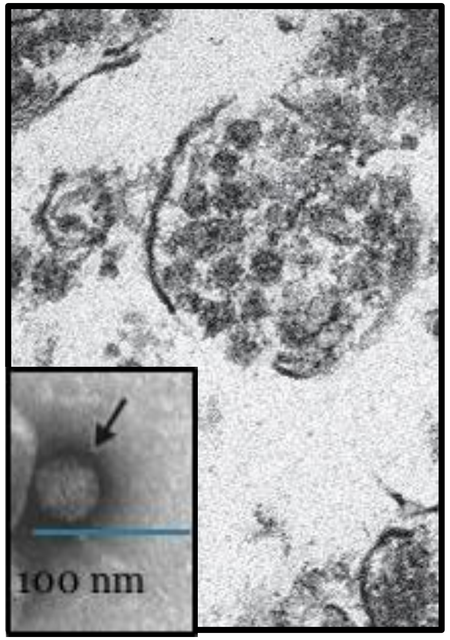
ZIKA has a familiar structure

Yellow Fever virus



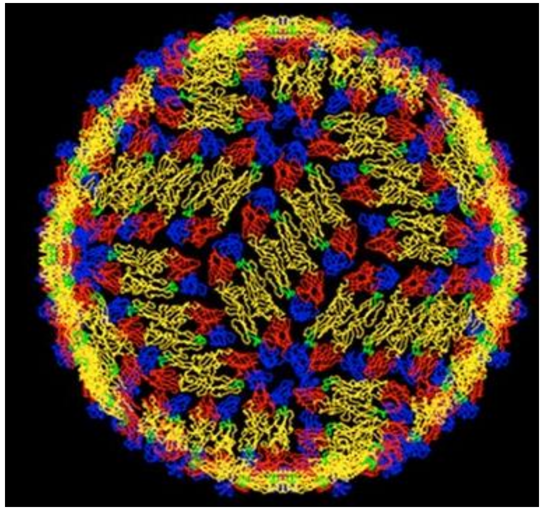
CDC

Zika virus



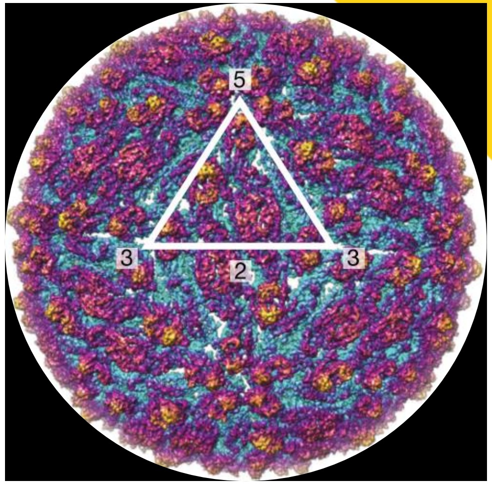
Mlakar, NEJM, 2016

Dengue virus

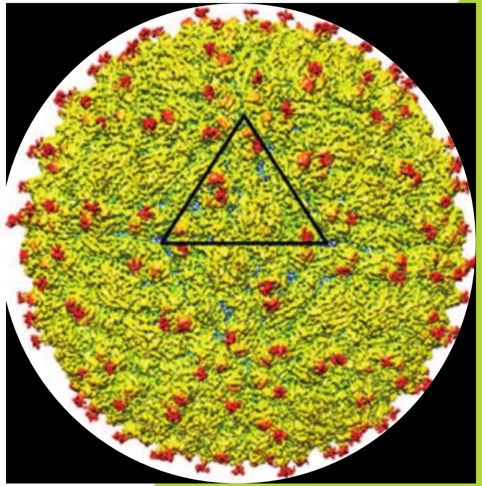


Kuhn, Cell, 2002

Zika Virus



Kostuychenko, Nature, 2016

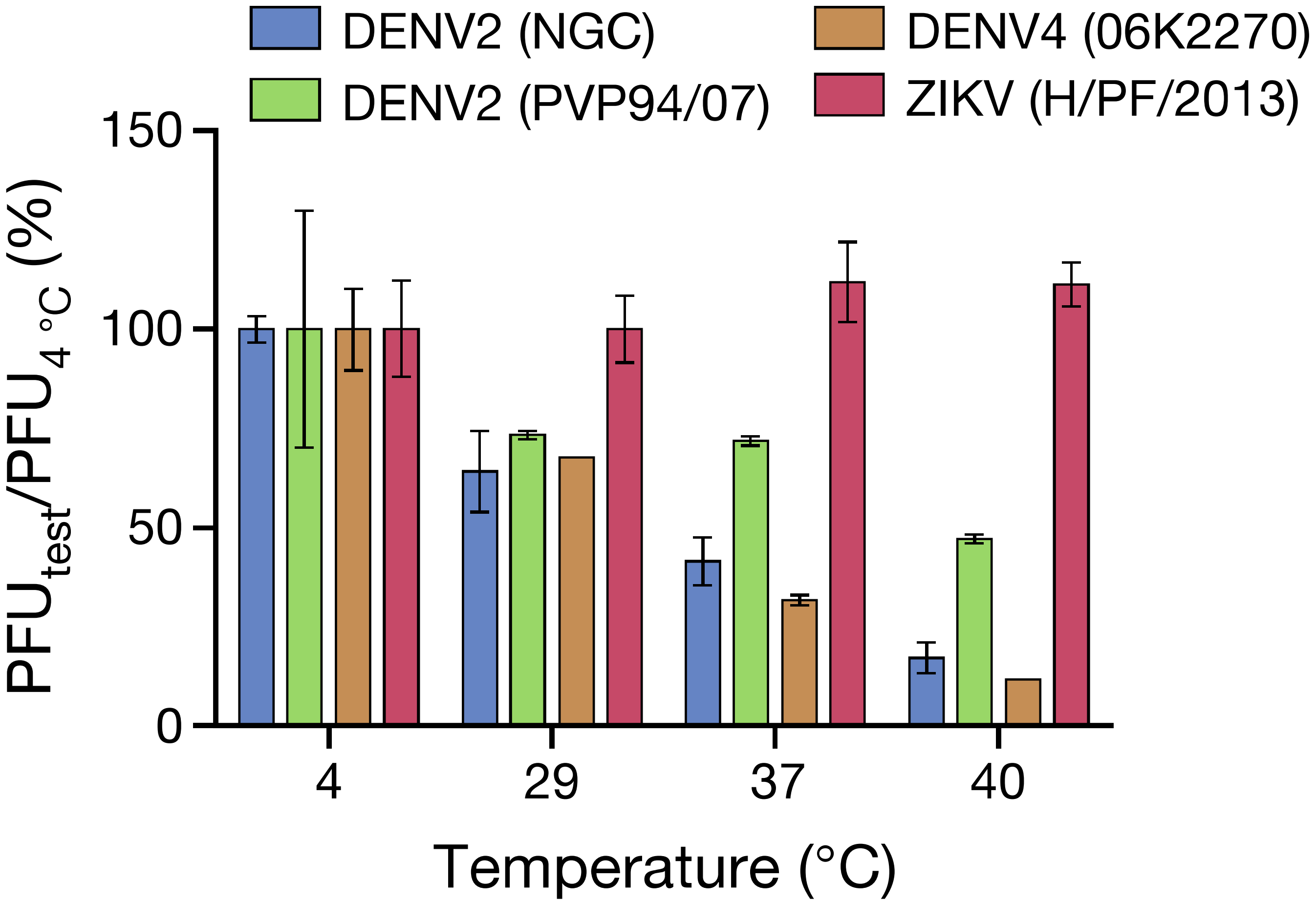


Sirohi, Science, 2016

Key points:

- Icosahedral (soccer ball)
- Unique sugar structure – receptor binding?
- Stability

ZIKA is a stable flavivirus

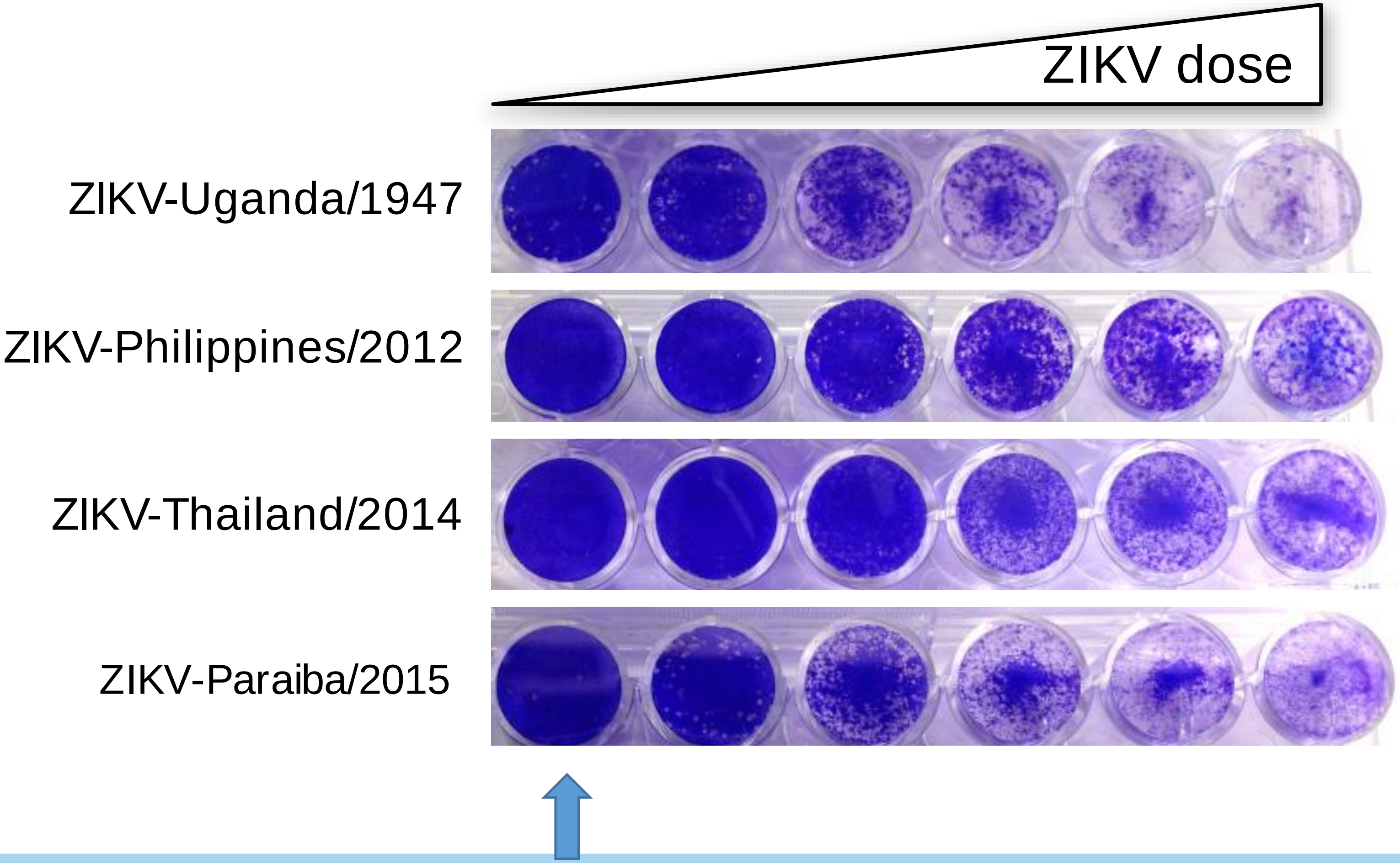


Key points:

- Dengue virus loses infectivity at higher temperatures
- ZIKV retains infectivity even at 40^oC
- Implications for unique transmission

Kostuychenko, Nature, 2016

ZIKA –cytopathic flavivirus



Dengue always looks like this even at high doses (cells fully intact)

- All ZIKV strains are cytopathic
- Dengue not cytopathic

Routes of ZIKA transmission

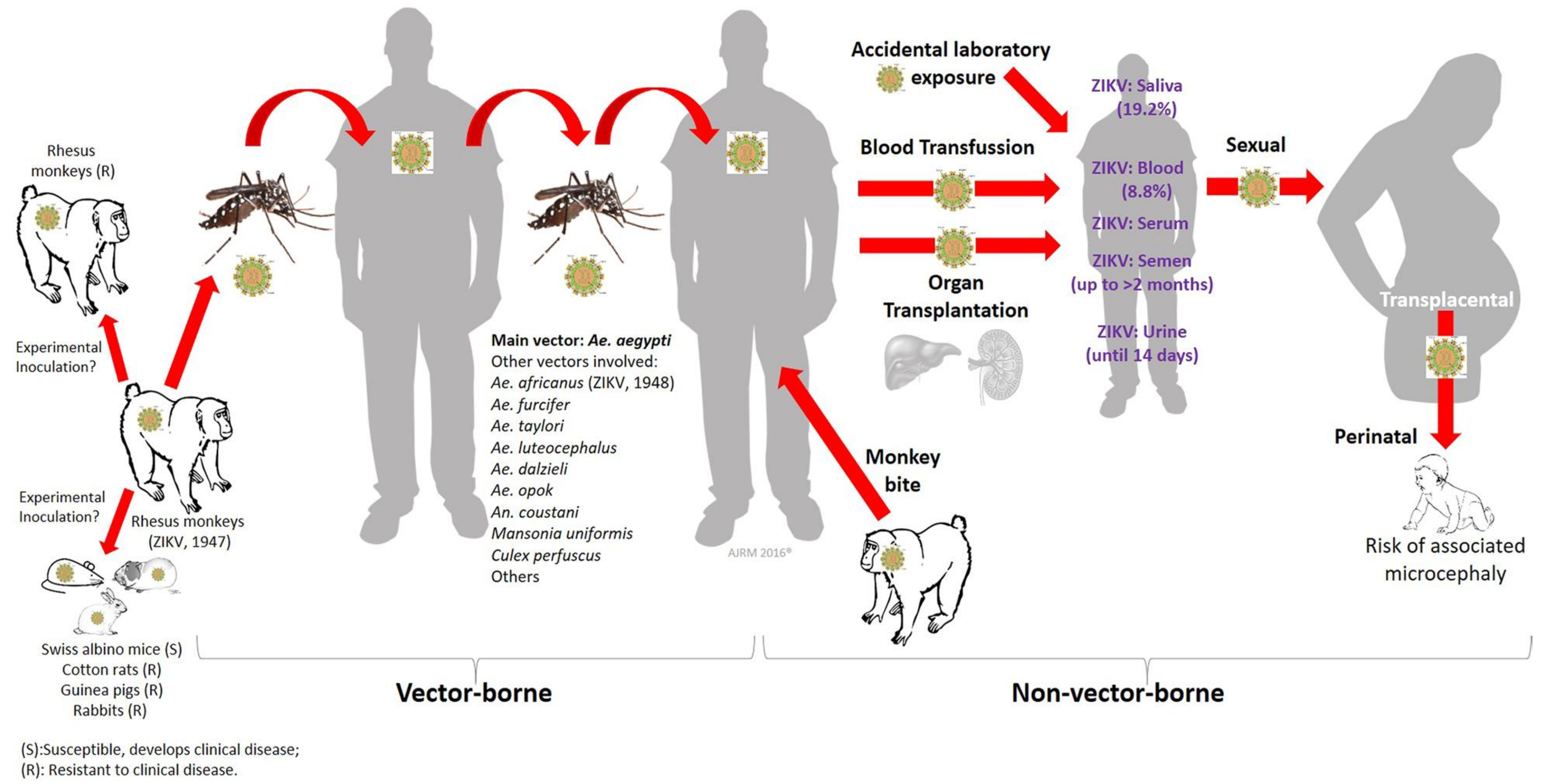
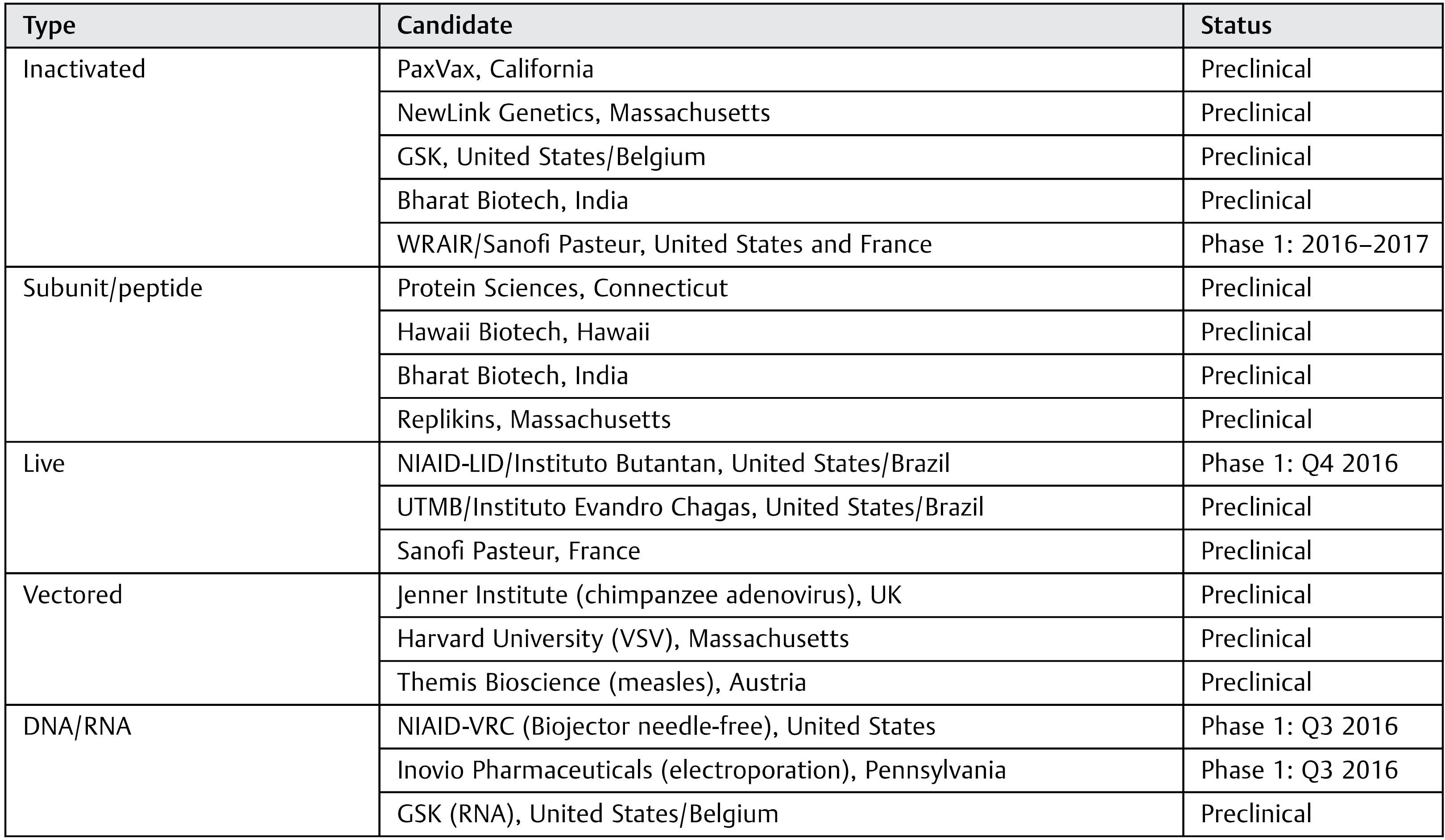


Fig. 1 Summary of reported forms of transmission of Zika virus

Rodriguez-Morales et al., *Ann Clin Microbiol Antimicrob* (2016) 15:13

ZIKA vaccine approaches

Table 1 Proposed Zika virus vaccine candidates/platforms



Type	Candidate	Status
Inactivated	PaxVax, California	Preclinical
	NewLink Genetics, Massachusetts	Preclinical
	GSK, United States/Belgium	Preclinical
	Bharat Biotech, India	Preclinical
	WRAIR/Sanofi Pasteur, United States and France	Phase 1: 2016–2017
Subunit/peptide	Protein Sciences, Connecticut	Preclinical
	Hawaii Biotech, Hawaii	Preclinical
	Bharat Biotech, India	Preclinical
	Replikins, Massachusetts	Preclinical
Live	NIAID-LID/Instituto Butantan, United States/Brazil	Phase 1: Q4 2016
	UTMB/Instituto Evandro Chagas, United States/Brazil	Preclinical
	Sanofi Pasteur, France	Preclinical
Vectored	Jenner Institute (chimpanzee adenovirus), UK	Preclinical
	Harvard University (VSV), Massachusetts	Preclinical
	Themis Bioscience (measles), Austria	Preclinical
DNA/RNA	NIAID-VRC (Biojector needle-free), United States	Phase 1: Q3 2016
	Inovio Pharmaceuticals (electroporation), Pennsylvania	Phase 1: Q3 2016
	GSK (RNA), United States/Belgium	Preclinical

Killed virus

Outer shell of virus

Live attenuated

Parts of ZIKA in another virus

Parts of ZIKA as genetic pieces

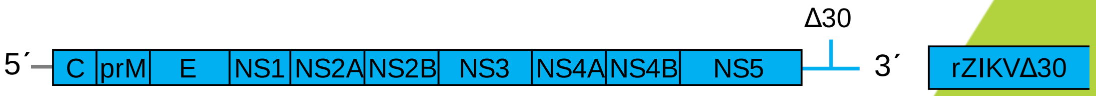
UVM and Johns Hopkins

Durbin, *Sem. Reprod Med* Sept. 2016

Experimental Zika viruses

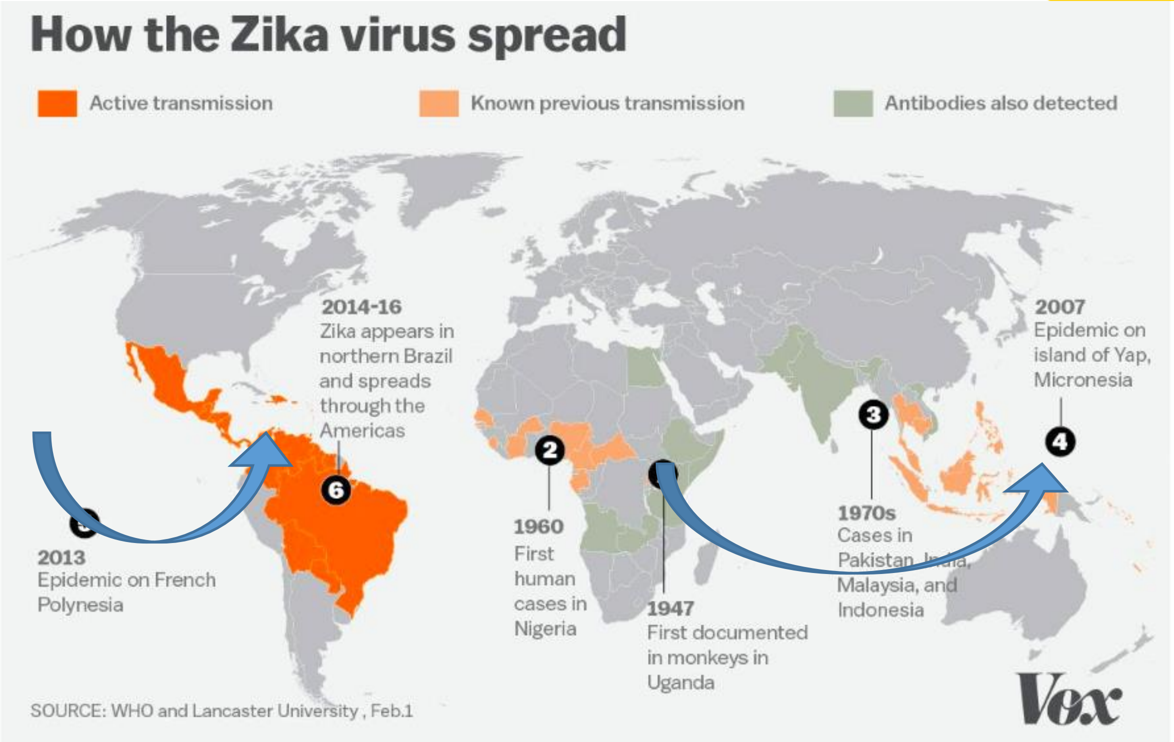
- ZIKA is known to replicate less than DENV
  Waggoner et al. *Clin Infect Dis* August 2016

- A genetic blueprint of ZIKA is now available
  Tsetsarkin, et al. (2016) mBio 23 Aug 2016

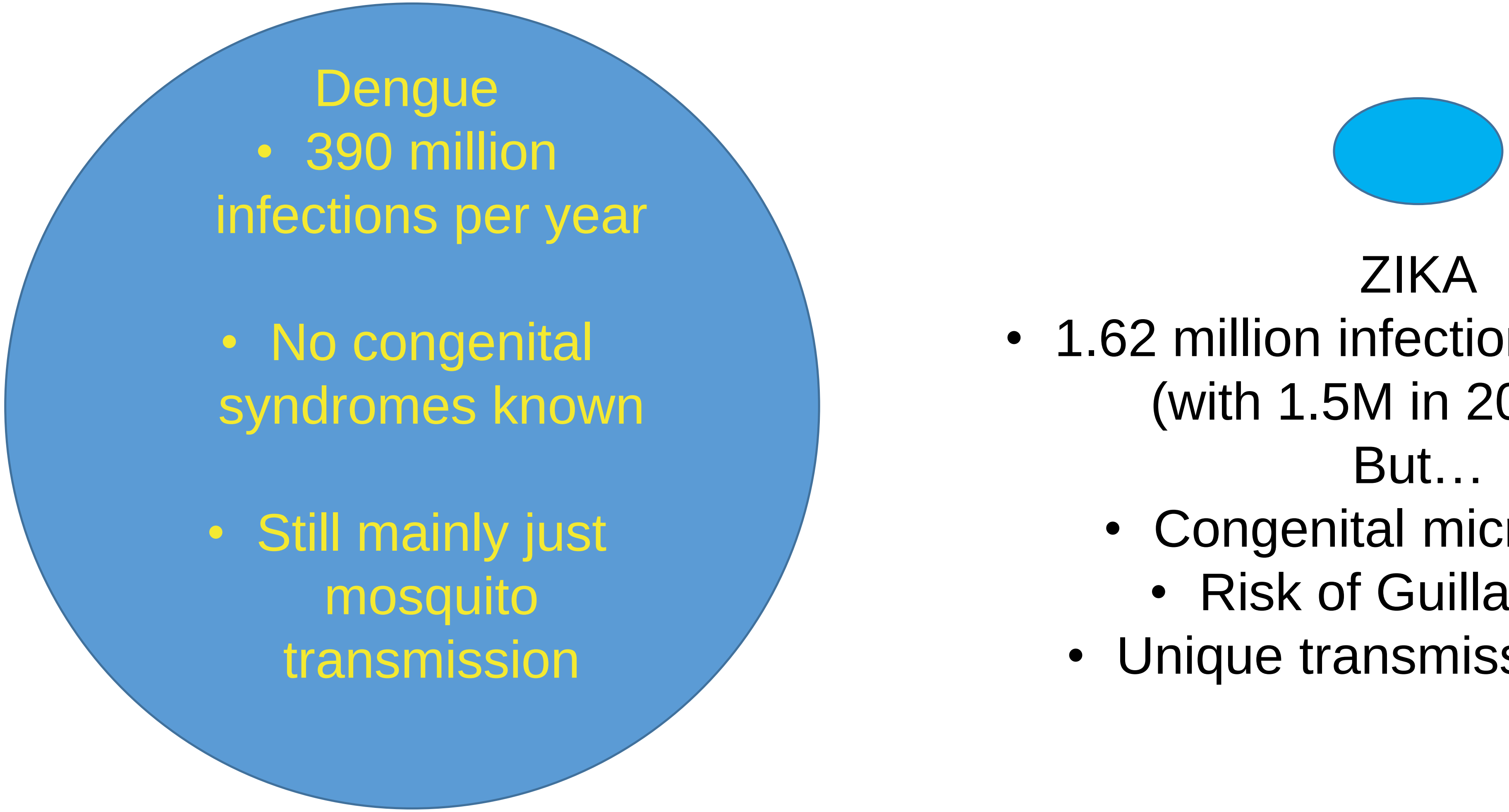


Challenges with rapid global vaccine development

- The infection has to be circulating and some go away (examples: Ebola, ZIKA?)
- ZIKA has typically been associated with small outbreaks (before this one)
- Hard to know exactly how virus behaves



Sustainability of a ZIKA vaccine



Dengue

- 390 million infections per year
- No congenital syndromes known
- Still mainly just mosquito transmission

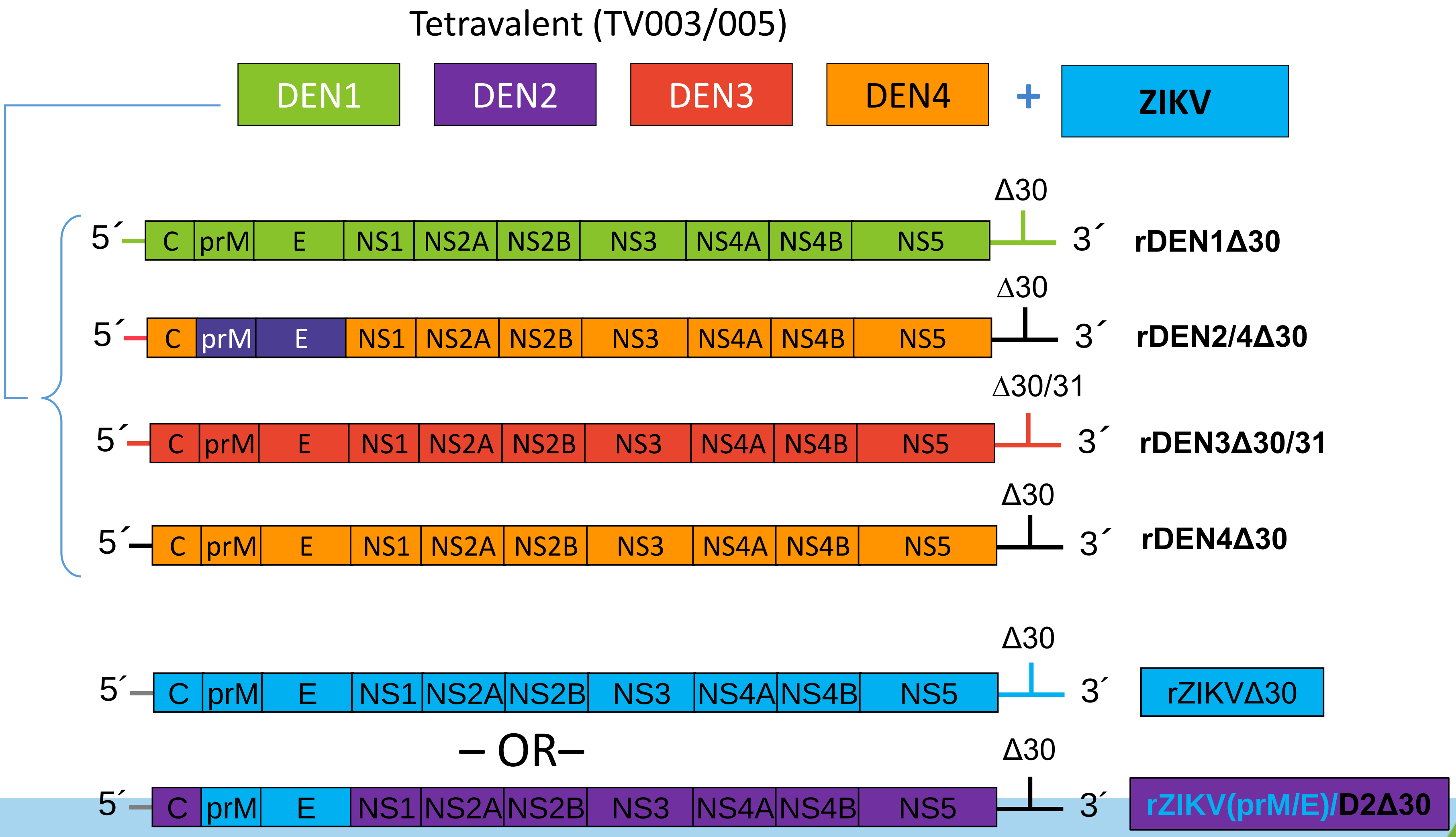
ZIKA

- 1.62 million infections since 1947 (with 1.5M in 2015-2016)

But…

- Congenital microcephaly
- Risk of Guillan-Barre
- Unique transmission modes

Proposed dengue/ZIKA combination vaccines



Tetravalent (TV003/005)

DEN1 DEN2 DEN3 DEN4 + **ZIKV**

Δ30
5´ C prM E NS1 NS2A NS2B NS3 NS4A NS4B NS5 3´ **rDEN1Δ30**

Δ30
5´ C prM E NS1 NS2A NS2B NS3 NS4A NS4B NS5 3´ **rDEN2/4Δ30**

Δ30/31
5´ C prM E NS1 NS2A NS2B NS3 NS4A NS4B NS5 3´ **rDEN3Δ30/31**

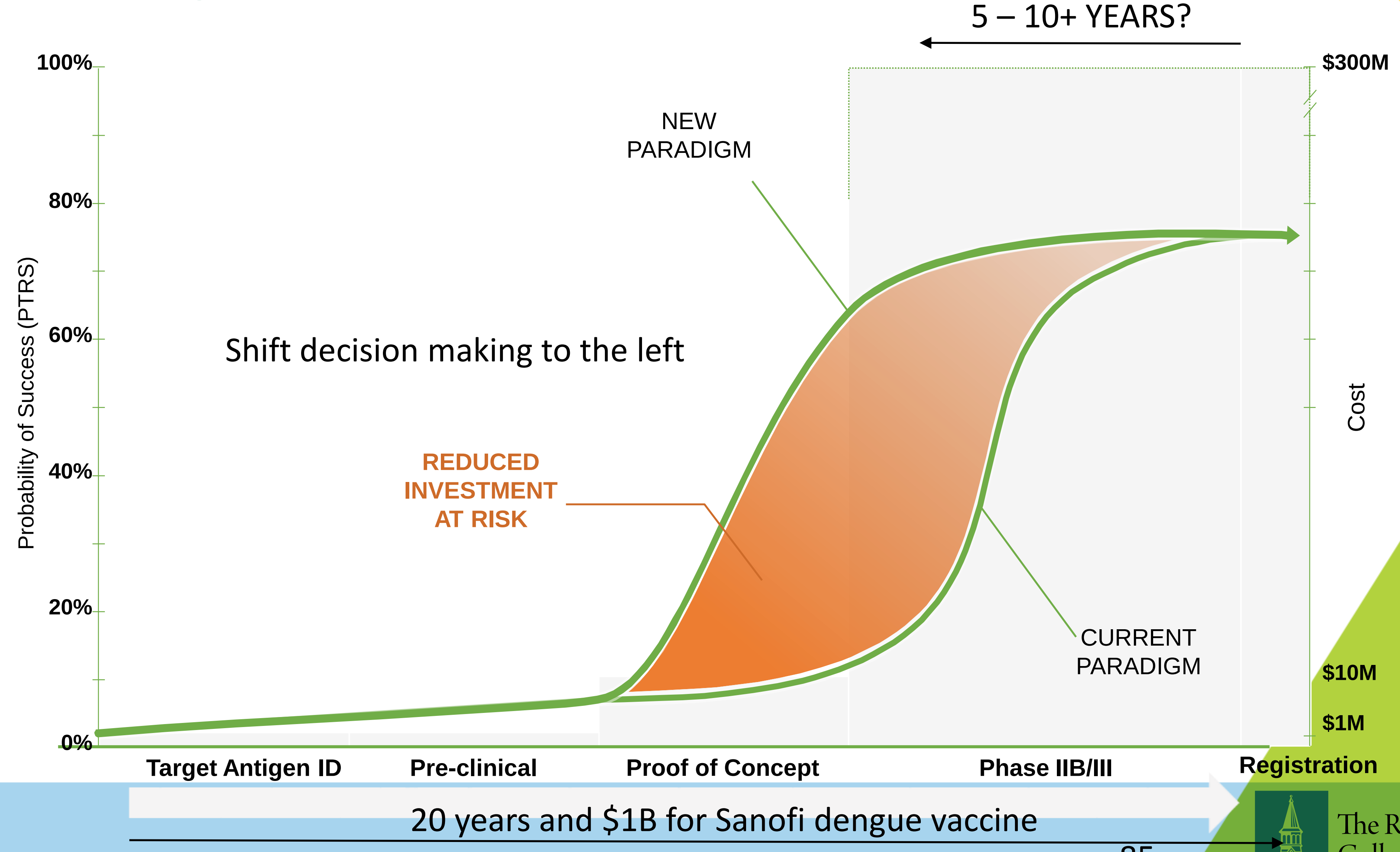
Δ30
5´ C prM E NS1 NS2A NS2B NS3 NS4A NS4B NS5 3´ **rDEN4Δ30**

Δ30
5´ C prM E NS1 NS2A NS2B NS3 NS4A NS4B NS5 3´ rZIKVΔ30

– OR–

Δ30
5´ C prM E NS1 NS2A NS2B NS3 NS4A NS4B NS5 3´ **rZIKV(prM/E)/D2Δ30**

The Challenge in Vaccine Development



Shift decision making to the left

20 years and $1B for Sanofi dengue vaccine

Vaccine Testing Center

University of Vermont College of Medicine

JOHNS HOPKINS
BLOOMBERG
SCHOOL of PUBLIC HEALTH

Center for Immunization Research
Anna Durbin
CIR clinic and lab teams

National Institute of Allergy and Infectious Diseases
Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.

Stephen Whitehead

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