



# **Arbovirus Infections: Trends, Recognition and Diagnosis**

**Beatriz Puzon-Quiambao, MD,  
FPPS, FPIDSP**



# Objectives

---

- To present current epidemiology of arbovirus infections: Japanese encephalitis, dengue, chikungunya, and Zika virus infections
- To discuss how to recognize and diagnose these infectious diseases
- To discuss recent or ongoing researches and highlight research gaps related to the topic

Arbovirus = arthropod borne viruses

Arthropod = insects, arachnids, crustaceans, myriapods



---

# Epidemiology



# Japanese encephalitis (JE)

---

- Most important form of viral encephalitis in Asia
- First documented case in 1871 in Japan
- JEV originated from an ancestral virus in the area of the Malay Archipelago → evolved into different genotypes → spread across Asia
- High CFR and frequent residual neuropsychiatric sequelae in survivors make JE a considerable public health problem in many Asian regions

Solomon et al. Asia J Virol. 2003



# JE Epidemiology



- Affects any age, but primarily affects children
  - Most people living in JE-endemic areas are infected before 15 years of age; usually, Asxtic
  - Most adults in endemic countries have natural immunity after childhood infection
- Two distinct epidemiologic patterns of JE
  - In **temperate** zones (northern part of the Korean peninsula, Japan, China, Nepal, and northern India), large epidemics occur in the **summer** months
  - In **tropical** areas (southern Vietnam, southern Thailand, Indonesia, Malaysia, the *Philippines* and Sri Lanka), cases occur more sporadically and peaks are usually observed during the **rainy** season

WHO position paper on JE, 2014  
Tobias et al, EID 2009. 15 (1)



# JE Global Situation

- In recent decades - JE **outbreaks in previously non-endemic areas** including India, Myanmar, Nepal, Sri Lanka, Thailand and Viet Nam
- **Decreasing incidence** in Japan, Korea, and some regions of China due to extensive **use of JE vaccines**, improvement of socioeconomic conditions and changing agricultural practices
- Annually - at least 50,000 cases of clinical disease and 10,000 deaths, mostly among children
- 24 countries in the WHO SEA and WPR have endemic JEV transmission
- More than 3 B people to risk of infection

WHO position paper on JE, 2014

## Japanese encephalitis, countries or areas at risk\*

\* Based on 2012 data

- Australia
- Bangladesh
- Brunei
- Burma
- Cambodia
- China
- Guam
- India
- Indonesia
- Japan
- Laos
- Malaysia
- Myanmar
- Nepal
- North Korea
- Pakistan
- Papua New Guinea
- **Philippines**
- Russia
- Saipan
- Singapore
- South Korea
- Sri Lanka
- Taiwan
- Thailand
- Timor-Leste
- Vietnam

 Countries or areas at risk

0 1,200 2,400 4,800 Kilometers

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization/CDC  
Map Production: Public Health Information  
and Geographic Information Systems (GIS)  
World Health Organization



© WHO 2012. All rights reserved



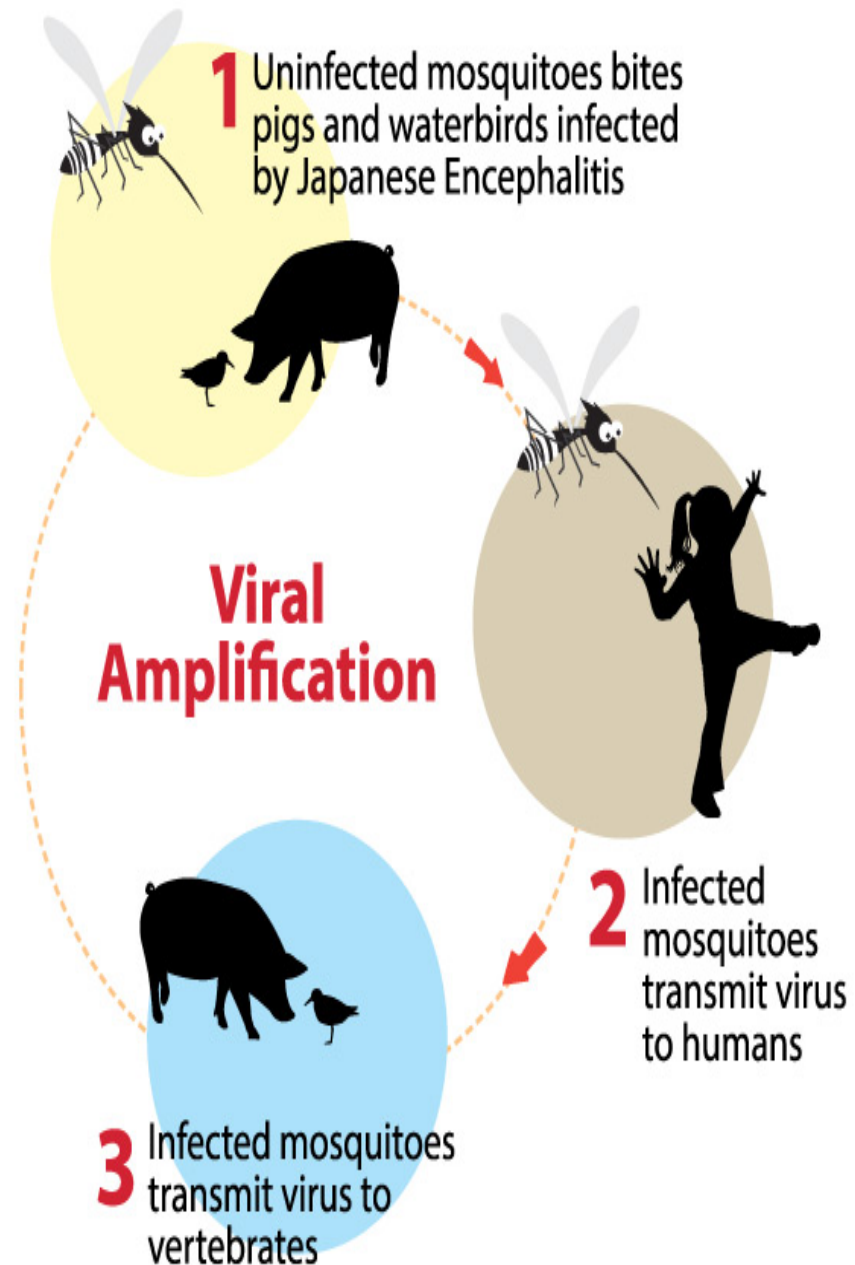
# JE Etiology & Transmission

- Single-stranded RNA flavivirus related to dengue, yellow fever and West Nile viruses
  - Genotypes I-IV isolated in many vectors & patients in Asia
  - Genotype V – Muar strain isolated in Malaysia
- Transmitted by *Culex tritaeniorhynchus* mosquitos from viraemic animals (pigs and aquatic birds) to humans
  - <1% to 3% virus infection rate in the mosquito vector
  - Culex breeds preferably in water pools and flooded rice fields
- Most human cases occur in rural areas; outbreaks have occurred also in periurban and urban populations

WHO position paper on JE, 2014; Li et al PLoS NTD 2011

# JE Transmission

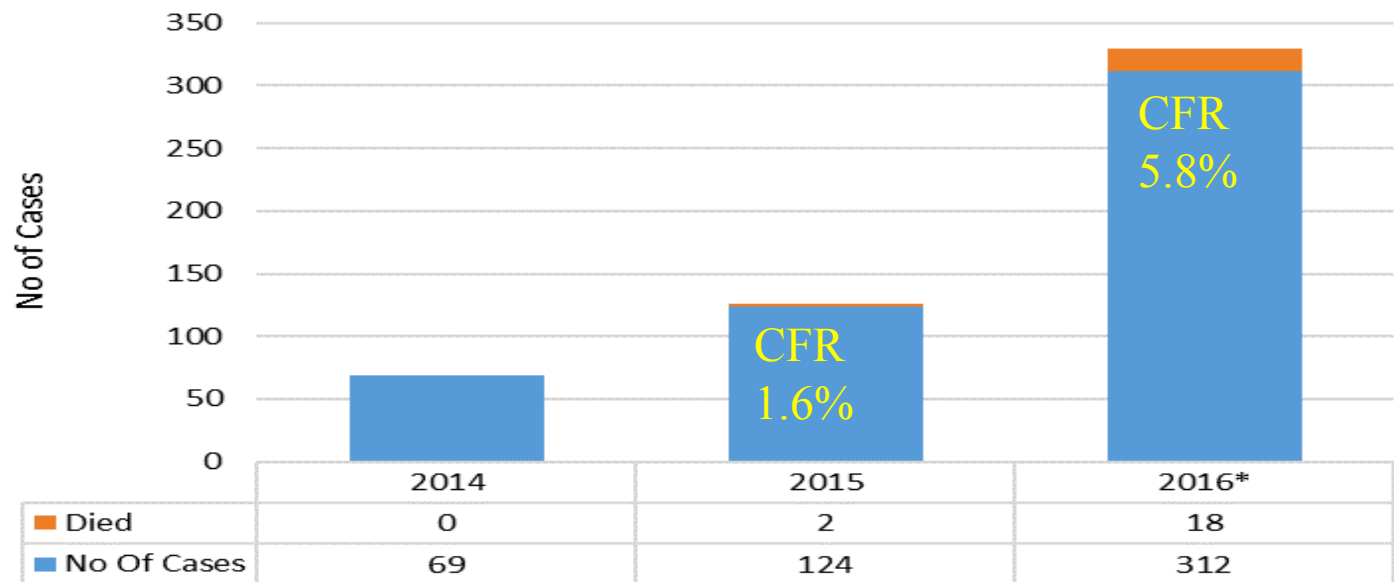
- Human beings are incidental hosts and are not considered a reservoir for viral transmission
  - Viremia is low and of short duration
- Because infected pigs act as amplifying hosts, domestic pig rearing is an important risk factor in the transmission to humans



# JE in the Philippines

- JEV Ab first identified in horses in 1943
- First reported case in 1950
- Season – year round

Confirmed Japanese Encephalitis Cases  
Philippines, 2014-2016\*



\*As of Dec 31, 2016

# JE in the Philippines

- JEV caused 16 - 40% of clinical encephalitis cases and 7-18% of meningitis/encephalitis cases
- 68% < 15 years
- 1M: 3F
- CFR – 6-7%

## LEGEND:

### Hospitals with JE cases

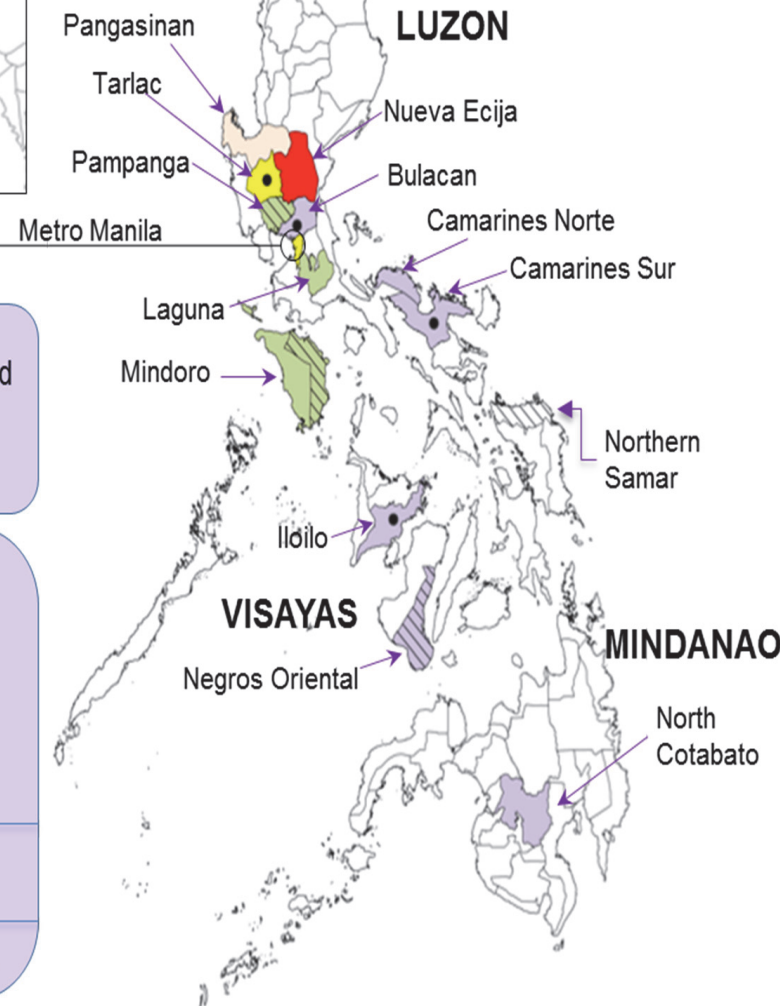
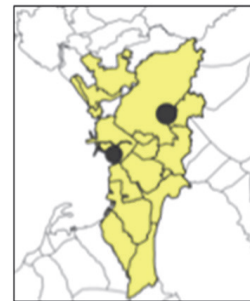
1 dot = Hospital with JE cases and no reported origin  
Dots are randomly distributed within the province boundaries

### Reported JE cases

- No Data
- 1 to 4 cases
- 5 to 19 cases
- 20 to 50 cases
- >50 cases

■ Suspected JE, test results not available

■ JEV Seroprevalence studies



Lopez, et al. PLoS NTD 2015



# Dengue

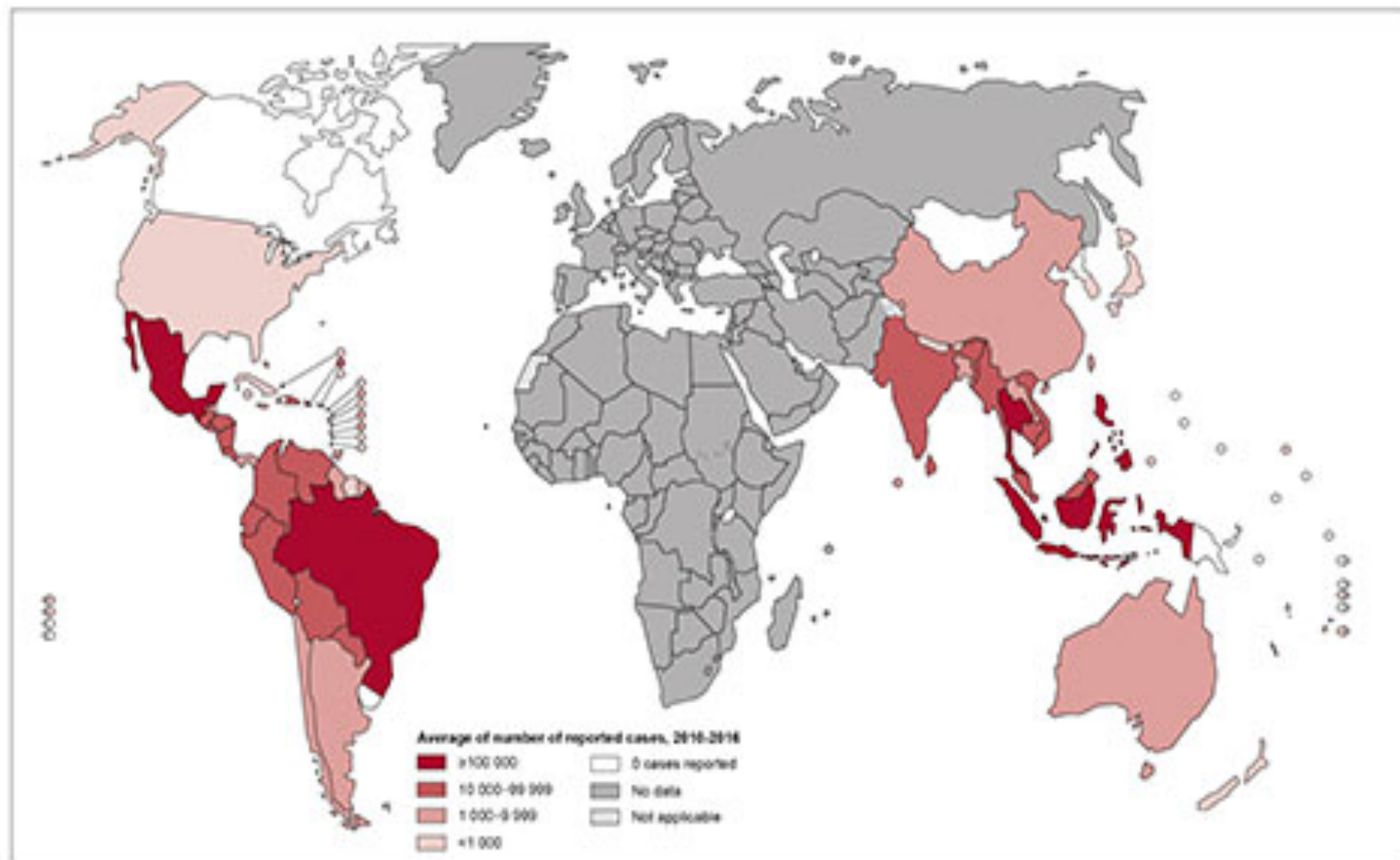


- Most rapidly spreading mosquito-borne viral disease in the world
- 1960's - only 9 countries experienced severe dengue epidemics
- In the past 50 years- 30-fold increased IR; Geographic spread to new countries; Rural → urban
- Current – endemic in > 100 countries worldwide
- Approx 2.5 B people live in dengue endemic countries (40 % of the world)
- Annually 390 M dengue infections; 96M Sxtic; 0.5 M severe dengue

<http://www.who.int/denguecontrol/epidemiology/en/>



## Distribution of dengue, worldwide, 2016



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. © WHO 2016. All rights reserved.

Data Source: World Health Organization  
Map Production: Control of Neglected  
Tropical Diseases (CNTD)  
World Health Organization



# Why is dengue on the rise?

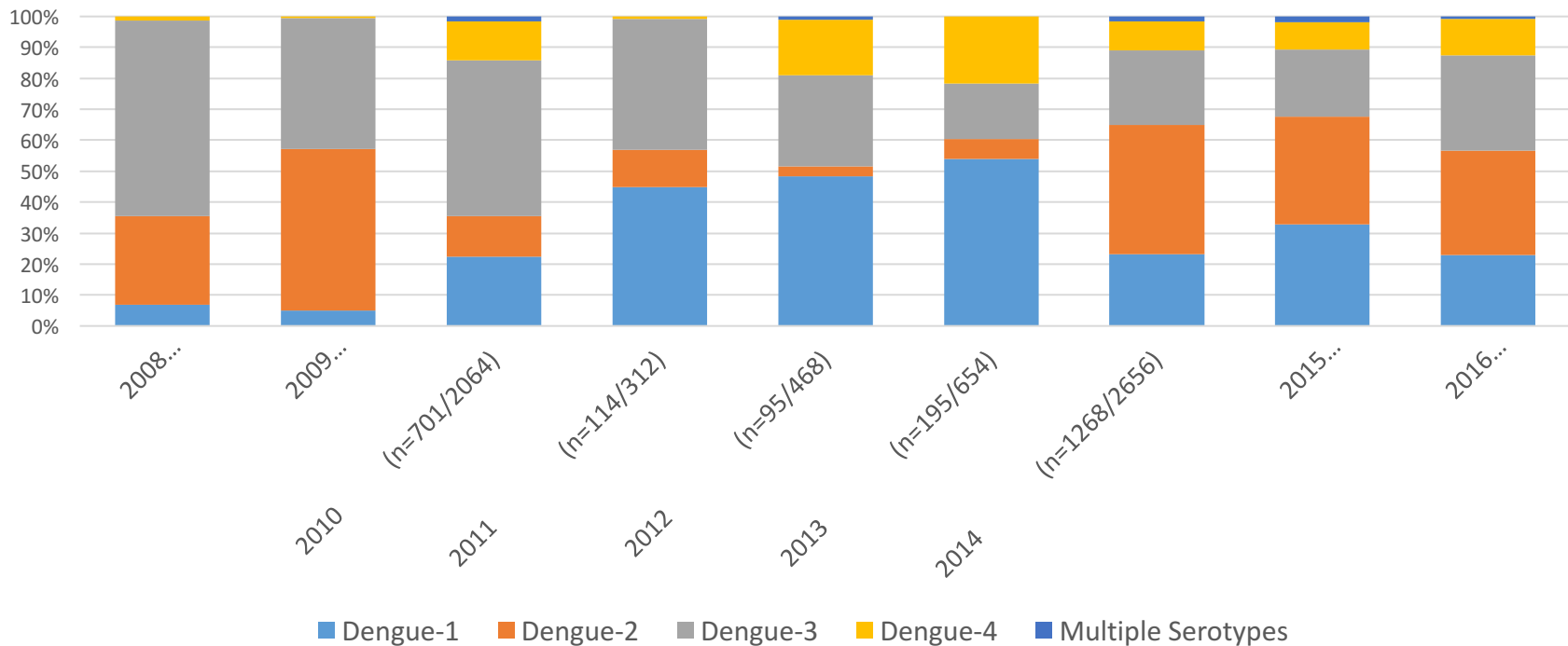
---

- Rapid urbanisation
  - lack of clean water and sanitation are a problem
  - Disease transmission rates increase when people live in close proximity to each other
- Climate change contributes to the rapid spread
  - Increased breeding grounds for mosquito vectors
- Increased international travel - travellers can carry the virus to other countries

# Dengue Etiology



- Single-stranded RNA virus, genus *Flavivirus*, family *Flaviviridae*
- 4 antigenically distinct but genetically related serotypes (DENV-1 to DENV-4)



# Dengue Transmission

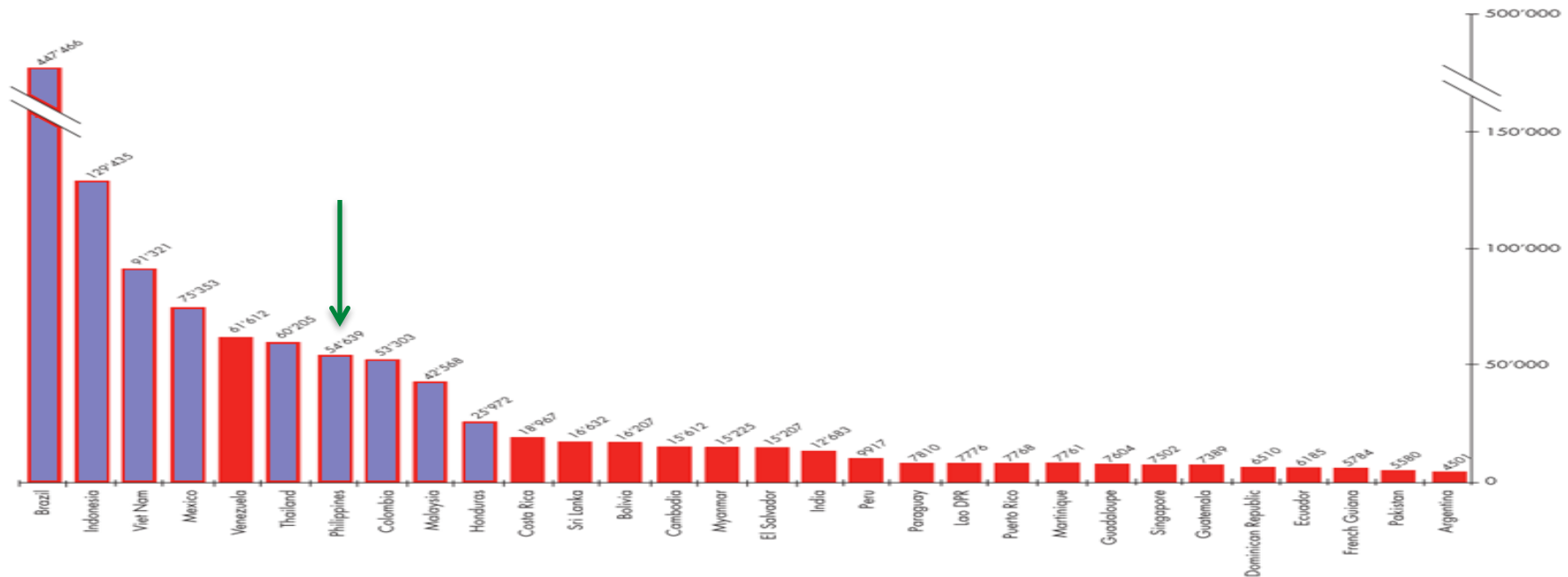


- Primarily to humans through the bite of an infected *Aedes* mosquito (*A aegypti*, *A albopictus*)
- May also occur through:
  - Transfusion of infected blood
  - Transplantation of infected organs or tissues
    - E.g. Peripheral blood stem cells (Punzel et al, EID 2014)
  - Human transmission
    - After occupational exposure in healthcare settings, e.g., needle stick injuries (Nemes et al, EID 2004)
    - Vertical transmission have been described in the literature (i.e., transmission from a dengue infected pregnant mother to her fetus in utero or to her infant during labor and delivery) (Tomashek et al, CDC Yellow book Chapter 3)

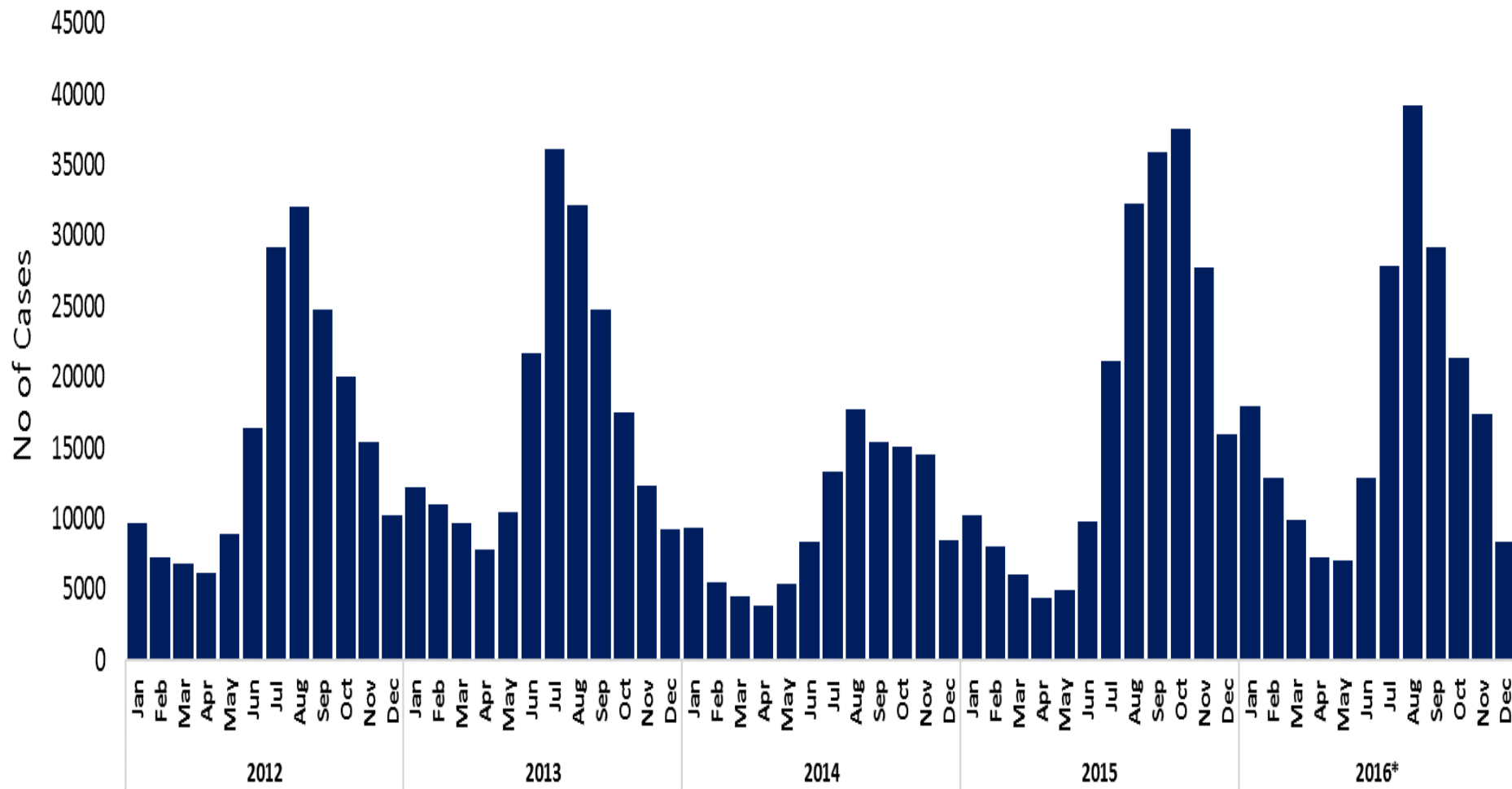
# Dengue in the Philippines



- First recorded dengue outbreak in the world occurred in Manila in 1953
- Philippines experienced the seventh highest number of dengue fever cases in the world between 2004 and 2010



# Suspect Dengue Cases by Morbidity Month, Philippines, 2012-2016\*

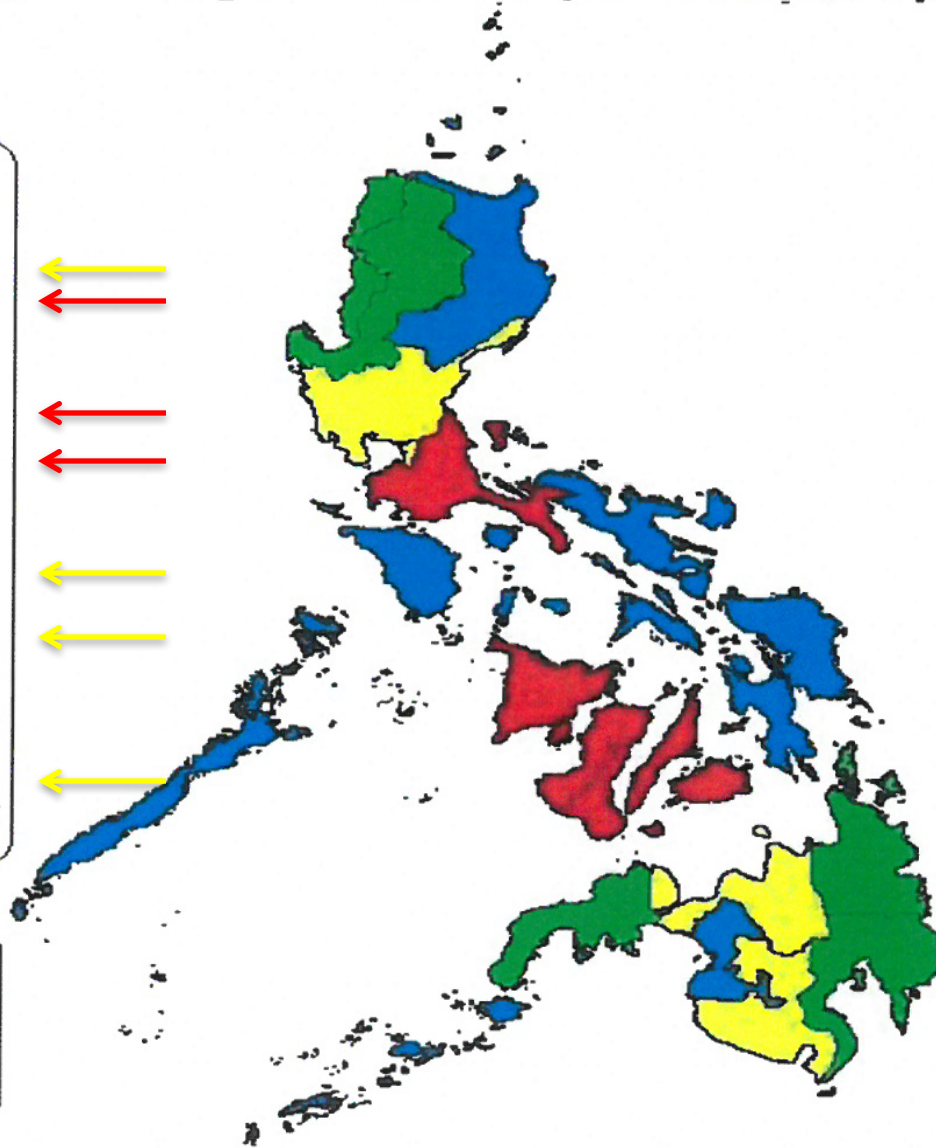


\*As of Dec 31, 2016

# Suspect Dengue Cases MW44 (N=176,411) 2016

Region	Cases
I	7,135
II	3,539
III	15,941
IVA	18,365
IVB	3,178
V	1,948
VI	23,424
VII	20,213
VIII	5,778
IX	6,150
X	13,523
XI	11,077
XII	14,041
ARMM	2,665
CAR	9,852
CARAGA	6,955
NCR	12,627
<b>PHILIPPINES</b>	<b>176,411</b>

Legend - Cases	
<span style="color: blue;">■</span>	1.00 - 6,000.00
<span style="color: green;">■</span>	6,000.01 - 12,000.00
<span style="color: yellow;">■</span>	12,000.01 - 18,000.00
<span style="color: red;">■</span>	18,000.01 +



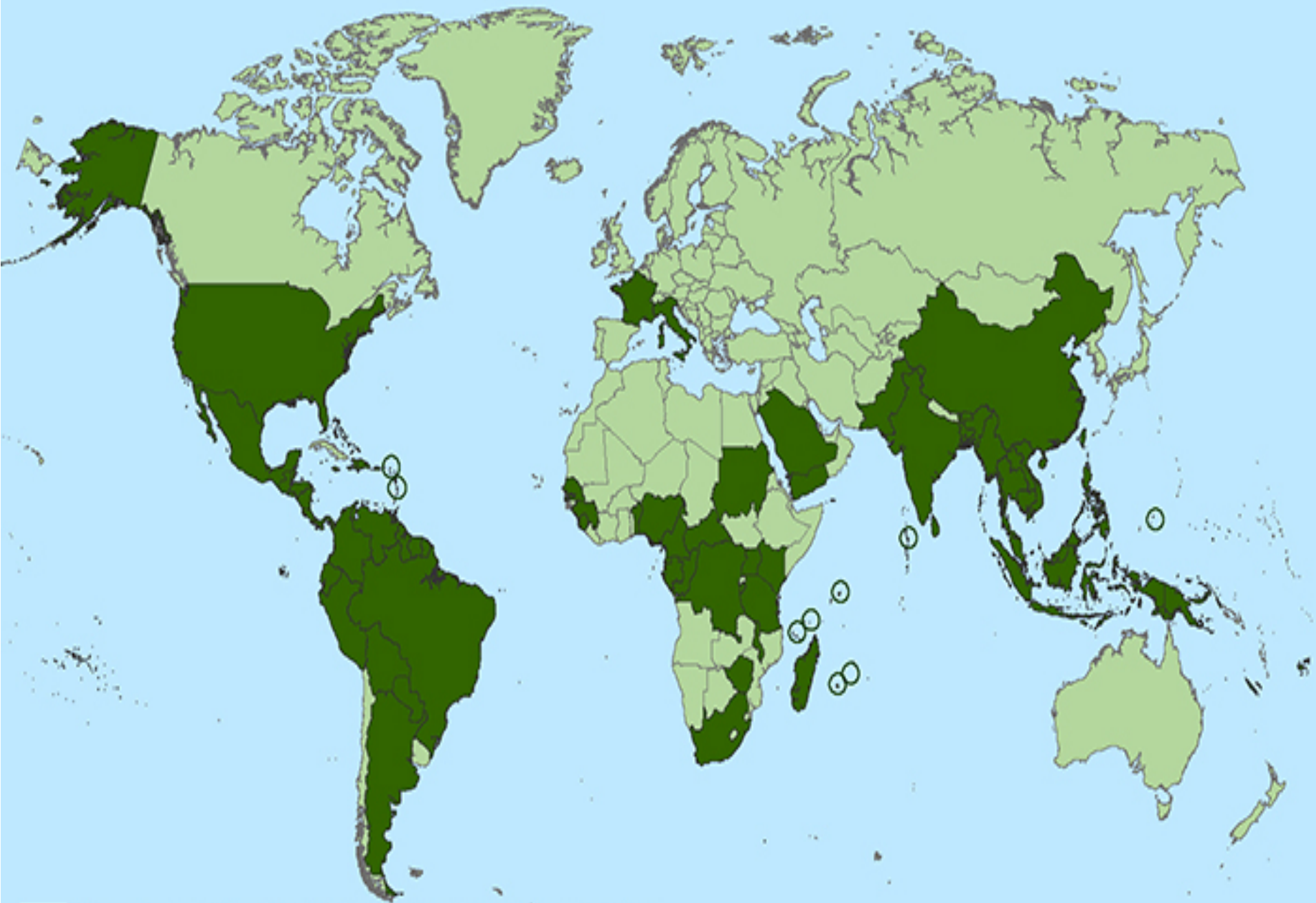


# Chikungunya

- “Chikungunya” – derived from a word in the Kimakonde language, meaning “*to become contorted*”, and describes the stooped appearance of sufferers with joint pain
- Benign, dengue-like syndrome characterized by abrupt onset of fever, arthralgia, maculopapular rashes and leukopenia
- Initially described in Tanzania and India in 1820’s
- CHIKV first isolated during an outbreak in southern Tanzania in 1952

Poirier et al, Bull WHO 2016





**■** Current or previous local transmission of chikungunya virus

# Etiology & Transmission

- Single-stranded RNA virus of Family *Togaviridae*, Genus *Alphavirus*
- Have geographically associated genotypes:
  - West African (Waf)
  - East/Central/South African (ECSA)
  - Asian genotypes
- Transmitted by mosquito vectors – *A. aegypti*, *A. africanus*, *A. Albopictus*
  - Same vectors as dengue but different geographic distribution since CHIKV infected *A. aegypti* mosquitoes transmit virus to vertebrates poorly



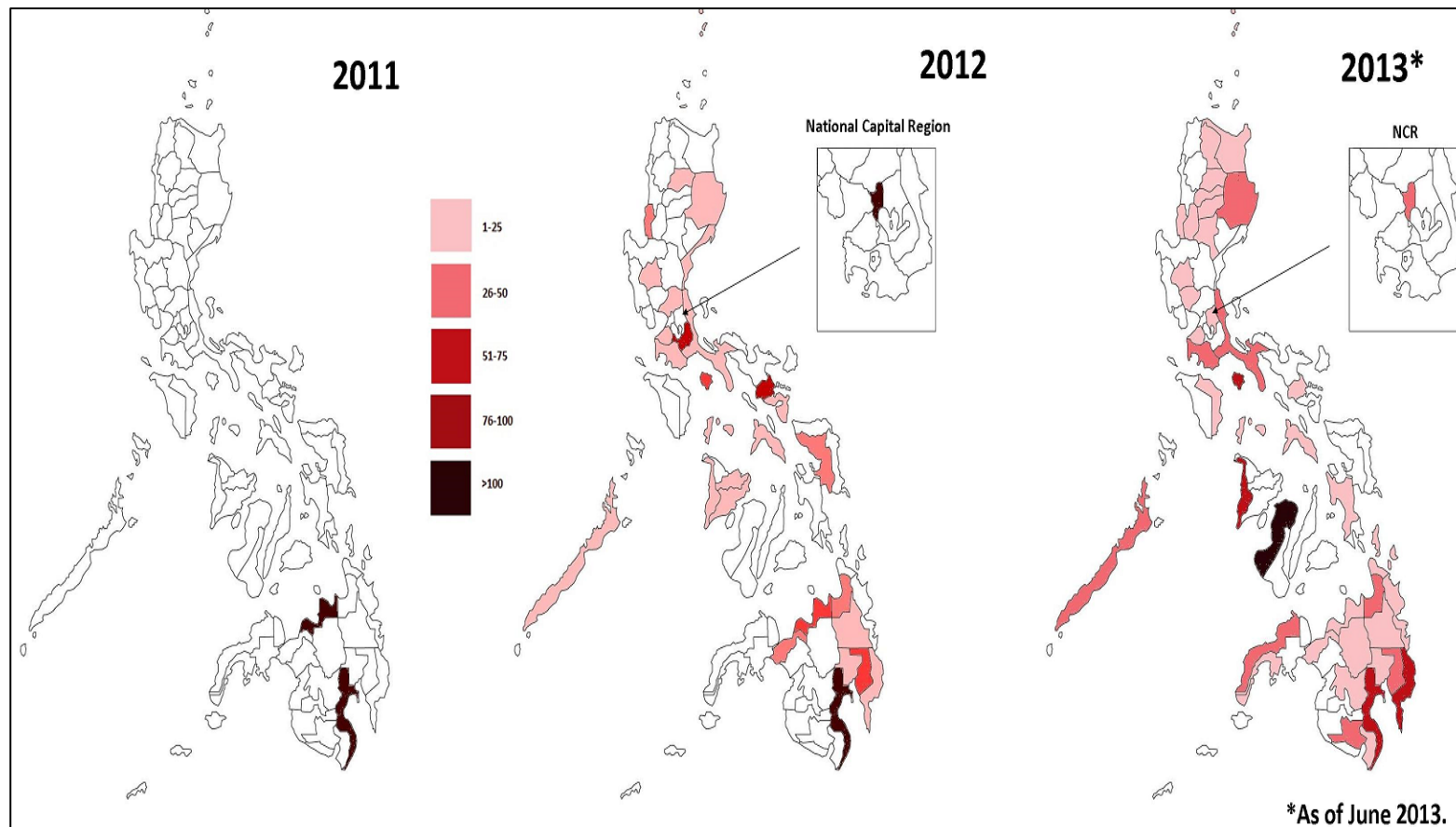
# Chikungunya in the Philippines

- Local outbreaks every 1-3 years: Manila (1967), Negros (1968), Peace corps volunteers (1986), Cavite (1996)
- Outbreak in Cebu, 2012-2014
  - Active fever surveillance for > 6 years old

Year	Prevalence per 100 person yrs	Prevalence of neut Ab	Sxtic Infections	% below 15 years old
Year 1	12.32 – 16.9	28%	19 %	44 %
Year 2	2.84	42%	28.6 %	33 %

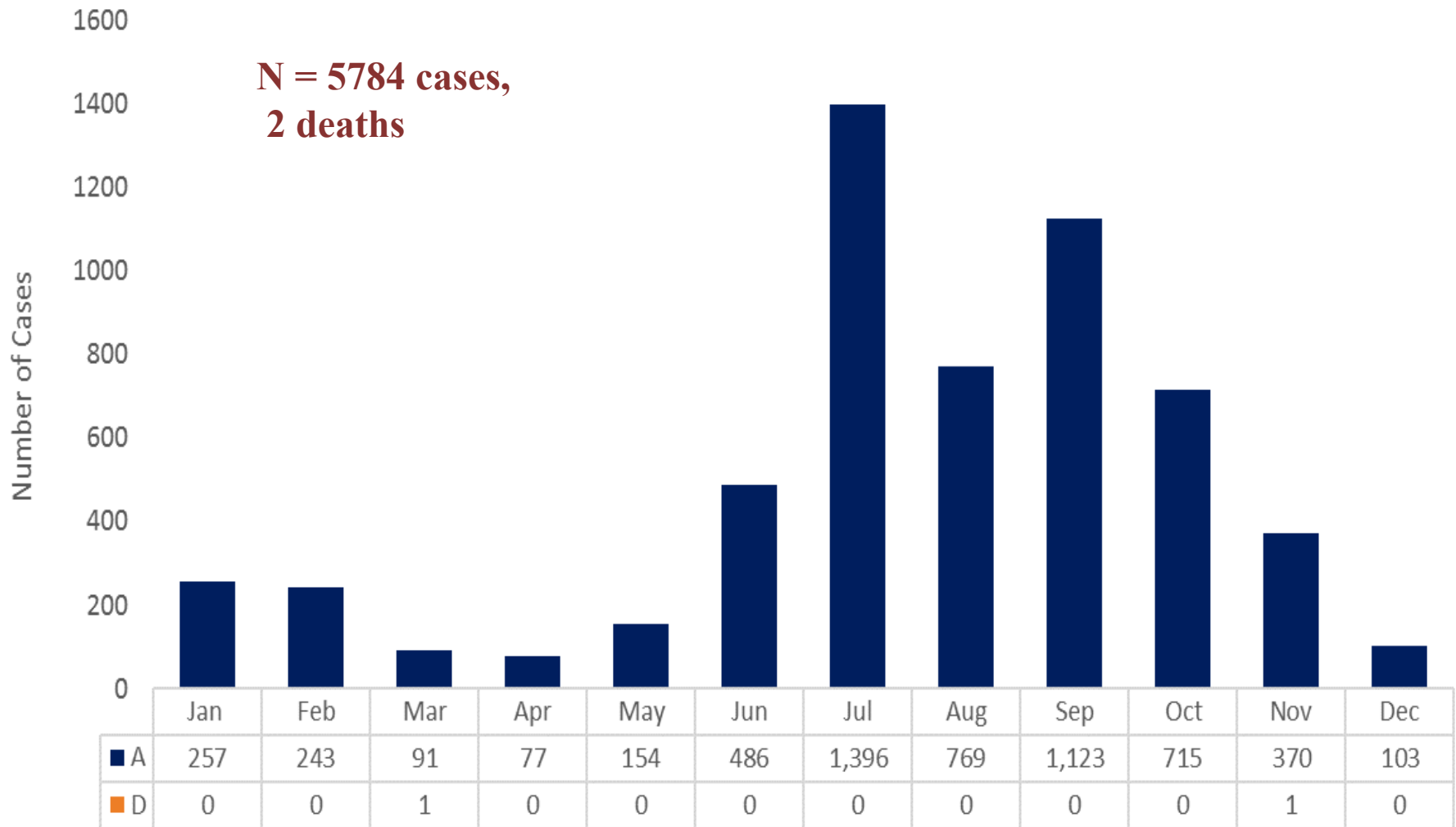
Srikiatkachorn, Alera et al, EID 2016

# Re-emergence of Chikungunya 2013



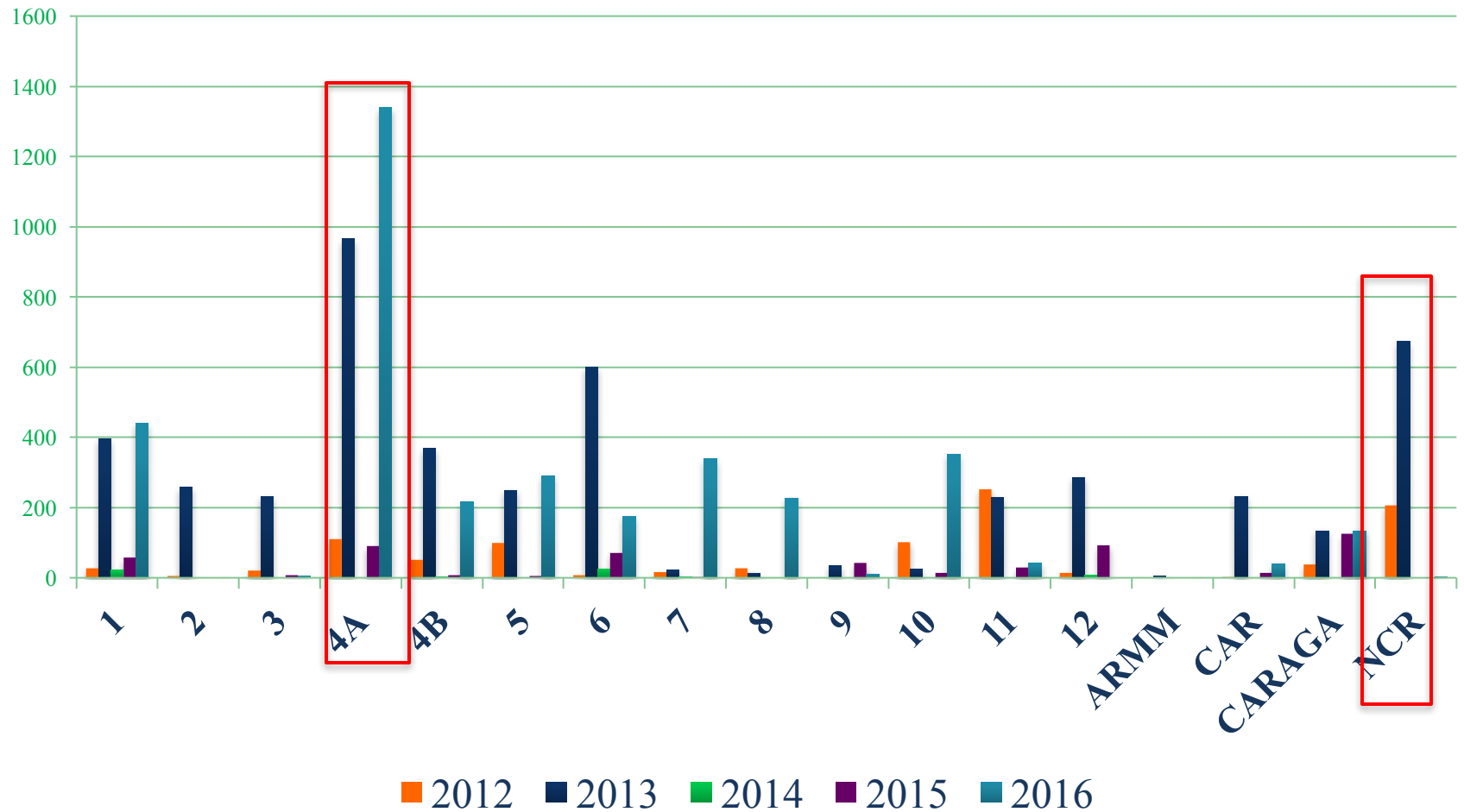
RITM-Tohoku Collaborative Research Center for EREID

# Suspect Chikungunya Cases by Morbidity Month Philippines, 2016

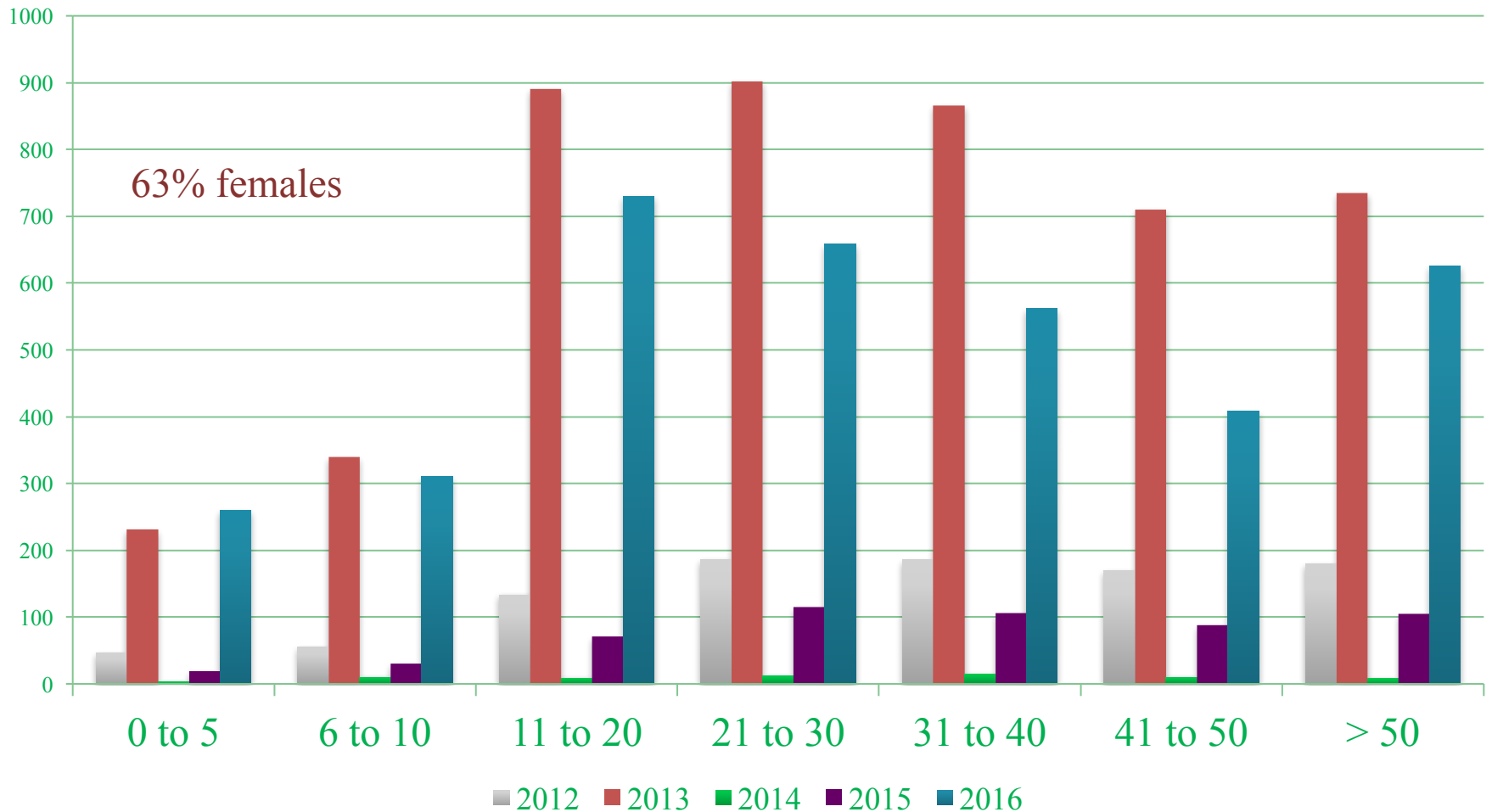


\*As of Dec 31, 2016

# Regional Distribution of Suspected CH... cases, 2012-2016

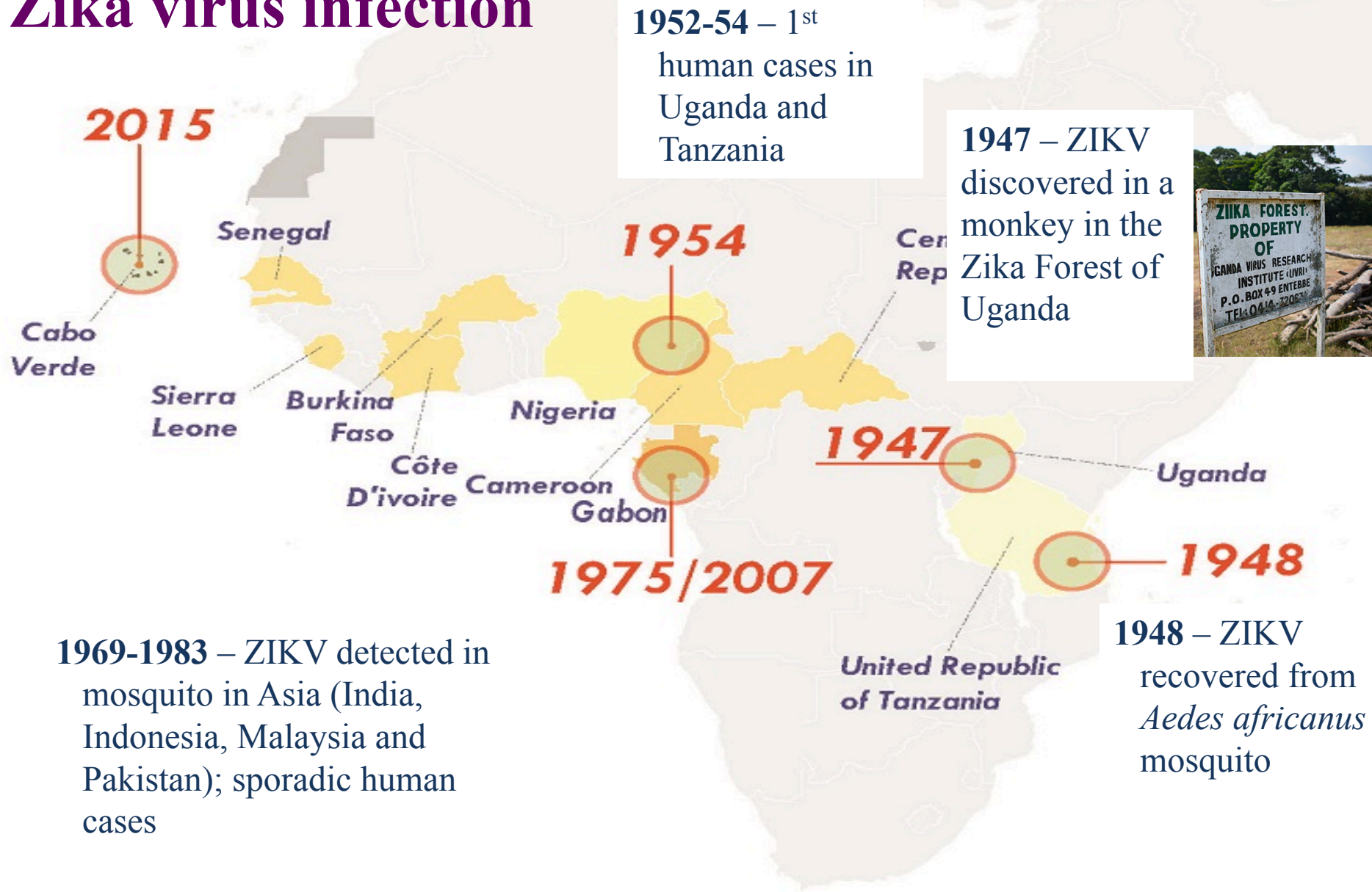


# Age Distribution of Suspected CHIKV cases, 2012-2016

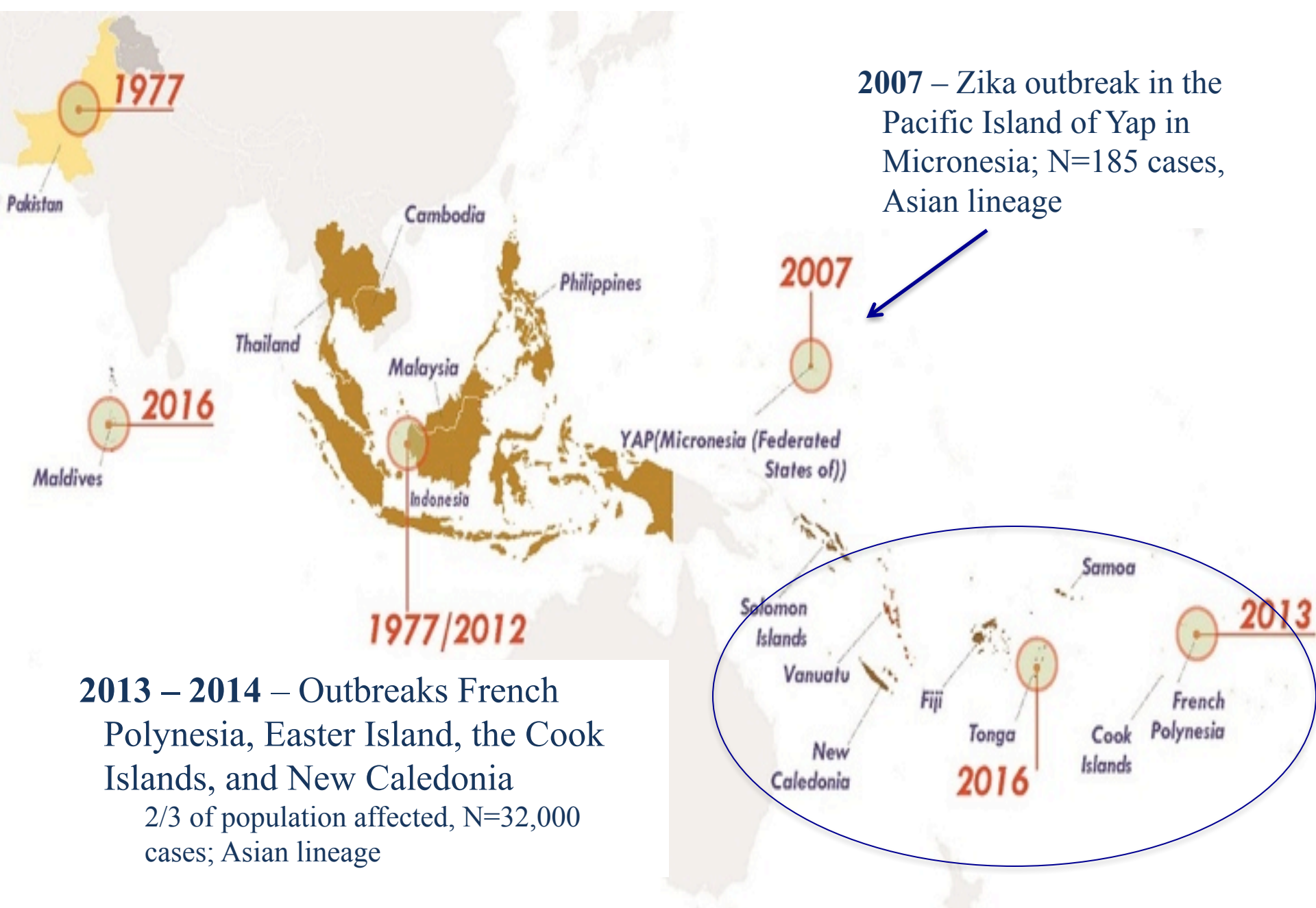




# Zika virus infection







2007 – Zika outbreak in the Pacific Island of Yap in Micronesia; N=185 cases, Asian lineage

2013 – 2014 – Outbreaks French Polynesia, Easter Island, the Cook Islands, and New Caledonia

2/3 of population affected, N=32,000 cases; Asian lineage

# Zika virus infection

---



- Feb-April 2015 – 7000 cases of illness with rash in Brazil; 13% dengue, negative for CHIKV, measles, rubella, parvovirus B19 and enterovirus; no Zika testing done
- May 2015 – ZIKV circulating in Brazil; Asian lineage
- July 2015 – Brazil reports neurological disorders (GBS) asso with Hx of infection (dengue, zika, Chik)
- Sept to Oct 2015 – outbreak in Capo Verde, Africa



# Zika virus infection

---

- Oct 2015 - unusually high rates of neurological disorders (microcephaly) in Brazilian newborns
- Nov 2015 – PAHO issues epid alert for reporting congenital microcephaly/CNS malformations
- Feb 1, 2016 - Zika declared PHEIC because of clusters of microcephaly and other neurological disorders in some affected areas
- April 2016 – Vietnam reports first locally acquired case
- May 2016 – WHO issues public health advice regarding the Olympics
- Nov 2016 – WHO declares end of PHEIC

# Global situation



- Human ZIKV infection appears to have changed in character while expanding its geographical range
  - from an endemic, mosquito-borne infection causing mild illness across equatorial Africa and Asia
  - to an infection causing, from 2007 onwards, large outbreaks, and from 2013 onwards, outbreaks linked with neurological disorders including Guillain-Barré syndrome and microcephaly across the Pacific region and the Americas

WHO situation report, 20 Jan 2017



# Global situation

---

- 76 countries and territories have reported evidence of mosquito-borne Zika virus transmission since 2007 (70 with reports from 2015 onwards)
- 13 - with person-to-person transmission
- 29 – increased incidence of GBS and/or lab confirmation of Zika virus infection among GBS cases

WHO situation report, 20 Jan 2017



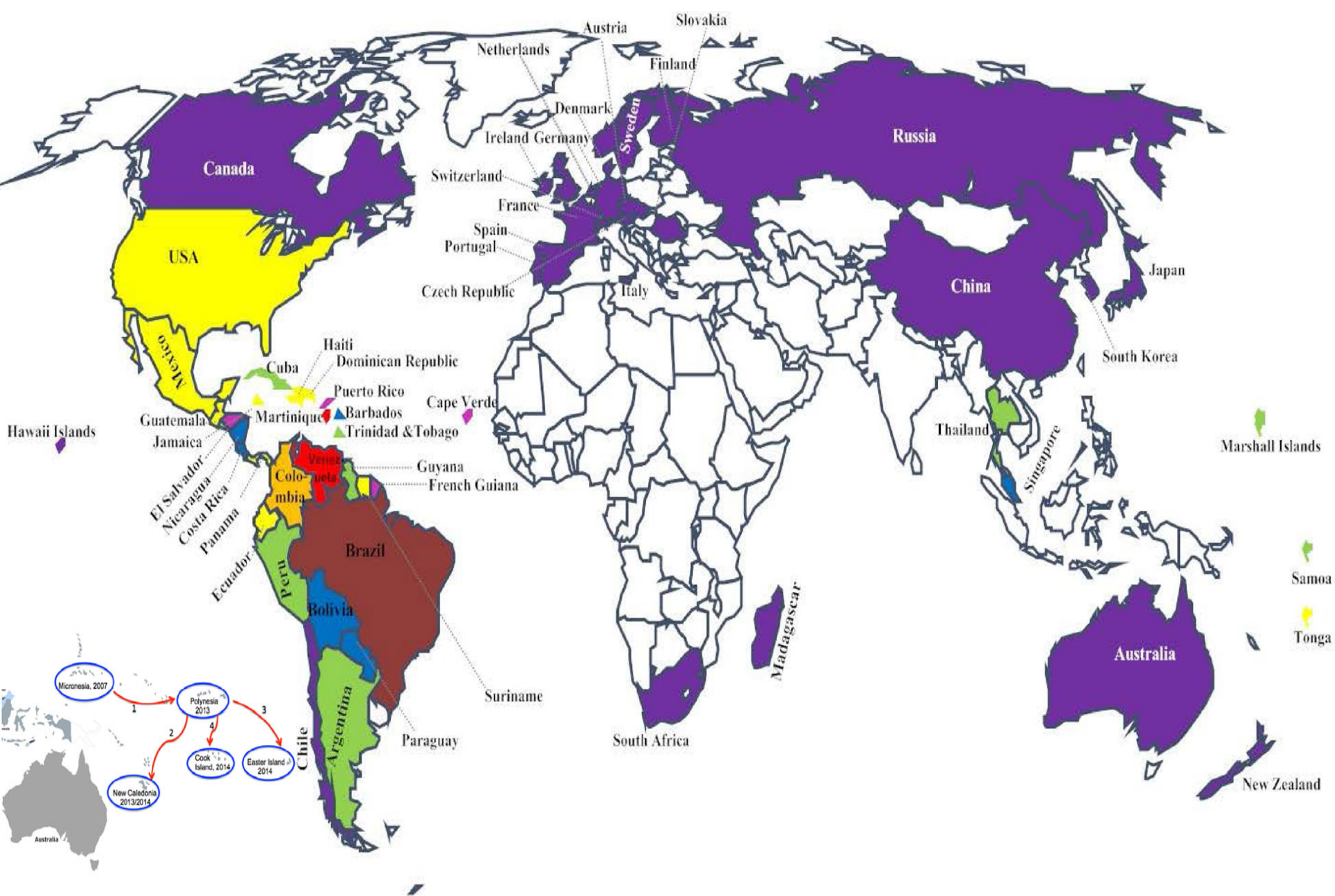
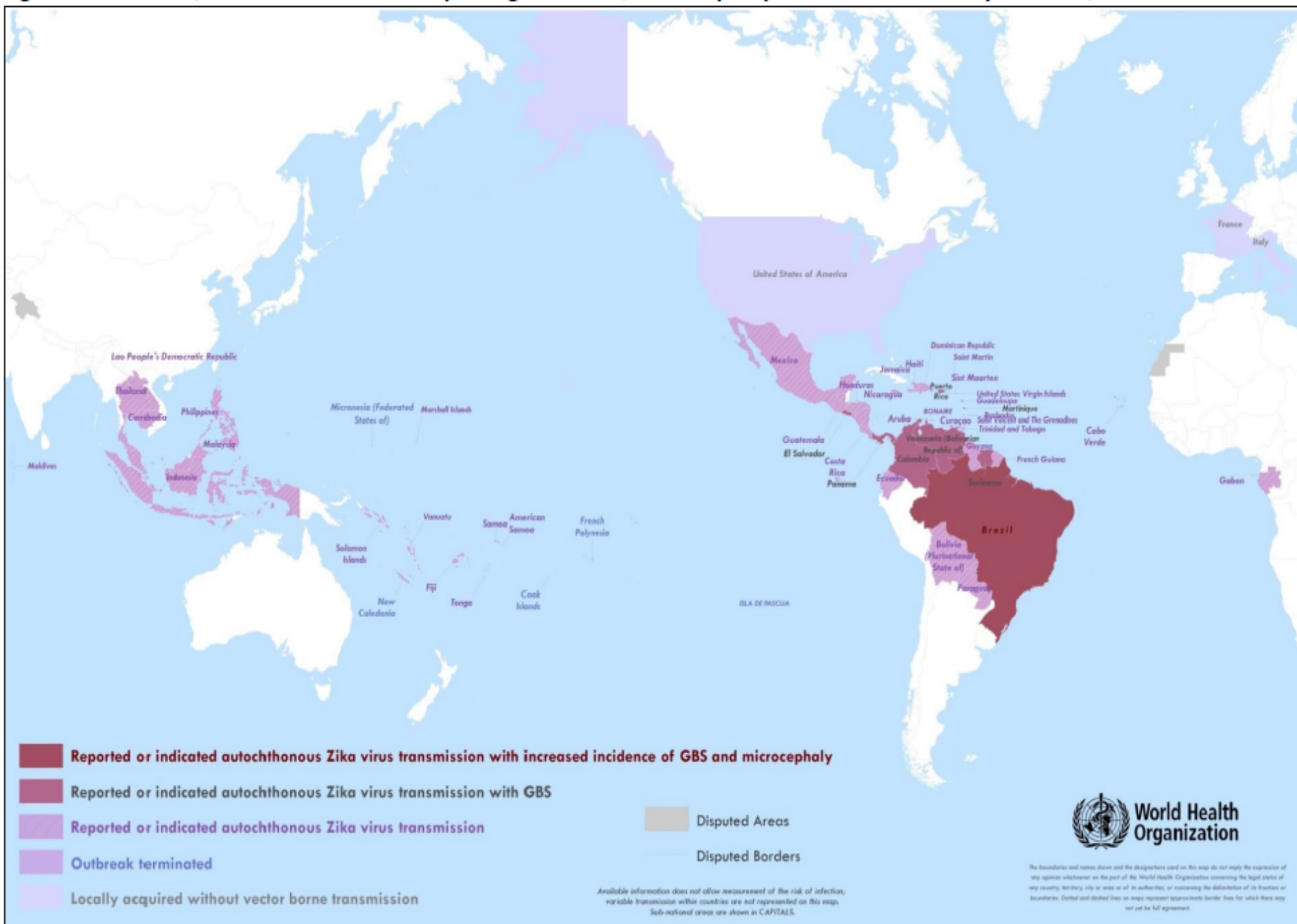


Figure 3. Countries, territories and areas reporting Zika virus, microcephaly and Guillain-Barré syndrome\*, 2007-2016.

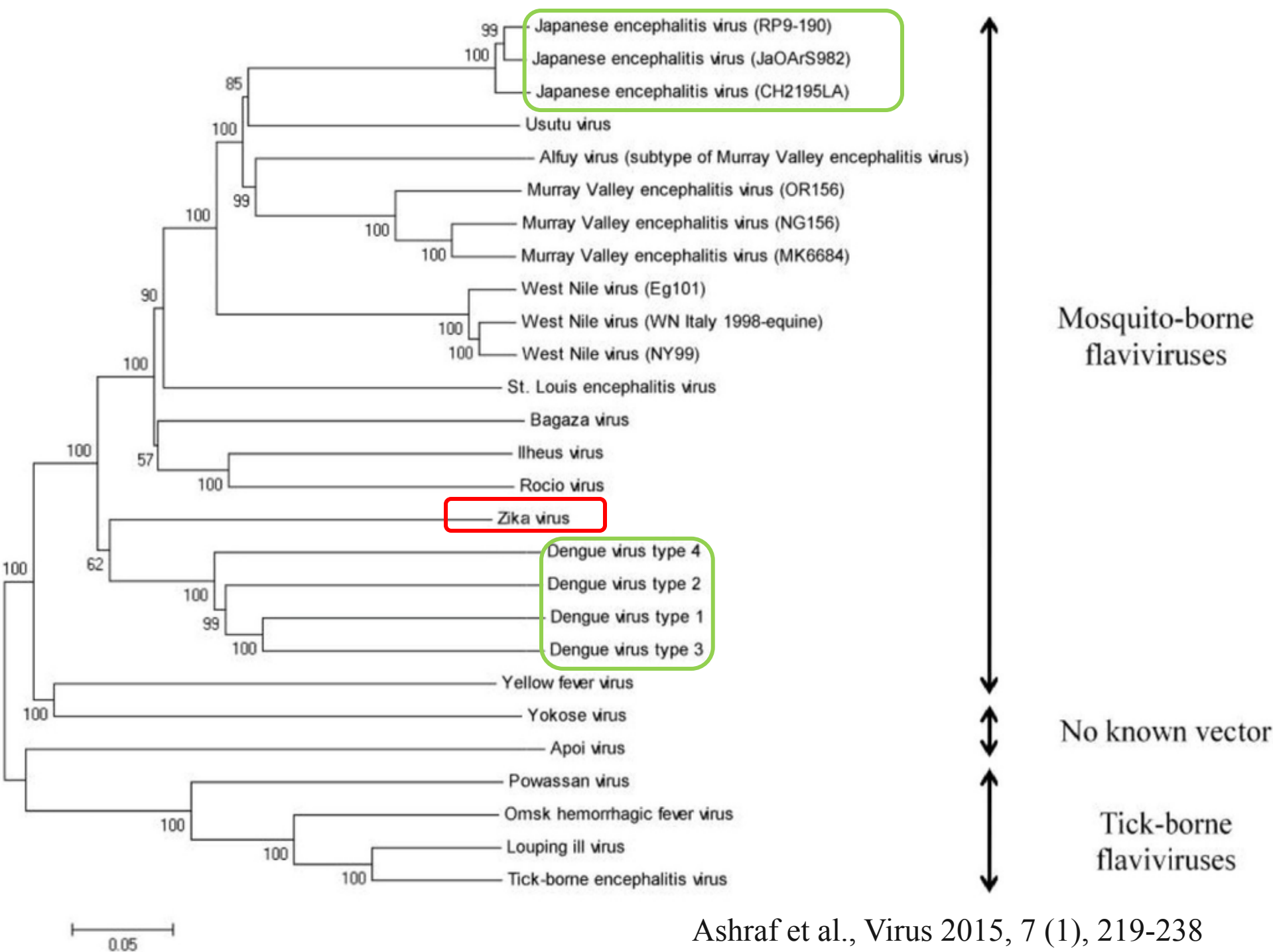


# Etiology

---

- Single stranded RNA virus belonging to the *Flaviviridae* family
- Neurotropic virus that particularly targets neural progenitor cells
- Has 2 distinct genotypes:
  - African
  - Asian
- High cross reactivity with DENV Ab
- Vector – *Aedes* mosquitoes (*Aedes aegypti*, *Aedes albopictus*, *Aedes africanus*, *Aedes hensilli*, *Aedes polynesiensis*, *Aedes furcifer*, *Aedes vitattus*)







# Transmission

---

- Primarily - bite of an infected *Aedes* mosquito
- Sexual transmission - has been detected in semen (69 days), blood, urine, amniotic fluids, saliva
  - Sex includes vaginal/anal/oral sex; sharing of sex toys
  - Zika can be passed through sex before Sx start, during, and after Sx end
  - Highest frequency from M to F
- Mother to child
  - ZIKV detected in amniotic fluid, fetal brain and serum of babies 4 days after birth
  - Congenital transmission - transmitted from a pregnant mother to her fetus during pregnancy
  - Perinatal transmission - around the time of birth

Basi & Tumban. *Virology Journal* 2016

<https://www.cdc.gov/zika/transmission/index.html>



# Transmission

- Blood transfusion
  - 1,505 asymptomatic blood donors (3%) reported to be positive for ZIKV by PCR in French Polynesia outbreak
  - 2 confirmed cases transmitted by BT in Brazil
  - ZIKV can persist in whole blood for 2 months
- Laboratory exposure – 4 reports
- Breastmilk
  - ZIKV has been identified in breast milk
  - No reports of infant ZIKV infection associated with breastfeeding
  - Benefits of breastfeeding outweigh the theoretical risk of ZIKV infection transmission through breast milk

Basi & Tumban. Virology Journal 2016

<https://www.cdc.gov/zika/transmission/index.html>



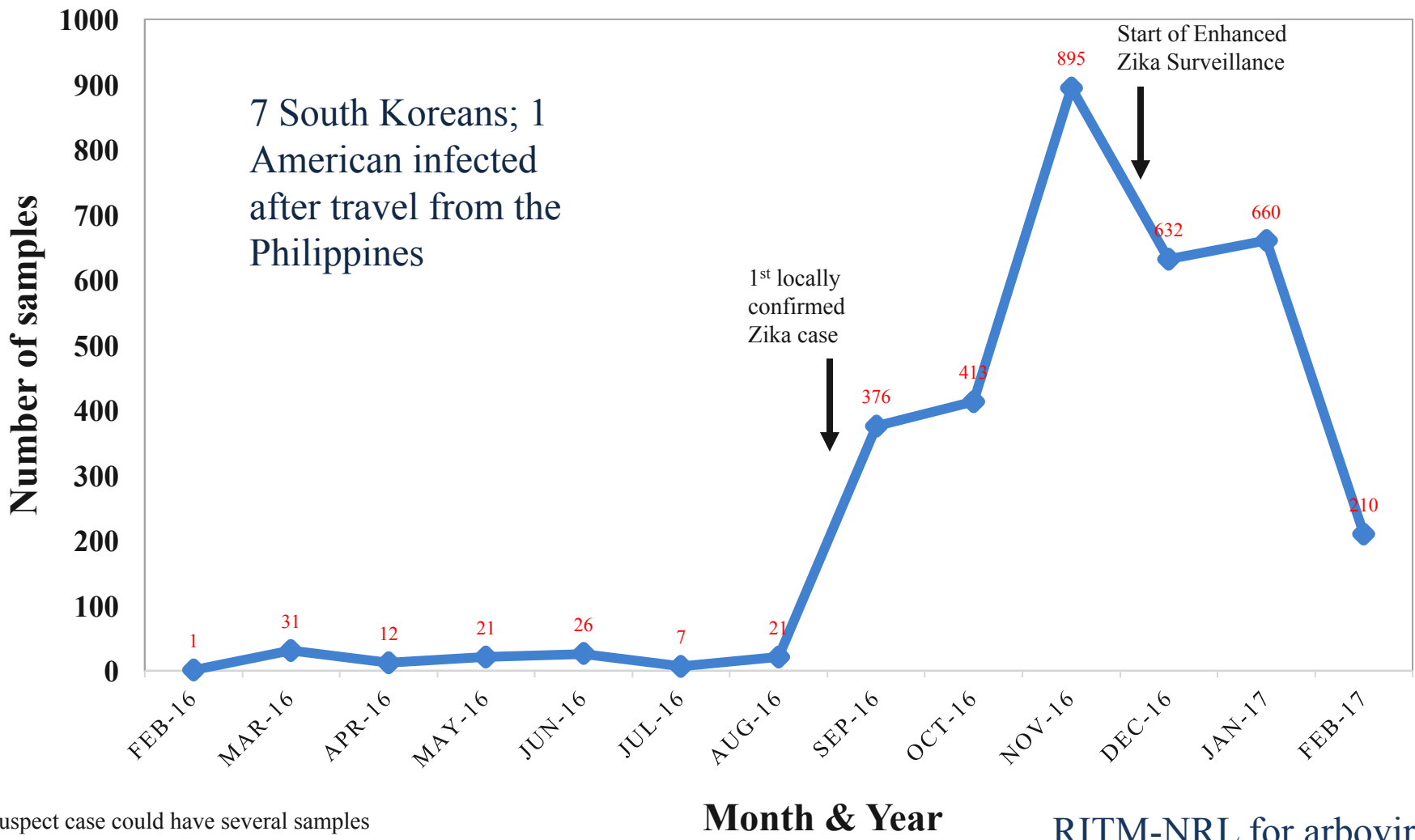
# Zika in the Philippines

---

- May 2012 – first case of ZIKV infection detected as part of active fever surveillance in Cebu
  - serologic testing for evidence of influenza, DENV, CHIKV, JE, and ZIKV infections
  - 15/M with fever, HA, conjunctivitis, sore throat, myalgia, stomach pain, anorexia, nausea and vomiting; NO RASH; no travel Hx
  - Lab confirmation - (+) ZIKV by rtPCR; phylogenetic analysis showed isolate belonged to ZIKV Asian lineage

Alera et al. EID April 2015; 21 (4)

# Distribution of Referred Samples\* for Zika Testing by Month, RITM, Feb. 18, 2016 to Feb. 10, 2017 (N=3,310)

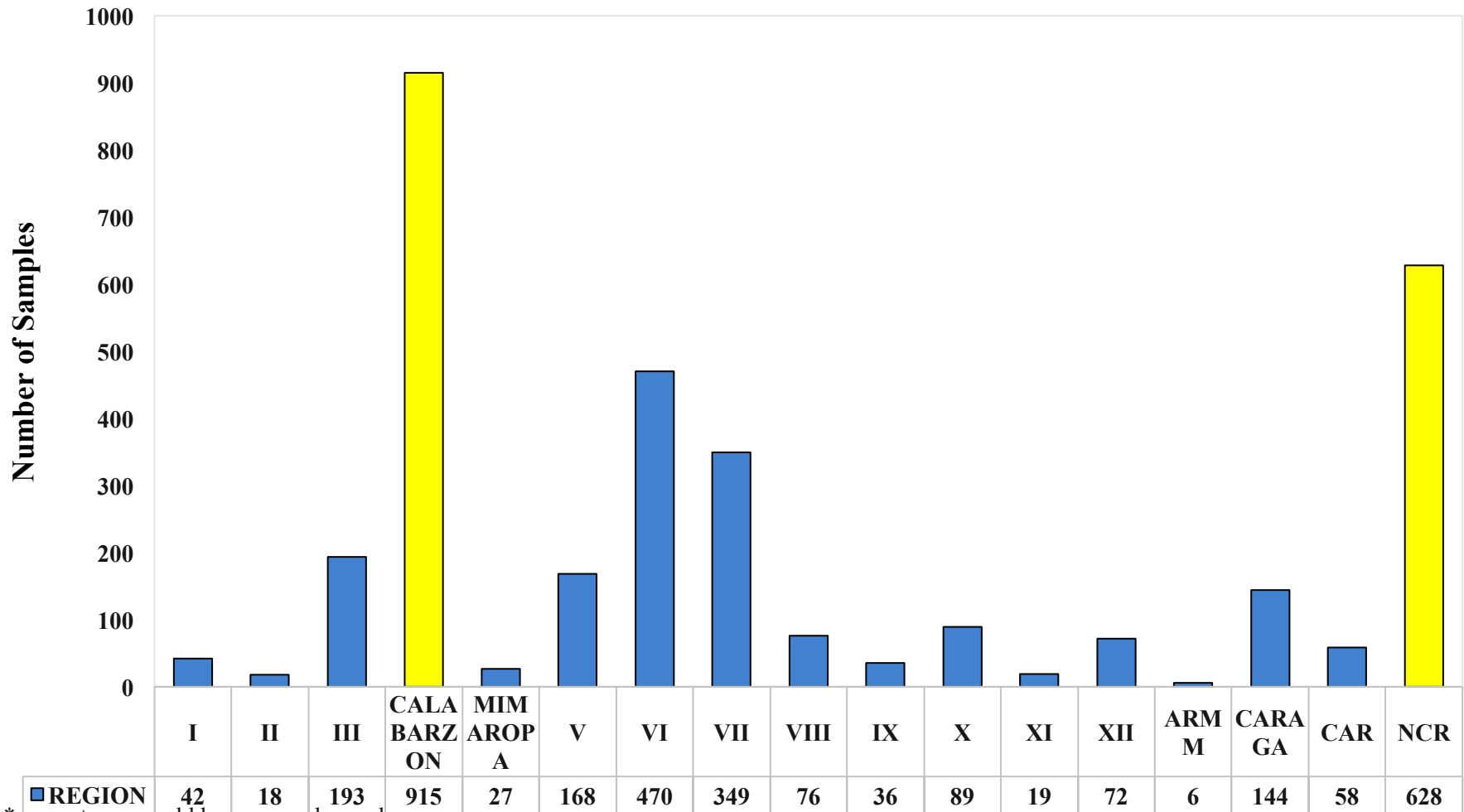


\*suspect case could have several samples

Month & Year

RITM-NRL for arboviruses

# Distribution of Referred Samples\* for Zika Testing by Region, RITM, Feb. 18, 2016 to Feb. 10, 2017 (N=3,310)



\*suspect case could have several samples

RITM-NRL for arboviruses  
RITM SRU

# Zika Suspect Cases from Referred Samples to RITM, Feb. 18, 2016 to Feb. 10, 2017 (n=1,952)



<b>Number of Suspect Cases</b>	<b>1,952</b>
Female	1244
Male	708

Pregnant suspect cases	64
Suspect cases w/ microcephaly	10
Suspect cases w/ GBS	8

# Profile of Locally Confirmed Zika Cases by PCR, Feb. 18, 2016 to Feb. 10, 2017 RITM (n=58)

---



- Age range: 7 – 59 years old (median=32 years)
- Female = 39 (67%); Male=19
- Pregnant = 7 cases (1 delivered, 1 miscarried)
- Asymptomatic = 3 (household members of confirmed cases)

RITM-NRL for arboviruses  
DOH-EB, RITM SRU



# List of Regions and Cities/Municipalities with **Confirmed Zika Cases** as of Feb 8, 2017 (n=58)



Region	City/Municipality	Province	No of Cases
III	Sto Tomas	Pampanga	2
NCR <b>20</b>	Caloocan, Manila, Pasay, QC, Las Pinas, Muntinlupa, Makati, Mandaluyong	Metro Manila	20
IV A	Calamba, Nagcarlan, Binan	Laguna	8
<b>19</b>	Bacoor, Dasmaringas, Ternate	Cavite	7
	Antipolo, Cainta	Rizal	4
VI	Iloilo, Oton	Iloilo	15
VII	Cebu	Cebu	1
	Sibulan	Negros Oriental	1



---

# Recognition and Diagnosis



# JE Clinical Manifestations

- IP – 4-14 days
- > 99% are subclinical; Sxtic infection is mild (fever and headache)
- Severe clinical illness - Approx 1 in 250 infections
  - Rapid onset of high fever, headache, neck stiffness, disorientation, coma, seizures, spastic paralysis and ultimately death
  - In children, GI pain/dysfunction in initial stage of the disease
    - Convulsions very common in children
  - CFR - as high as 30 – 35 %
  - Of survivors, 20%–50% suffer permanent intellectual, behavioural or neurological problems

[www.cdc.gov/japaneseencephalitis](http://www.cdc.gov/japaneseencephalitis)  
WHO position paper on JE, 2014

# Case definition for AES Surveillance



- **AES (Suspected JE) Case:** a case of **Acute Encephalitis Syndrome (AES)** is defined as a person of any age, with the acute onset of fever **and at least one** of the following :
  - Change in mental status (e.g.confusion, disorientation, coma or inability to talk)
  - New onset of seizures (excluding simple febrile seizures)
- ***Clinical case: a case that meets the suspect case definition***

DOH PIDSR

# Case definition for AES Surveillance



- ***Probable JE:*** an AES case that occurs in close geographical and temporal relationship to a laboratory-confirmed case of JE, in the context of an outbreak
- ***Laboratory-confirmed JE:*** an AES case that has been laboratory confirmed as JE

## **Laboratory Confirmation:**

- Presence of JE virus-specific IgM Ab in a single sample of CSF or serum, as detected by an IgM-capture ELISA

IgM-capture ELISA detects specific IgM in CSF/blood within 4-7 days of onset of disease



# Differential diagnosis

---

- CNS infections - bacterial meningitis, TB cerebral malaria
- Other encephalitides - caused by other arboviruses and herpesviruses
- Sivamani et al. Neurology India 2017
  - Case report of dengue and JE in a patient
  - Dengue encephalitis should be considered in the DDx of any encephalitis in tropical countries



# JE Laboratory Diagnosis

- IgM ELISA

## JE testing at RITM, 2014-2016

Year	No. tested	% positive
2014	656	11.7
2015	1497	16.7
2016	2069	26.5

RITM NRL for arboviruses

# Dengue Clinical Manifestations

---

- IP – 4–10 days after the bite from an infected mosquito
- High fever (40°C/104°F) accompanied by 2 of the following symptoms
  - severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash
- Sx usually last for 2–7 days
- Severe dengue – warning signs
  - severe abdominal pain, persistent vomiting, rapid breathing, bleeding, fatigue, restlessness

<http://www.who.int/mediacentre/factsheets/fs117/en/>



# Case definition: Dengue without warning signs

---



- *Suspect*
  - A previously well person with acute febrile illness of 2-7 days duration plus **two** of the following:
    - Headache, Body malaise, Myalgia, Arthralgia, Retro-orbital pain, Anorexia, Nausea, Vomiting, Diarrhea, Flushed skin, Rash (petechial, Herman's sign)
- *Probable*
  - *A suspect case plus:*
  - Laboratory test, at least CBC (leucopenia with or without thrombocytopenia) and/or Dengue NS1, antigen test or dengue IgM antibody test (optional)
- *Confirmed:*
  - Viral culture isolation

# Case definition: Dengue with warning signs



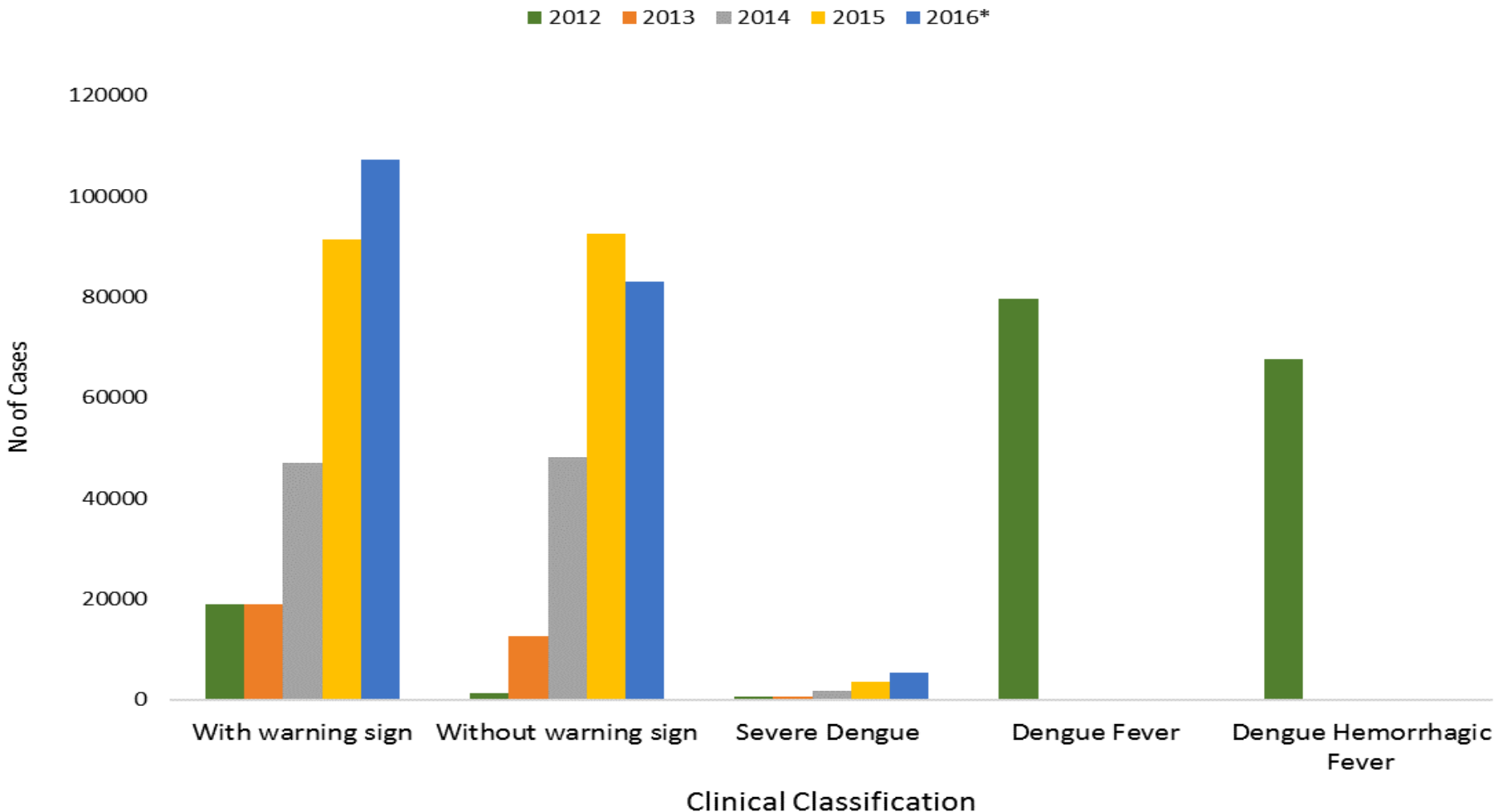
- A previously well person with acute febrile illness of 2-7 days duration plus any **one** of the following:
  - Abdominal pain or tenderness
  - Persistent vomiting
  - Clinical signs of fluid accumulation
  - Mucosal bleeding
  - Lethargy, restlessness
  - Liver enlargement
  - Laboratory: increase in Hct and/or decreasing platelet count



# Case definition: Severe Dengue

- A previously well person with acute febrile illness of 2-7 days duration and any of the clinical manifestations for dengue with or without warning signs:
- Plus any of the following:
  - **Severe plasma leakage leading to**
    - Shock
    - Fluid accumulation with respiratory distress
  - **Severe bleeding**
  - **Severe organ impairment**
    - Liver: AST or ALT  $\geq 1000$
    - CNS: seizures, impaired consciousness
    - Heart: myocarditis
    - Kidneys: renal failure

# Suspect Dengue Cases by Clinical Classification Philippines, 2012-2016\*



\*As of Dec 31, 2016



# Dengue Laboratory Confirmation

- Real time PCR

## Dengue testing at RITM, 2014-2016

Year	# tested	# positive	Positivity rate*
2014	2413	1165	48.3%
2015	6463	3044	47.1%
2016	5587	2100	37.6%

RITM NRL for arboviruses

# JE versus Dengue



Points	Japanese encephalitis	Dengue
Age	Children	All ages
Season	Rainy	Rainy
Duration of illness	Fever 2-3 days	Fever 2-7 days
Prodrome Sx	Sudden onset of fever, chills, HA, myalgia	High fever, severe HA, myalgia, <b>arthralgia, N/V, rash</b>
Clinical features	<b>Rapid progression to deep coma</b> , focal deficit, seizures	<b>Altered sensorium</b> , focal deficit, seizures
Blood changes	Leukopenia	<b>Thrombocytopenia</b> , leukopenia
Metabolic/circulatory changes	Uncommon	Common
CSF changes	<b>Inc WBC (lymph)</b>	<b>Normal</b>
Diagnosis	IgM AB in serum/CSF	Dengue PCR, NS1, IgM
Outcome	MR 20-30%	Variable; high MR w/o supportive Tx

# Chikungunya Clinical Manifestations



- Classical symptoms:
  - Fever
  - Maculopapular Rash
  - Persistent joint pains
- Less hemorrhagic tendencies compared to Dengue
- Sequelae - persisting arthralgia/arthritis, alopecia, depression



A. Edematous rash of the face



B. Edematous polyarthritis of the hands



I. End of the acute stage. Swollen hands and fine desquamation



J. Hyperpigmentation



O. Erythema that blanches with pressure



D. Periarticular swelling and joint effusion in knees



K. Tenosynovitis in hands



L. Tenosynovitis in ankle



E. Maculopapular rash in trunk and extremities



F. Maculopapular rash in extremities, including palms



M. Elbow hygroma



N. Swollen and stiff hands in a 55-year-old man who was infected 5 years earlier



# SIGNS AND SYMPTOMS OF CHIKUNGUNYA CASES

## PHILIPPINES, AS OF OCTOBER 1, 2016

	No	Yes	Total
<b>Fever</b>	346	3289 90%	3635
<b>Skin Manifestation</b>	1007	2628 72%	3635
<b>Arthritis</b>	1059	2576 71%	3635
<b>Arthralgia</b>	1660	1975 54%	3635
<b>Head Ache</b>	1758	1877 52%	3635
<b>Myalgia</b>	2064	1571	3635
<b>Back Pain</b>	2220	1415	3635
<b>Nausea</b>	3041	594	3635
<b>Periarticular Edema</b>	3089	546	3635
<b>Asthenia</b>	3109	526	3635
<b>Vomiting</b>	3322	313	3635
<b>Mucosal Bleeding</b>	3608	27	3635



# Chikungunya Case Definition

- **Suspected case**

- a patient with acute onset of fever, rash (over limbs or trunk) and severe arthralgia or arthritis not explained by other medical conditions

- **Confirmed case**

- a suspect case with any of the following CHIK specific tests:
  - Detection of viral RNA by RT-PCR
  - Detection of IgM in a single serum sample (collected during acute or convalescent phase)
  - Four-fold increase in CHIKV-specific antibody titers (samples collected at least two to three weeks apart)
  - Viral isolation



# CHIKV Laboratory Confirmation

- IgM ELISA

## CHIKV testing at RITM, 2014-2016

Year	# tested	# positive	Positivity rate*
2014	820	293	35.7%
2015	548	202	36.8%
2016	1833	1051	57.3%

RITM NRL for arboviruses



# Chikungunya vs dengue

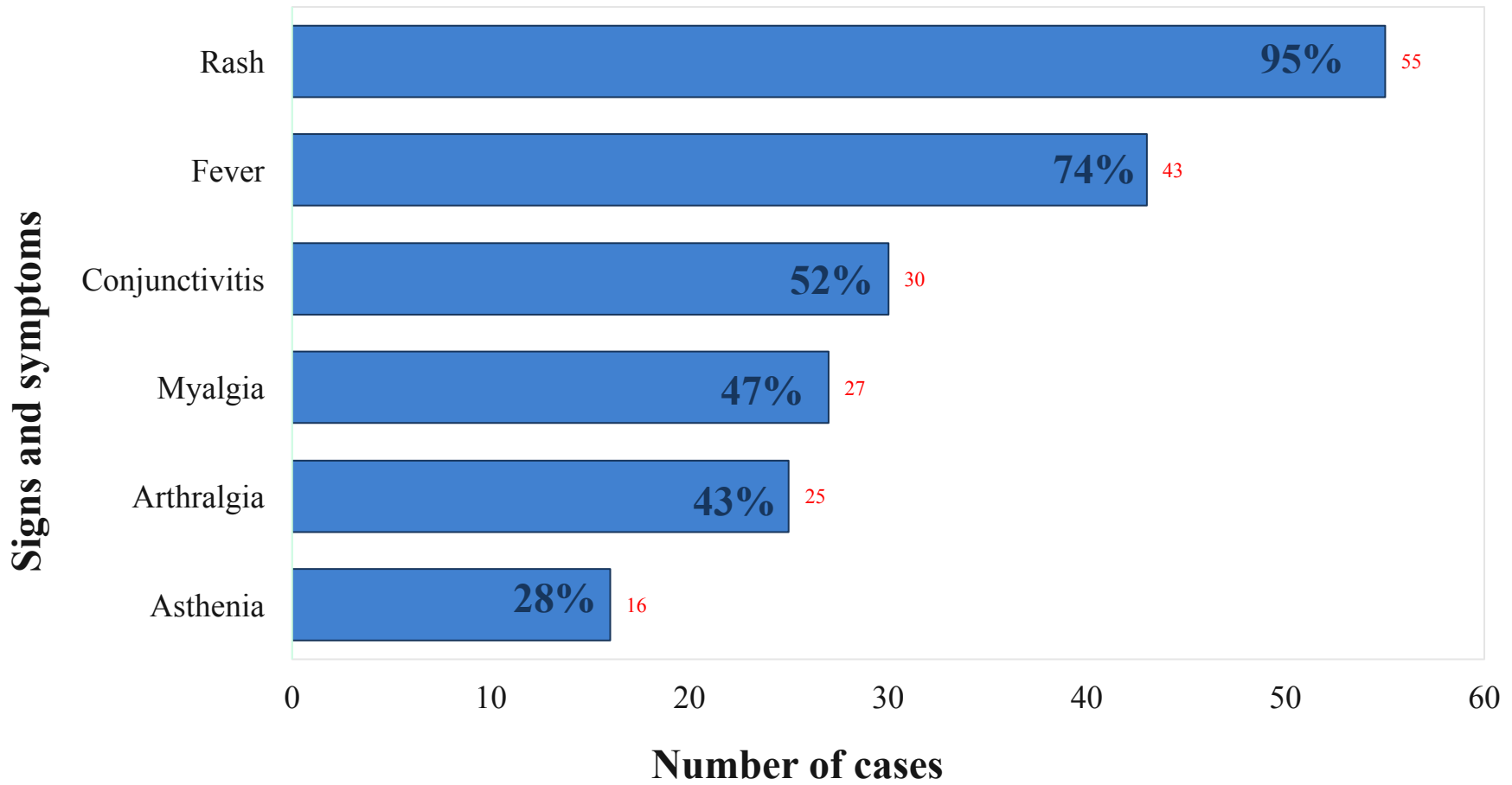
- Difficult to distinguish CHIK and DENV infections based on clinical findings alone
  - CHIKV more likely to cause high fever, severe polyarthralgia, arthritis, rash, and lymphopenia
  - DENV more likely to cause neutropenia, thrombocytopenia, hemorrhage, shock, and death
- CHIKV and DENV transmitted by the same mosquitoes
  - The viruses can circulate in the same area and cause occasional co-infections in the same patient
- Patients with suspected CHIKV should be managed as dengue until dengue has been ruled out

# Zika Clinical Manifestations

- Acute onset of mild fever, maculopapular rash, non-purulent conjunctivitis, muscle and joint pain, malaise or headache for 2-7 days

Symptom	Brazil N=57	Puerto Rico N=30
Rash	98%	77%
Headache	67%	
Fever (37.5 – 38.5 °C)	67%	73%
Arthralgia	58%	73%
Myalgia	49%	77%
Retro orbital pain	40%	
Conjunctivitis	39%	
Joint swelling	23%	

# Signs and Symptoms of PCR Confirmed Zika Cases, Feb. 8, 2016 to Jan. 25, 2017 (n=58)





# Zika Complications

- Neurological problems such as GBS
  - 98-100% of patients diagnosed with GBS during the French Polynesia ZIKV outbreak had anti-ZIKV Ab compared to 56 % of patients without GBS
- Miscarriage and Congenital Zika Syndrome
  - Pattern of birth defects found among fetuses and babies infected with Zika virus during pregnancy
  - Maternal ZIKV infection → placental infection and injury → transmission of the virus to the fetal brain → kills neuronal progenitor cells and disrupts neuronal proliferation, migration, and differentiation → slows brain growth and reduces viability of neural cells → severe congenital abnormalities

Costelo et al. Bull WHO 2016

Basi & Tumban. Virology Journal 2016



# Congenital Zika infection

---

- 5 features:
  - Severe microcephaly where the skull has partially collapsed
  - Decreased brain tissue with a specific pattern of brain damage
  - Damage to the back of the eye
  - Joints with limited range of motion, such as clubfoot
  - Too much muscle tone restricting body movement soon after birth

[https://www.cdc.gov/zika/healtheffects/birth\\_defects.html](https://www.cdc.gov/zika/healtheffects/birth_defects.html)



# Zika Case definition

---

## *Suspected case*

- A. patient with **Skin Rash** and one of the following:
- Fever (reported, measured, within the past 5 days prior to consultation)
  - Arthralgia
  - Arthritis
  - Conjunctivitis
- 
- All newly diagnosed GBS

Dm2016-0116-A, DOH, July 2016



# Zika Case definition

---

## *Suspected case*

B. A mother whose fetus, newborn or infant has any neurological condition listed below that cannot be explained by other etiologies:

- Head circumference less than the -3 Standard Deviation (<-3SD) or occipitofrontal circumference < 3rd percentile on standard growth charts OR
- Disproportionately small head compared to infant's length, OR
- Intra-cranial calcifications

C. A fetus, newborn or infant whose mother had confirmed or presumed infection with ZIKV during pregnancy

Dm2016-0116-A, DOH, July 2016

# Zika Case definition

---

## *Probable case*

A suspected case with Zika virus who tested positive for IgM serology for Zika virus

## *Confirmed case*

A suspected or probable case of ZIKV virus (ZIKV) who tested positive by:

- Real-time PCR (Polymerase Chain Reaction)
- Virus isolation in any body fluid

Dm2016-0116-A, DOH, July 2016



# Zika Laboratory Diagnosis

- Collect sample during acute phase (3-7 days after Sx onset) - serum, CSF, amniotic fluid, saliva, urine
- RT-PCR – test of choice
- Serologic test
  - ELISA IgM/IgG or plaque-reduction neutralization test (PRNT)
- Test concomitantly for dengue, CHIKV
- Congenital ZIKV infection
  - Test cord blood, CSF, placenta within first 2 days
  - Neuro-imaging
    - Pre-natal – obstetric ultrasound
    - Post-natal – CT scan or MRI

Nunez et al, J Pediatr (Rio J) 2016

# Distribution of Referred Samples of Zika Tested by PCR\*\*, RITM, Feb. 18, 2016 to Feb. 10, 2017 (n=2,049)



Type of Sample	ChikV	Den 1	Den 2	Den 3	Den 4	Zik V	Negative	Pending
Serum 1	76	9	7	15	2	13	589	4
Serum 2	1	0	0	0	0	0	6	0
Urine 1	26	70	16	21	1	62	1447	35
Urine 2	0	0	0	1	0	2	7	0

\*\*Serum 2 and Urine 2 were collected from confirmed cases

RITM NRL for arboviruses  
RITM SRU

# Differentiation between DENV, CHIKV and ZIKV infections



Feature	Dengue	Chikungunya	Zika
Incubation Period	4-7 days	3-7 days	3-12 days
Sex predilection	None	F > M	None
Age group	All ages	All ages	All ages
% Symptomatic	20-60%	70%	20%
Recovery	6-7 days	7-10 days	4-7 days
Sequelae	Depression, asthenia, bradycardia	Chronic joint pain	Recovery
Vaccine	Yes	No	No

<http://www.biovecblog.org/blog/symptom-differences-between-zika-chikungunya-and-dengue-fever>  
 Feigin and Cherry. Textbook of Pediatr Infect Dis 6<sup>th</sup> ed

# Differentiation between DENV, CHIKV and ZIKV infections



S/Sx	Dengue	Chikungunya	Zika
Fever	High grade; biphasic	High grade	Low grade
Rash	+	++	+++
Headache	<b>++ severe</b>	+	+
Arthralgia	+++ Moderate	<b>++++ Severe</b>	++, mild
Pruritus	+	+	++/+++
Conjunctivitis/ conj injection	-	+	+++
Myalgia	+++	+	+
Limb edema	-	-	++

<http://www.biovecblog.org/blog/symptom-differences-between-zika-chikungunya-and-dengue-fever>  
 Feigin and Cherry. Textbook of Pediatr Infect Dis 6<sup>th</sup> ed



# Differentiation between DENV, CHIKV and ZIKV infections



S/Sx	Dengue	Chikungunya	Zika
Shock	+/-	-	-
Bleeding	++	+/-	-
Other S/Sx	Nausea/vomiting, retro orbital pain, <b>taste aberration, anorexia, LN</b>	LN, <b>convulsions</b>	GI, retro orbital pain, sore throat
Neutropenia	+++	+	-
Hemoconc	+++	-	-
Thrombocytopenia	+++	+	-

Feigin and Cherry. Textbook of Pediatr Infect Dis 6<sup>th</sup> ed;

Beltran-Silva et al. Rev Med Gen Mex 2016, in press

<http://www.biovecblog.org/blog/symptom-differences-between-zika-chikungunya-dengue-fever>



---

# Research



# JE Research

---

- Re-emergence of JE in South Korea, 2010-2015
  - Shift in age distribution toward older groups (unvaccinated or Ab have waned) after initiation of vaccination programs

Sunwoo et al, EID 2016

- Genotype V JE virus is emerging
  - XZ0934, isolated from *Culex tritaeniorhynchus* in China

Li et al, PLoS NTC 2011

- Low Protective Efficacy of current JE vaccine against Genotype V
  - Only 25 % of children who received current JE vaccine developed neutralizing Ab to JEV genotype V

Cao et al, PLoS NTC 2016



# Dengue Research

- Early Dengue classifier
  - Dxtic algorithm using the patient's age, total WBC and platelet count at presentation → 75 % sensi/speci for Dx of dengue; addition of NS1 rapid test improved sensi to > 90%

PLoS Negl Trop Dis 2015;9:e0003638

- Antivirals - chloroquine, balapiravir (polymerase inhibitor developed for Tx of hepatitis C) and celgosivir (a cellular glucosidase inhibitor)
  - no evidence of reduction in plasma viremia or prevention of Cx  
PLoS Negl Trop Dis 2010; Lancet Infect Dis 2014; JID 2013;
- High dose steroids given during febrile phase to prevent or attenuate severe disease
  - trend for hyperglycemia; no evidence of efficacy in preventing DSS

PLoS Negl Trop Dis 2013;7:e2592



# Ongoing Dengue Researches

---

- Identification of clinical and laboratory warning signs with high predictive power for patients at high risk of progression
- Role of prophylactic platelet transfusion
- Lovastatin - anti-inflammatory and endothelial-stabilising properties



# Chikungunya Research

- Prolonged shedding of Chikungunya virus in semen and urine
  - Semen and urine specimens were positive for CHIKV after 30 days of symptoms onset

IDCases 6 (2016) 100–103

- Anti-virals against CHIKV and other alphaviruses
  - Abamectin and ivermectin > berberine reduced levels of viral RNA synthesis as well as downregulate CHIKV protein expression

Antiviral Research 126 (2016) 117e124

- Mefenamic acid in combination with ribavirin shows significant effects in reducing CHIKV infection

- MEFE in combination with RIBA exhibited significant anti-CHIKV activity by impairing viral replication in vitro and in vivo

Antiviral Research 127 (2016) 50e56



# Zika Research

- Microcephaly and Zika virus: a clinical and epidemiological analysis of the Brazil outbreak
  - clear temporal association between the increased reporting of cases of microcephaly and the ZIKV epidemic in Brazil

Nunez et al, J Pediatr (Rio J) 2016

- Zika Virus from Southeast Asian Perspective
  - ZIKV circulating throughout SEA for 6-60 years
  - Current pattern of cases observed is consistent with spillover from sylvatic transmission cycles
  - ZIKV has undergone marked change and may return to SEA in a devastating form

Wikan and Smith. Asian Pacific J of Trop Med 2017

# Co-Infection with DENV, CHIKV and ZIKV

---



- 2013 – 28/106 (26%) suspected dengue cases were also positive for CHIKV in India
- 15/18 (83%) dengue confirmed cases were positive for CHIKV

Saswat et al. *Infection, Genetics and Evolution* 2015

- 33 y/o pregnant woman from Colombia, G3P1 co-infected with DENV, CHIKV and ZIKV at 14.6 weeks AOG; S/Sx included non-purulent bilateral conjunctivitis, maculopapular rash, HA, mild-to-intense arthralgia, limb edema

Villamil-Gomez et al. *Int J Infect Dis* 2016



# Enhancement of DENV and ZIKV Infections



- **Anti-DENV** HMAbs, cross-react, do not neutralize, and **greatly enhance ZIKV infection** in vitro
- Pre-existing DENV immunity may enhance ZIKV infection in vivo and may lead to increased disease severity  
*Paul et al. Clinical and Translational Immunology (2016) 5 e117*
- Ab directed against Zika virus envelope protein domain I/II can enhance DENV infection

*Stettler et al. Science 2016, 353 (6301): 823-826*



# Research Gaps

---

- Disease epidemiology
  - Risk factors, cohort studies, impact of climate change
- Development of diagnostic tests – point of care tests
- Vector control and surveillance
  - Insecticide resistance
- Vaccine development
  - Long term effectiveness
  - Safety – pregnant
- Case management
  - Treatment algorithms
  - Early warning signs
  - Antivirals

# Summary

---



- Mosquito-borne disease epidemics have become more frequent and diverse and are likely to continue for several years
- Improved surveillance and response measures:
  - To mitigate the already heavy burden on health systems
  - To limit further spread to other parts of the world

Roth et al. Euro surveillance 2014; 19 (41)



**Thank you**