Keeping Microbes at our Doorstep

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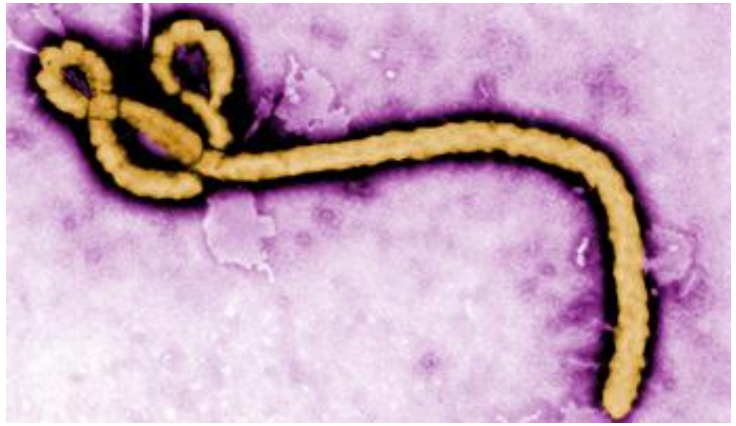
AMMI CANADA-CACMID Plenary on Global Health, Vancouver March 31, 2016

Infection Control

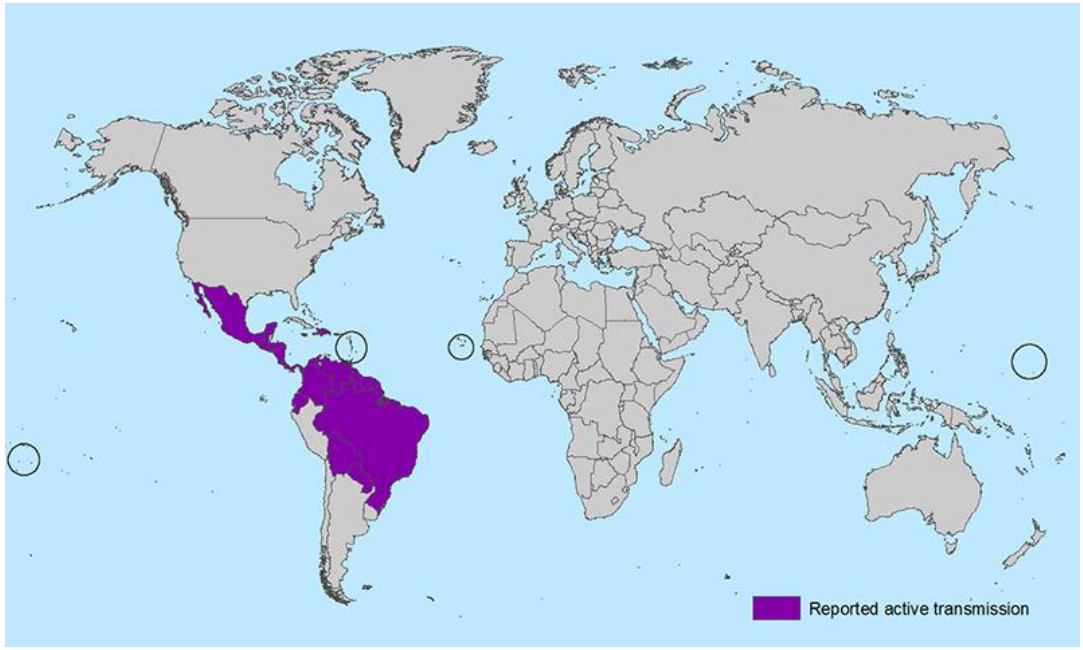


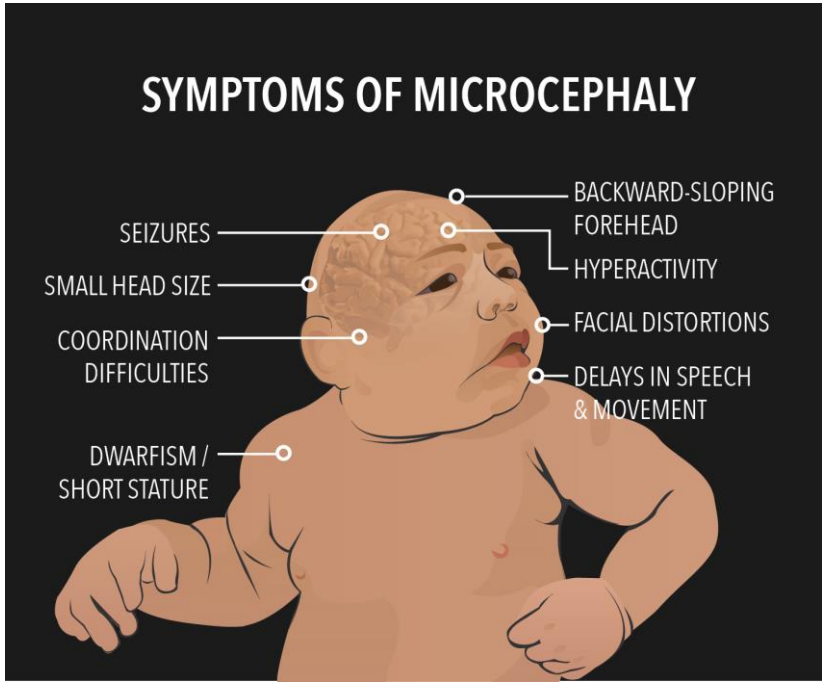
Objectives

- The challenge of anticipating risk
- Proving causality
- Preventing Transmission
- Lines of research inquiry

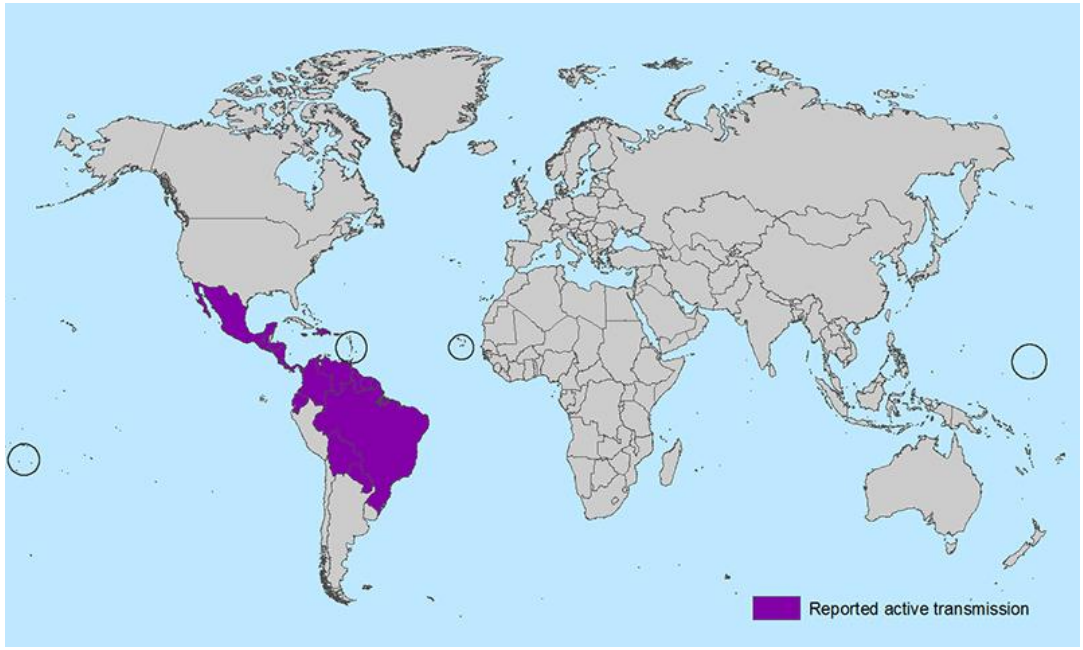
“As we know, there are known knowns; there are things we know we know. We also know there are known unknowns; that is to say we know there are some things we do not know. But there are also **unknown unknowns – the ones we don't know we don't know…it is the latter category that tend to be the difficult ones**”

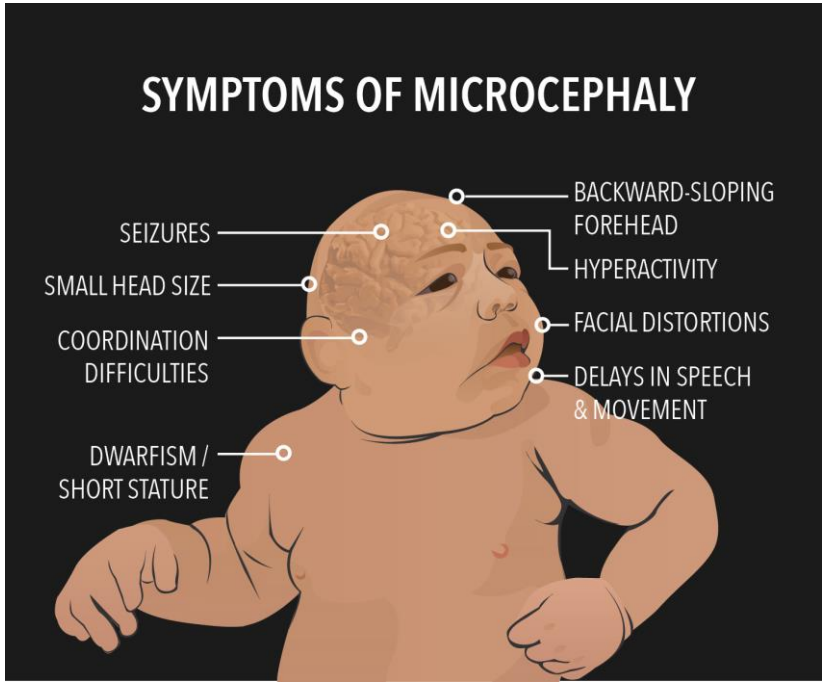
Zika Virus Outbreak





“Known Unknown or Unknown Unknown?”





Yap Island Outbreak – 2007

Table 1. Clinical Characteristics of 31 Patients with Confirmed Zika Virus Disease on Yap Island during the Period from April through July 2007.

Sign or Symptom	No. of Patients (%)
Macular or papular rash	28 (90)
Fever*	20 (65)
Arthritis or arthralgia	20 (65)
Nonpurulent conjunctivitis	17 (55)
Myalgia	15 (48)
Headache	14 (45)
Retro-orbital pain	12 (39)
Edema	6 (19)
Vomiting	3 (10)

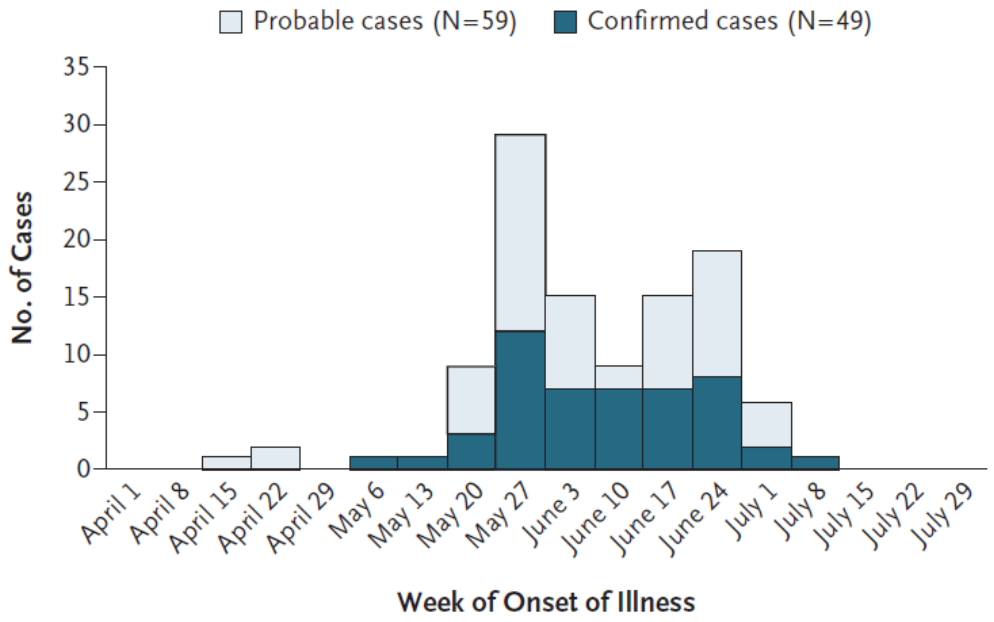


Figure 2. Confirmed and Probable Cases of Zika Virus Disease on Yap among Persons Seeking Health Care, According to Week of Onset of Illness during the Period from April through July 2007.

Outbreak in French Polynesia – 2013

- About 10,000 cases were registered
- 70 were severe cases, including neurological (GBS, meningoencephalitis) or autoimmune (thrombocytopenic purpura, leukopenia) complications
- 42 registered cases of GBS
- The vectors were Aedes aegypti and Aedes polynesiensis
- Two cases positive ZIKAV RT-PCR performed on serum collected within four days post-delivery

Other reports of Adverse Outcomes of Pregnancy after Mosquito-borne Flaviviral Infections

- Spontaneous abortion after JEV infection
- Neonatal dengue has followed congenital dengue infection
- SLEV, JEV, WNV infection of pregnant mice has caused abortion and stillbirth, as well as neonatal hydrocephalus
- Abortion, stillbirth, neonatal death reported in offspring of pregnant sheep infected with WNV

Chye JK et al Clin Infect Dis. 1997;25:1374 –1377 8; Kerdpanich A et al Southeast Asian J Trop Med Public Health. 2001; 32:488 – 493 9; Poli L et al. Bull Soc Pathol Exot. 1991;84:513–521 10; Andersen AA et al. Infect Immun. 1975;12:1173–1183 11; Mathur A et al 1981;34: 26 –29 13; Barnard BJ et al Onderstepoort J Vet Res. 1986;53:235–238

Birth Outcomes Following West Nile Virus Infection of Pregnant Women in the United States: 2003-2004

- 2003-2004 women in the US with WNV infection during pregnancy were reported to CDC
- Data on pregnancy outcomes were collected
- 77 women infected with WNV, 71/77 delivered live infants; 4 miscarriages, 2 elective abortions
- 54/55 negative for anti-WNV IgM from cord serum

O'Leary DR et al. Pediatrics 2006;117:e537-45

Birth Outcomes Following West Nile Virus Infection of Pregnant Women in the United States: 2003-2004

TABLE 1 Characteristics of Live-Born Infants of 71 WNV-Infected Pregnant Women

Measurement	N[a]	Mean (Range) or *n* (%) With Condition
Gestational age, wk	71	39 (26–43)
APGAR score 1 min	59	8 (1–9)
Apgar score at 5 min	59	9 (6–10)
Length, cm	61	50.2 (33.5–55.8)
Weight, kg	63	3.3 (0.8–4.8)
Head circumference, cm	58	34.2 (23.5–37)
Adverse outcome		
Low birth weight[b]	63	3 (4.8)
Preterm birth[c]	71	4 (5.6)
Major birth defect[d]	66	7 (10.6)
Aortic coarctation with bicuspid aortic valve	66	1 (1.5)
Cleft palate	66	1 (1.5)
Down syndrome	66	1 (1.5)
Lissencephaly	66	1 (1.5)
Microcephaly[e]	58	2 (3.4)
Polydactyly	66	1 (1.5)
Other[f]	66	7 (10.6)
Glycogen storage disease type 1	66	1 (1.5)
Neonatal death	66	2 (3.0)
Skin tags	66	1 (1.5)
Umbilical hernia	66	4 (6.1)

The number of live-born infants includes 1 set of twins.

[a] Indicates the number of infants for whom information was available.

[b] Defined as <2500 g.

[c] Defined as delivery at <37 weeks' gestational age.

[d] The mother of the infant with lissencephaly had a WNV-like illness in the third trimester, which was not identified as WNV infection until after delivery. If this case were excluded, then 6 (9.2%) of 65 infants had major birth defects.

[e] Defined as head circumference >2 SD below mean; 1 of 2 microcephalic infants had several congenital abnormalities including multiple cerebral abnormalities, micrognathia, epicanthic folds, clinodactyly, splenomegaly, and cardiomegaly.

[f] One infant had both skin tags and an umbilical hernia.

Birth Outcomes Following West Nile Virus Infection of Pregnant Women in the United States: 2003-2004

TABLE 2 **Frequencies of Birth Abnormalities Among Live-Born Infants of 71 WNV-Infected Pregnant Women**

Condition	Among 72 Live Births, % (95% CI)	General Population, %	Source
Major birth defects			
All major defects[a]	10.6 (5.2–20.3)	5.5[d]	CDC, unpublished data, 2005; ref 24[d]
Aortic coarctation	1.5 (0.3–8.1)	0.03	Ref 20
Cleft palate	1.5 (0.3–8.1)	0.04	Ref 21
Down syndrome	1.5 (0.3–8.1)	0.13	Ref 22
Lissencephaly	1.5 (0.3–8.1)	0.001	Ref 23
Microcephaly[b]	3.5 (1.0–11.7)	2.28	Ref 24
Polydactyly	1.5 (0.3–8.1)	0.2	Ref 25
Other adverse outcomes			
Glycogen storage disease type 1	1.5 (0.3–8.1)	0.0005	Ref 26
Spontaneous abortion	9.1 (3.4–21.2)[c]	15.0	Ref 27
Low birth weight	4.8 (1.6–13.1)	7.9	Ref 28
Preterm	5.6 (2.2–13.6)	12.3	Ref 28

CI indicates confidence interval.

[a] The mother of the infant with lissencephaly had a WNV-like illness in the third trimester, which was not identified as WNV infection until after delivery. If this case was excluded, then 6 of 65 infants (9.2%) had major birth defects.

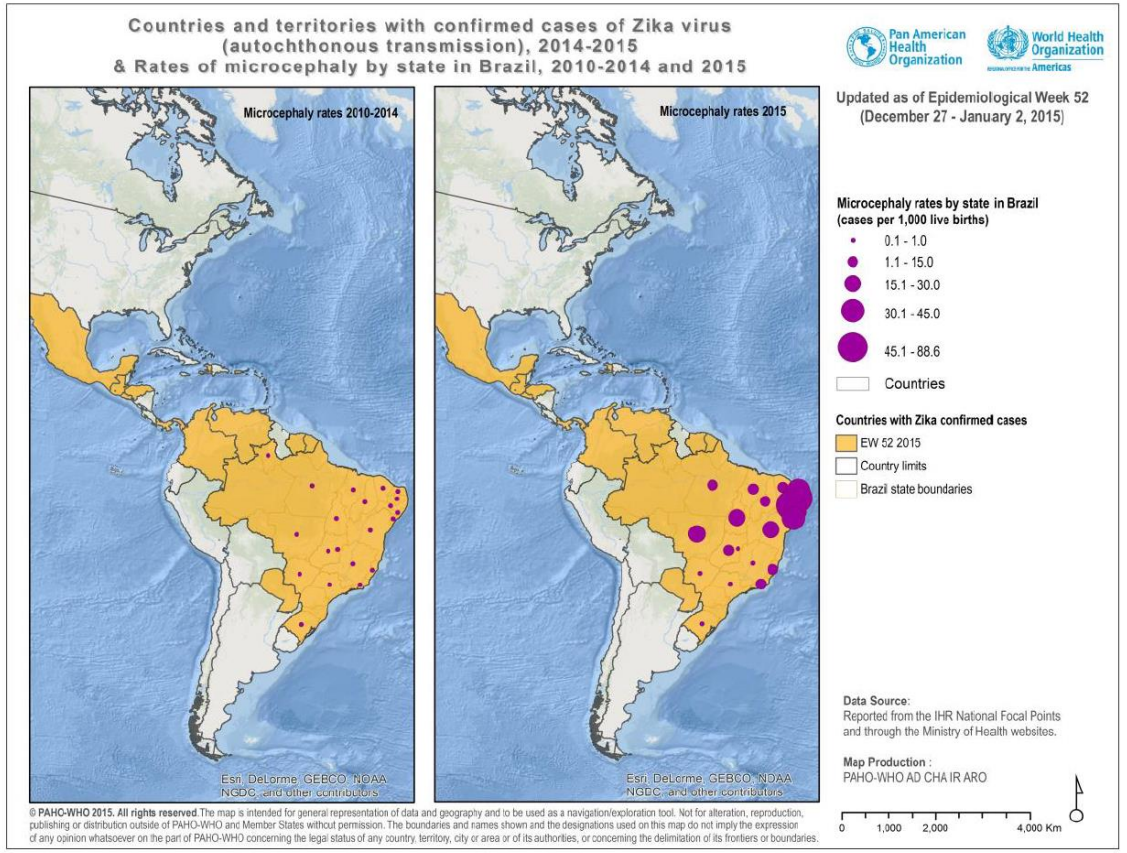
[b] Defined as head circumference >2 SD below mean for term infants.

[c] Calculated among 44 women with WNV illness onset estimated at <20 weeks' gestation.

[d] Estimated as 3.29% from the Metropolitan Atlanta Congenital Defects Program (CDC, unpublished data, 2005), which includes only physician-diagnosed microcephaly (0.06% of births), plus 2.28% to include infants with microcephaly defined as head circumference <2 SD below mean[24]; 3.25 − 0.06% + 2.28% = 5.47% ~ 5.5%.

Criteria for Causality

- Strength
- Consistency
- Specificity
- Temporality
- Biological gradient
- Plausibility
- Coherence
- Experiment
- Analogy



Zika Virus Associated with Microcephaly

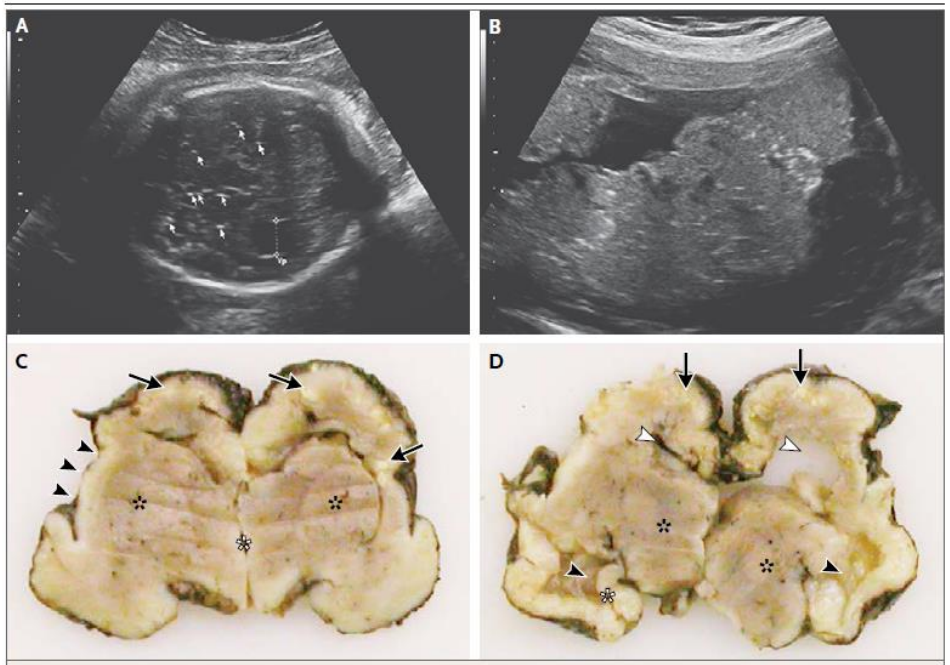


Figure 1. Prenatal Ultrasonographic Images and Photographs of Coronal Slices of Brain.

Panel A shows numerous calcifications in various parts of the brain (some marked with arrows) and the dilated occipital horn of the lateral ventricle (Vp, marked with a measurement bar) as seen on transverse ultrasonography. Panel B shows numerous calcifications in the placenta. Panel C shows multifocal cortical and subcortical white calcifications (arrows) and almost complete loss of gyration of the cortex. The basal ganglia are developed but poorly delineated (black asterisks), and the sylvian fissures are widely open on both sides (arrowheads on the left). The third ventricle is not dilated (white asterisk). Panel D shows dilated body of the lateral ventricles (white arrowheads); the left is collapsed. Temporal horns of the lateral ventricles (black arrowheads) are also dilated. The thalami (black asterisks) and the left hippocampus (white asterisk) are well developed, whereas the contralateral structure is not recognizable owing to autolysis.

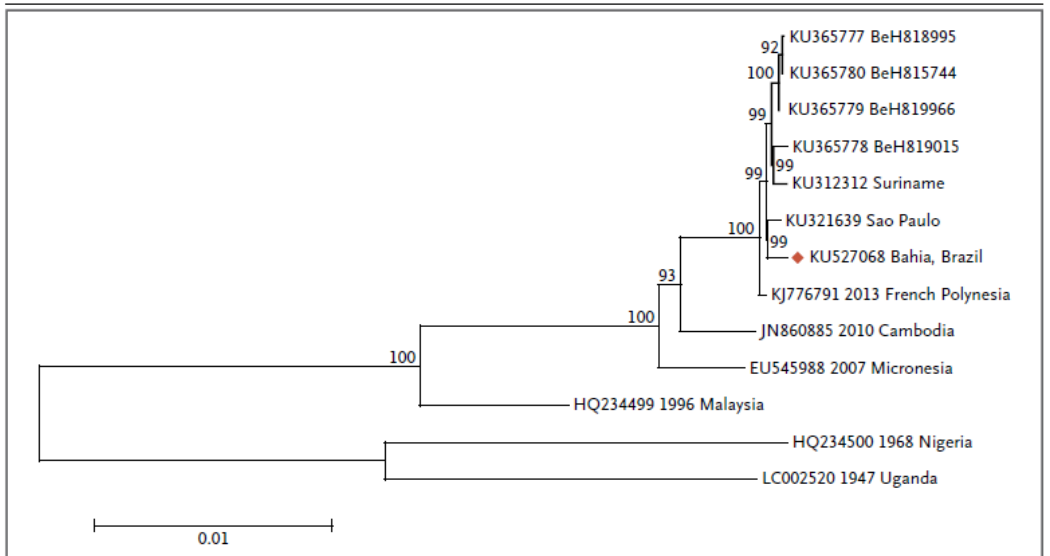


Figure 4. Phylogenetic Analysis of the Complete Genome of Zika Virus.

The evolutionary history was inferred by means of the neighbor-joining method under a GTR+G+I substitution model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) is shown next to the branches. The GenBank accession number, year of isolation, and country of origin are indicated on the ZIKV branches for all strains except for those identified in 2015 and 2016. ZIKV strain Bahia, Brazil (KU527068), was obtained in this study. The complete genome sequence was recovered from fetal brain tissue. The 0.01 scale bar denotes the genetic distance in nucleotide substitutions per site.

J. Mlakar et al., Zika virus associated with microcephaly. N. Engl. J. Med. 2016; 10.1056/NEJMoa1600651

Table 1. Baseline Demographic and Clinical Characteristics of Women in the Pregnancy Cohort.

Variable	ZIKV-Positive Women (N=72)	ZIKV-Negative Women (N=16)	P Value*
Demographics			
Age — yr			0.94†
Median (IQR)	29 (26–34)	28 (26–33)	
Range	17–46	20–36	
Other family members ill — no./total no. (%)	36/64 (56.2)	5/16 (31.2)	0.10
Partner ill — no./total no. (%)	12/57 (21.1)	1/16 (6.2)	0.27
Use of repellent — no./total no. (%)	19/47 (40.4)	3/10 (30.0)	0.73
History of dengue — no./total no. (%)	22/70 (31.4)	9/16 (56.2)	0.08
Socioeconomic status — no./total no. (%)‡			
Income ≤2× minimum wage	24/65 (36.9)	2/13 (15.4)	0.20
Income >2 to ≤5× minimum wage	26/65 (40.0)	5/13 (38.5)	1.00
Income >5× minimum wage	15/65 (23.1)	6/13 (46.2)	0.10
Week of gestation at time of infection			
Median (IQR)	20 (14–26)	17 (10–23)	0.60†
Range	5–38	7–39	
Distribution — no. (%)			
>4 to ≤13 wk	17/72 (23.6)	5/16 (31.2)	0.53
>13 to ≤26 wk	38/72 (52.8)	8/16 (50.0)	1.00
>26 to ≤39 wk	17/72 (23.6)	3/16 (18.8)	1.00
Symptoms — no./total no. (%)			
Rash§	72/72 (100.0)	16/16 (100.0)	
Any			0.47†
Median duration	4	5.5	
Range	2–14	2–60	
Macular	37/72 (51.4)	8/16(50.0)	1.00
Maculopapular	32/72 (44.4)	2/16 (12.5)	0.02
Other	3/72 (4.2)	6/16 (37.5)	0.001
Pruritus	69/72 (95.8)	14/15 (93.3)	0.54
Arthralgia or arthritis	46/72 (63.9)	7/16 (43.8)	0.16
Conjunctival injection	42/72 (58.3)	2/15 (13.3)	0.002
Headache	38/72 (52.8)	9/16 (56.3)	1.00
Fatigue or malaise	35/72 (48.6)	7/16 (43.8)	0.79
Retro-orbital pain	34/69 (49.3)	5/16 (31.3)	0.27
Myalgia	30/72 (41.7)	8/16 (50.0)	0.59
Lymphadenopathy	29/72 (40.3)	1/15 (6.7)	0.015
Localized	15/29 (51.7)	0/1	1.00
Generalized	14/29 (48.3)	1/1 (100.0)	1.00
Paresthesia	27/58 (46.6)	4/10 (40.0)	0.75
Edema	23/64 (35.9)	4/16 (25.0)	0.56

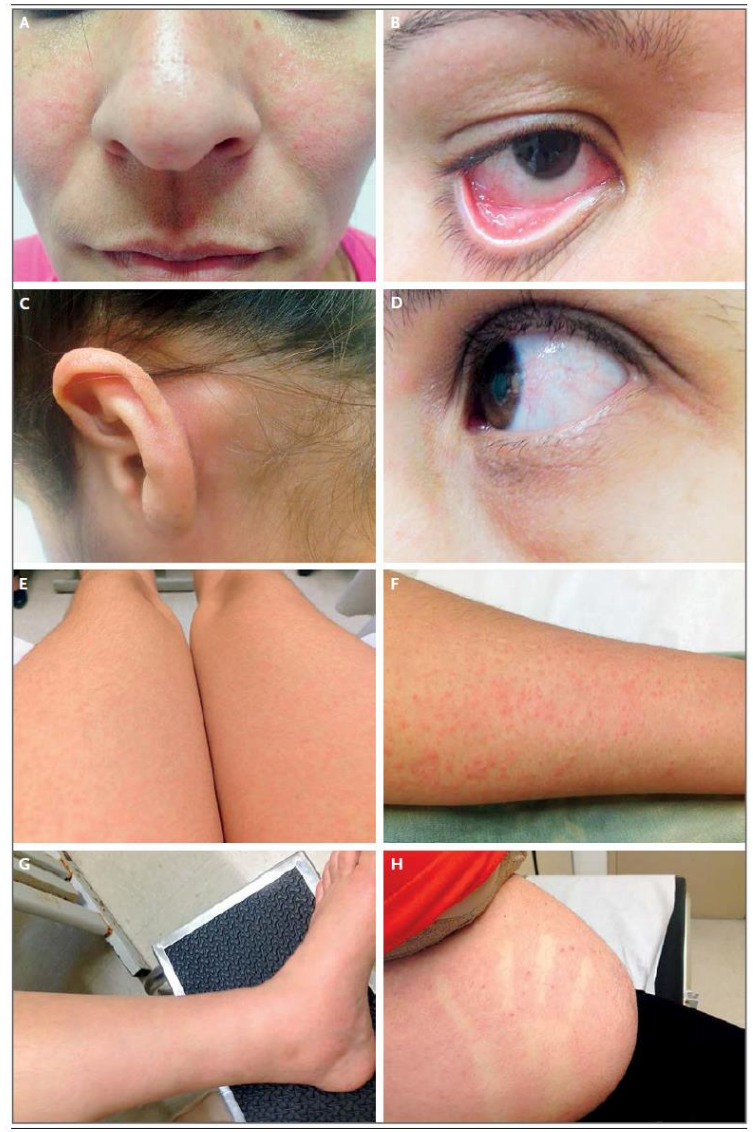

A
B
C
D
E
F
G
H

Table 2. Ultrasonographic Features of Fetuses and Findings at Birth.*

Fetus No.	Week of Gestation at Infection	Week of Gestation at Ultrasound Examination	Abnormal Findings on Doppler Ultrasonography	Findings at Birth
19	8	35	Microcephaly, cerebral calcifications, abnormal middle cerebral artery, intrauterine growth restriction	Microcephaly, cerebral calcifications on CT, global cerebral atrophy, macular lesions
40	8	20	Choroid plexus cyst, cerebellar atrophy (transverse diameter <5th percentile)	Still in utero
24	12	29	Microcephaly, cerebral calcification, Blake's cyst, agenesis vermis, club foot, intrauterine growth restriction	Still in utero
41	12	24	Mega cisterna magna (>95th percentile)	Still in utero
39	21	30	Cerebellar and cerebral right periventricular calcifications	Still in utero
17	22	26	Middle cerebral artery flow <5th percentile	Still in utero
12	22	27	Microcephaly, placental insufficiency as assessed by Doppler study, oligohydramnios, intrauterine growth restriction	Small for gestational age, head circumference proportional to body size, macular lesions
10	25	30	Normal first ultrasonogram, fetal death detected at 36 weeks on repeat ultrasonogram	Stillbirth
36	26	35	Microcephaly, abnormal umbilical artery flow (>95th percentile on the pulsatile index), intrauterine growth restriction	Small for gestational age, head circumference proportional to body size
38	27	35	Cerebral calcifications, ventriculomegaly, brachycephaly	Still in utero
2	30	34	None	Normal at birth
3	31	33	None	Normal at birth
53	32	38	Fetal death	Stillbirth
23	35	40	Anhydramnios, intrauterine growth restriction	Normal growth measure, poor sucking reflex, EEG abnormalities

* EEG denotes electroencephalogram, and CT computed tomography.

“The Case against Zika virus”

- Case reports are never free of confounders
- By definition, they represent selection bias
- “.. looking for ZIKV in microcephalic infants is like looking for dropped car keys in the light of a streetlamp”
- To observe the full picture, one must examine neurologically normal infants born to mothers infected with ZIKV during pregnancy

Bouvier N. Science Trans Med 2016

Slam Dunk (?) Epidemiological Study for Causality

- Cohort study – follow up of pregnant women infected/not infected with Zika with microcephaly as outcome
- Case control study – comparing exposure to Zika virus in infants with microcephaly to infants with no microcephaly

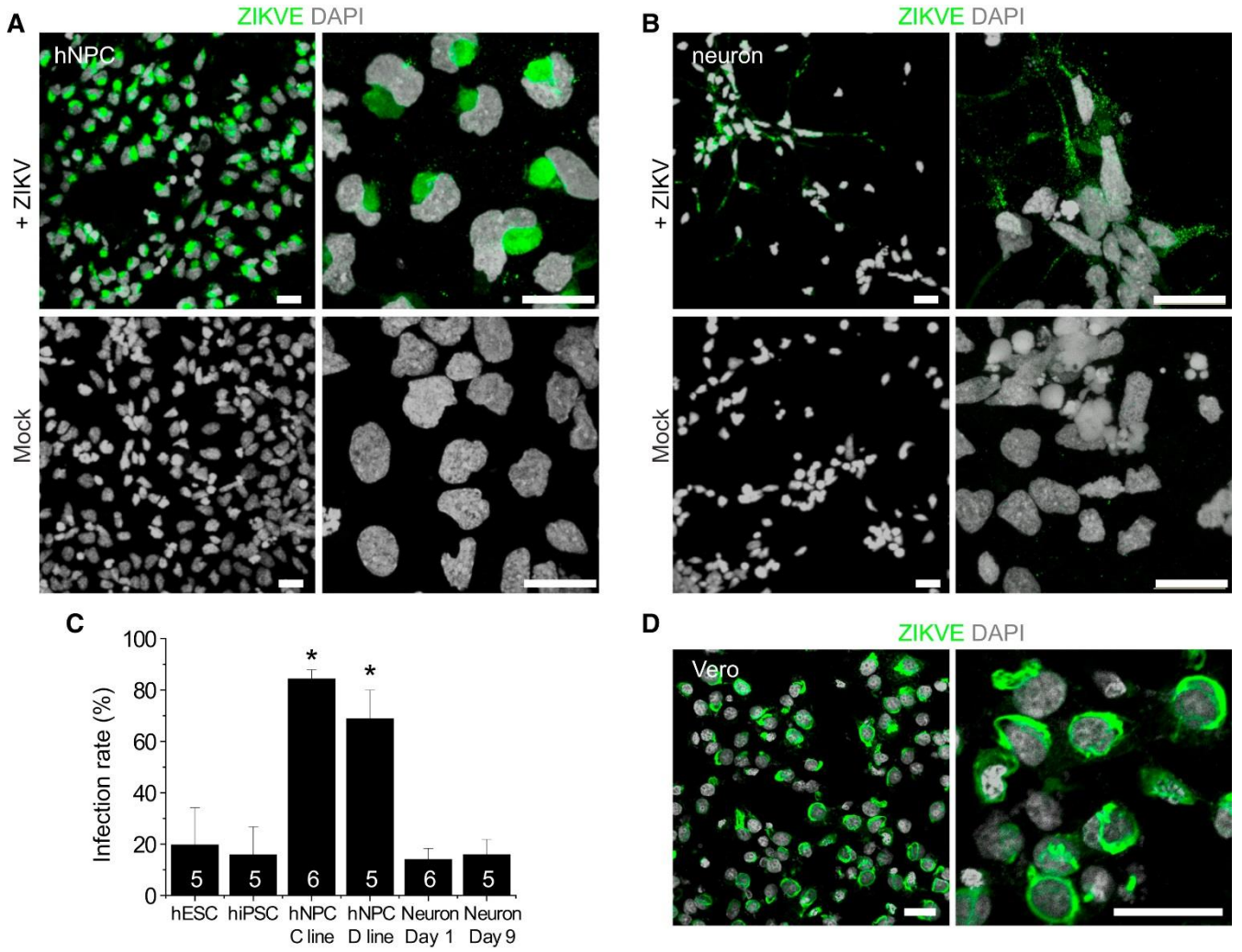
Association between Zika virus and microcephaly in French Polynesia, 2013–15: a retrospective study

	Baseline prevalence of microcephaly per 10 000 neonates	Number of microcephaly cases per 10 000 women infected in the period of risk	Risk ratio (95% CI)	p value	AICc for model fit
Trimester 1	2 (0–8)	95 (34–191)	53·4 (6·5–1061·2)	0·0007	0
Trimesters 1 and 2	2 (0–8)	50 (17–101)	26·4 (3·0–352·0)	0·0015	1·37
Trimesters 1, 2, and 3	2 (0–9)	42 (13–86)	20·8 (2·1–424·1)	0·0032	2·73

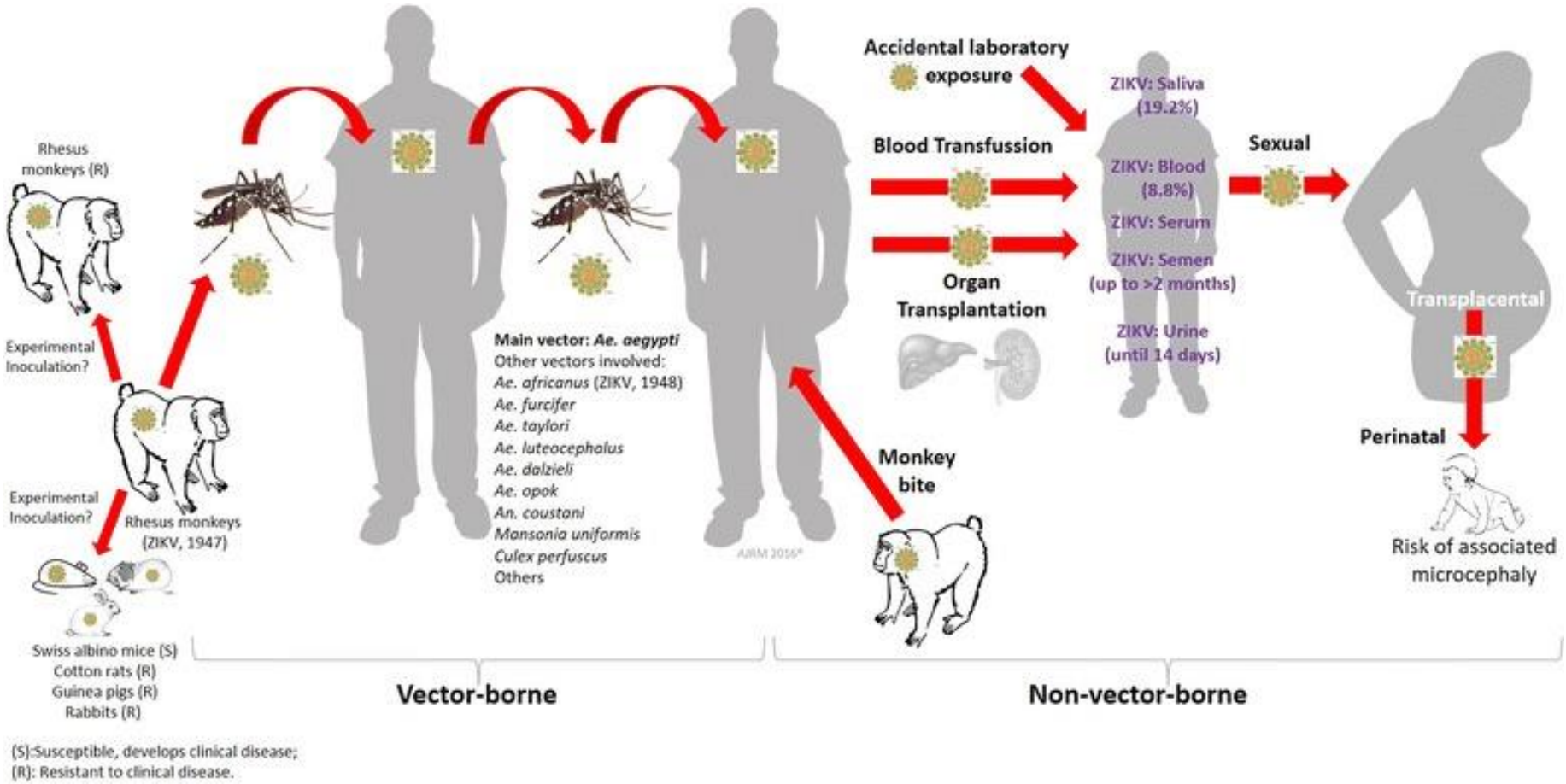
Cauchemez et al, Lancet 2016 in press

WHO Situation Report March 17, 2016

“The mounting evidence from observational, cohort and case-control studies indicates that Zika virus is highly likely to be a cause of microcephaly, GBS and other neurologic disorders. Among the tasks ahead are to further quantify the risk of neurologic disorders following Zika virus infection, and to investigate the biological mechanisms that lead to neurologic disorders.”

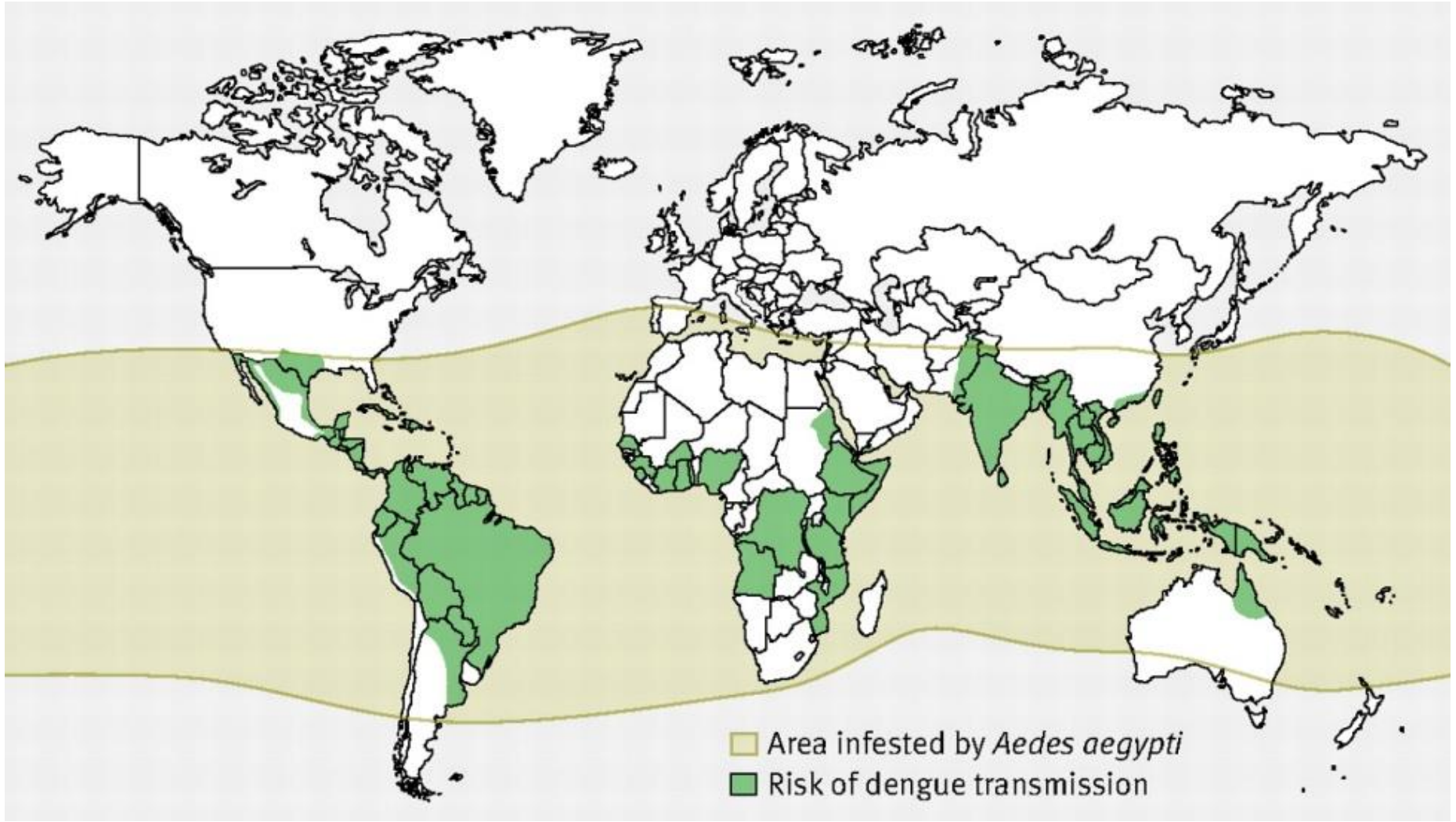


Tang H et al, Cell Stem cellDOI: 10.1016/j.stem.2016.02.016



Ann Clin Microbiol Antimicrob. 2016;15:13.

Aedes aegypti Breeding Sites


Area infested by *Aedes aegypti*
Risk of dengue transmission

Zika as a Hospital Infection Control Problem

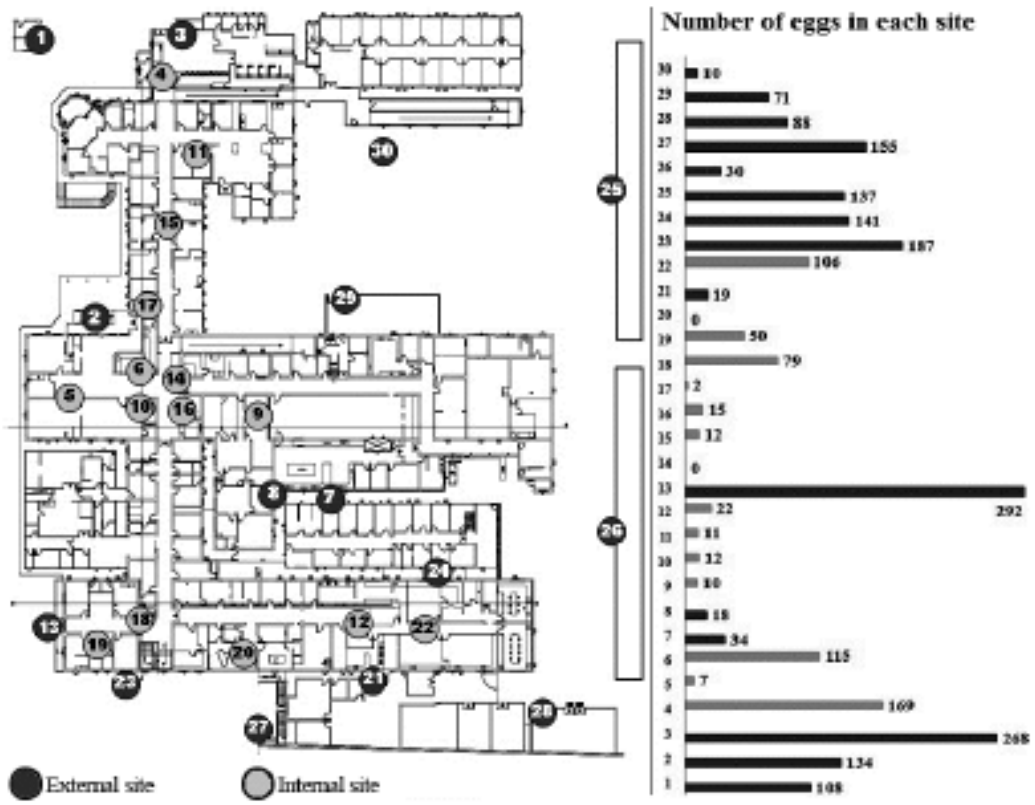


Figure 2. Number of *Aedes aegypti* eggs, humidity (A) and temperature (B) from January to December 2009 in a hospital in the city of Cuiabá, Mato Grosso, Brazil

Figure 2. Number of *Aedes aegypti* eggs, humidity (A) and temperature (B) from January to December 2009 in a hospital in the city of Cuiabá, Mato Grosso, Brazil

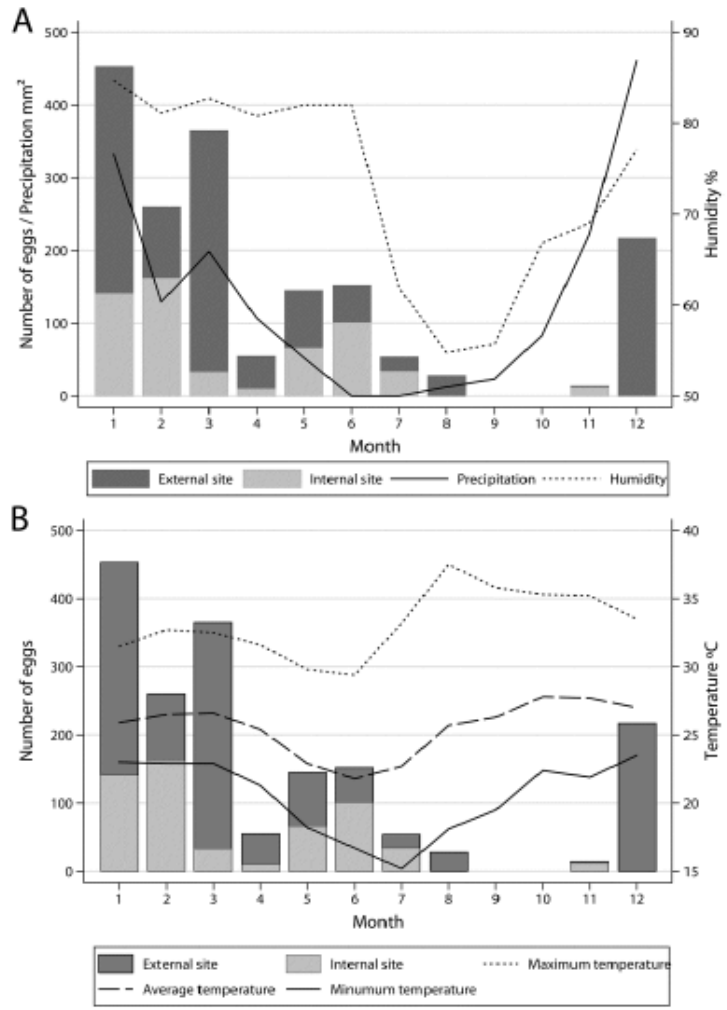
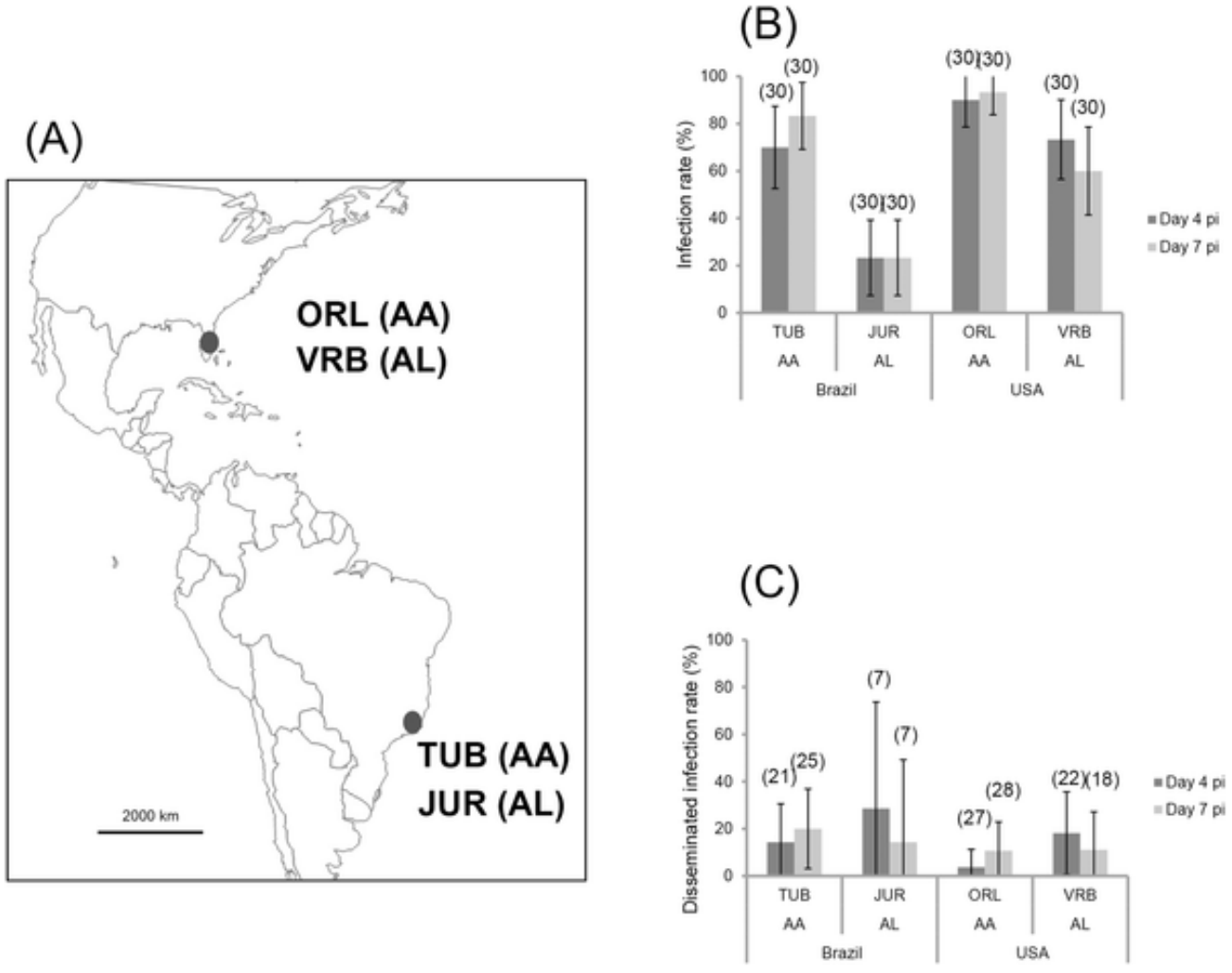


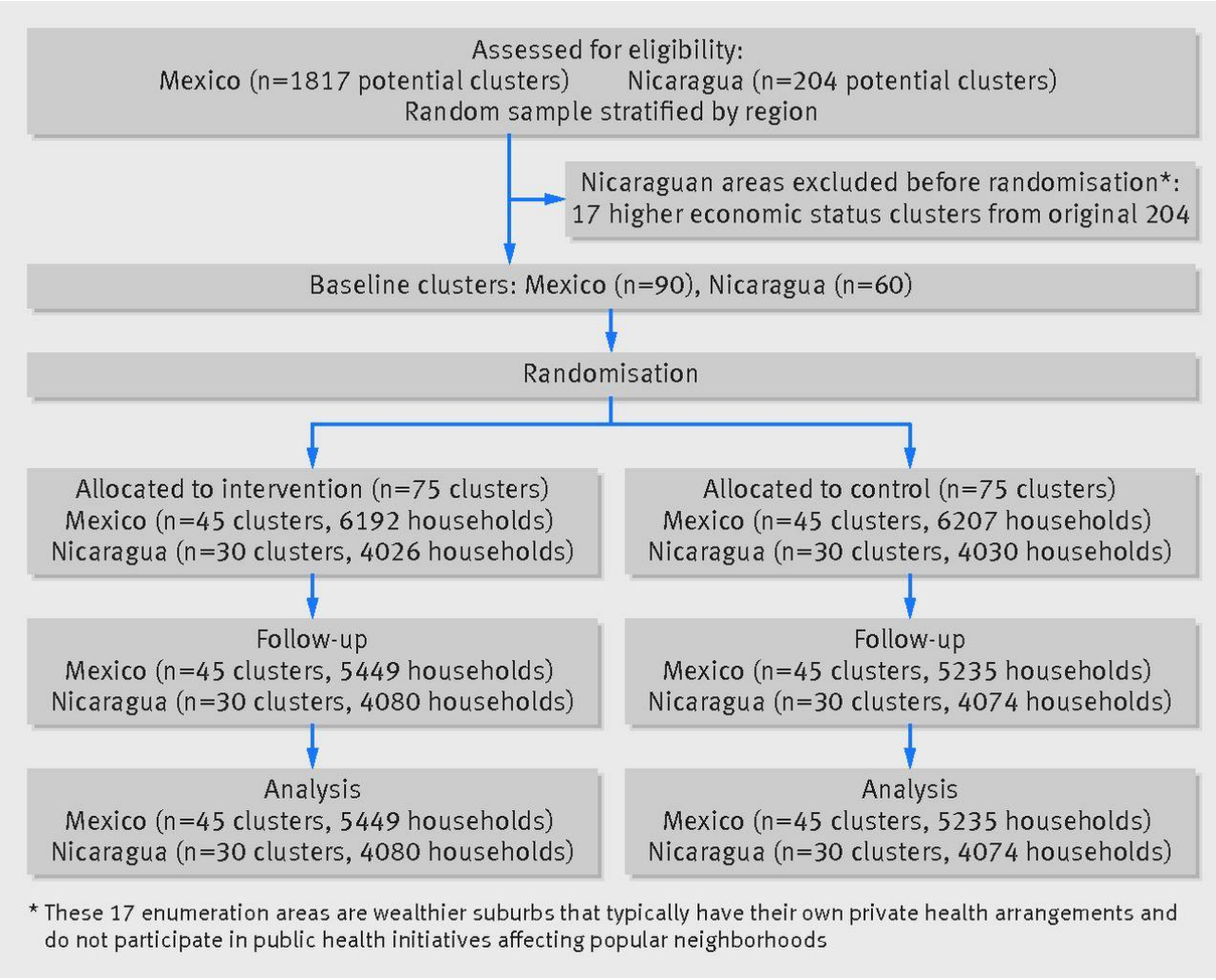
Fig 1. Mosquito populations (A), viral infection (B), dissemination (C) at days 4 and 7 after challenge of Aedes aegypti and Aedes albopictus from Continental America (Brazil and United States) with ZIKV provided at a titer of 107 TCID50/mL.



Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, et al. (2016) Differential Susceptibilities of Aedes aegypti and Aedes albopictus from the Americas to Zika Virus. PLoS Negl Trop Dis 10(3): e0004543. doi:10.1371/journal.pntd.0004543
http://journals.plos.org/plosntds/article?id=info:doi/10.1371/journal.pntd.0004543

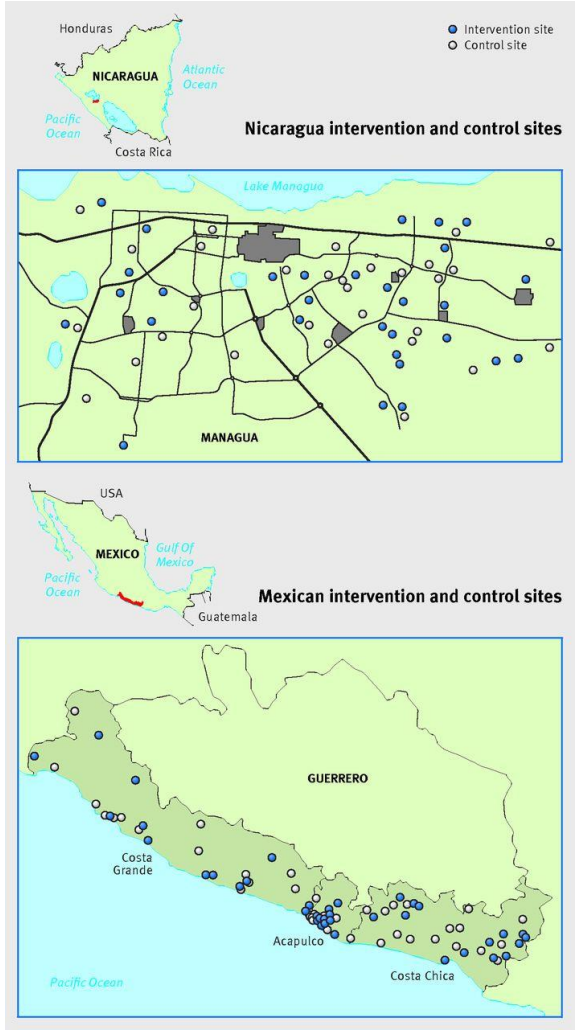
PLOS | NEGLECTED TROPICAL DISEASES

Identification and flow of clusters and households in study of community mobilization in Nicaragua and Mexico for dengue prevention



Neil Andersson et al. BMJ 2015;351:bmj.h3267

Areas covered by study of evidence based community mobilization for dengue prevention in Nicaragua and Mexico



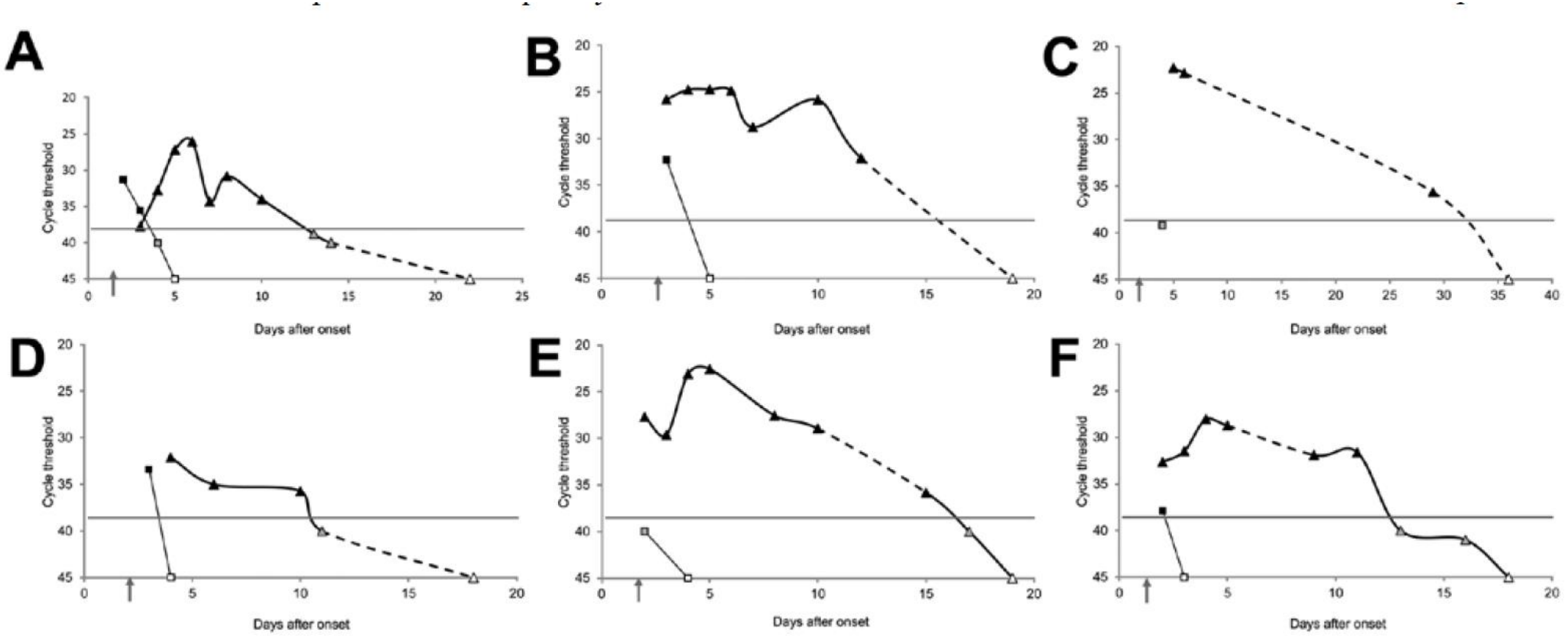
Neil Andersson et al. BMJ 2015;351:bmj.h3267

Cluster RCT of Interventions to Prevent Dengue

Table 3 | Cluster analysis for primary and secondary outcomes and intention to treat, with cluster as unit of analysis (risk difference (RD) across clusters, relative risk reduction (RRR), and intraclass correlation coefficient (ICC))*

	Mean in intervention clusters (n=75)	Mean in control clusters (n=75)	RD (95% CI)	RRR† (95% CI)	P value (df) for cluster *t* test	ICC‡
Primary outcomes						
Serology§: household evidence of recent dengue virus infection, children aged 3-9, ≥2× increase of IgG across paired samples	11.3%	14.6%	−3.3 (−4.9 to −1.7)	29.5 (3.8 to 55.3)	0.038 (148)	0.031
Self reported dengue illness: households reporting in past year/responding households	5.7%	7.1%	−1.4 (−2.1 to −0.7)	24.7 (1.8 to 51.2)	0.039 (148)	0.021
House index: houses infested with larvae or pupae/houses inspected	13.6%	19.6%	−6.0 (−7.1 to −5.0)	44.1 (13.6 to 74.7)	0.001 (148)	0.075
Container index: containers with larvae or pupae/containers inspected	5.3%	8.0%	−2.7 (−3.9 to −1.5)	36.7 (24.5 to 44.8)	0.001 (148)	0.078
Breteau index: containers with larvae or pupae/houses inspected	19.7%	30.2%	−10.5 (−17.6 to −3.4)	35.1 (16.7 to 55.5)	0.001 (148)	0.061
Pupae per person index: No of pupae/residential population ×100	9.2%	17.5%	−8.3 (−13.4 to −3.2)	51.7 (36.2 to 76.1)	0.001 (148)	0.068

Detection of Zika Virus in Blood and Saliva: duration



Gourinat et al, EID 2015

Detection of Zika Virus in Blood and Saliva: duration

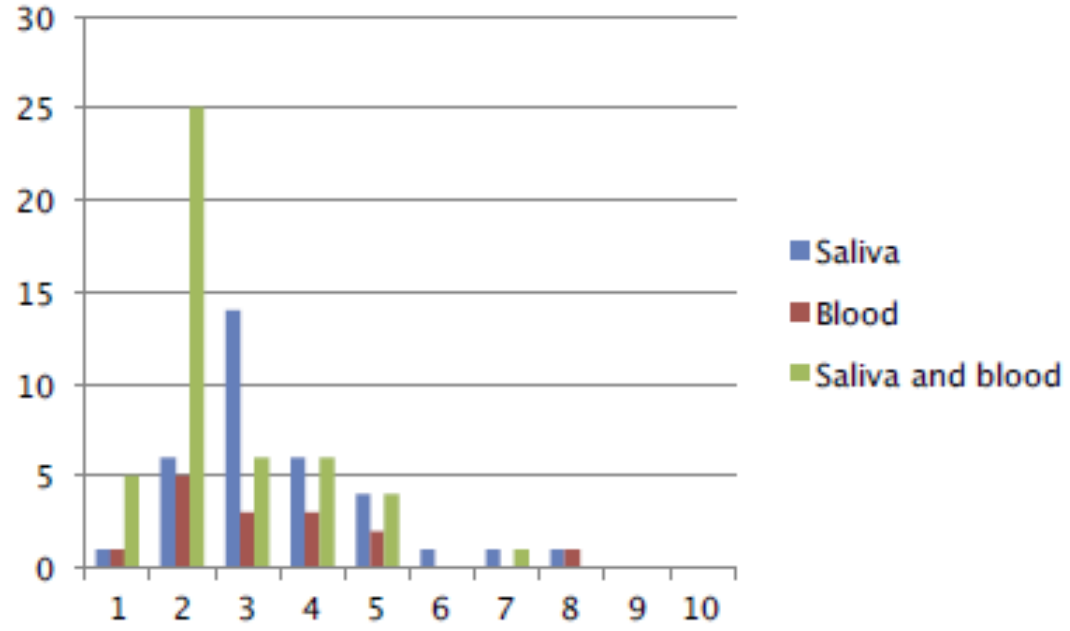
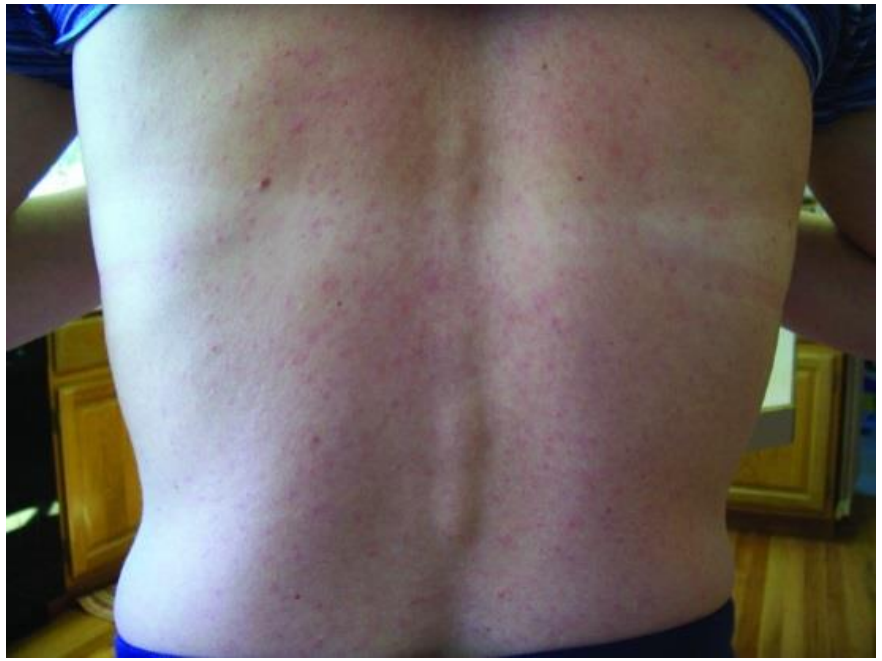


Fig. 1. Proportion of positive samples (Y axis in %) according to the number of days after symptoms onset (X axis) for the 182 patients with saliva, blood or both samples tested by ZIKV RT-PCR.

Musso et al, J Clin Virol 2015; 68:53-55

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

- Clinical and serologic evidence indicate that 2 U.S scientists contracted Zika virus infections while working in Senegal in 2008
- One of the scientists transmitted Zika to his wife after his return home
- Direct contact is implicated as the transmission route, most likely as a sexually transmitted infection



Foy BD et al, EID 2011; 17: 880-882

Transmission of Zika Virus Through Sexual Contact with Travelers to Areas of Ongoing Transmission — Continental United States, 2016

- During February 6–22, 2016, two confirmed and four probable cases of Zika virus sexual transmission were reported to CDC by health officials from multiple states
- Median patient age was 22.5 years (range 19–55 yrs), and several women were pregnant
- In all cases where type of sexual contact was documented, the contact included condomless vaginal intercourse and occurred when the male partner was symptomatic or shortly after symptoms resolved

MMWR March , 2016; 65:215-216

Zika Virus and Semen

- Case report of a 32 year old man who returned to France from Brazil and French Guyana diagnosed with Zika by PCR
- Semen sample collected 2 weeks after the diagnosis detected RNA viral loads of 8.6 $\log_{10}$ copies/ml, compared to 3.1 in urine and 2.8 in blood
- Case report from French Polynesia outbreak, 2 weeks after initial symptoms, Zika detected by PCR in semen
- Semen positive at 27 and 62 days after symptom onset (CT 29 and 33), infectious virus not cultured

Mansuy JM et al, Lancet Infect Dis 2016
Musso D et al, EID 2015; 21: 552
Atkinson B et al, EID 2016

MMWR Report- March 25, 2016

Morbidity and Mortality Weekly Report

Preventing Transmission of Zika Virus in Labor and Delivery Settings Through Implementation of Standard Precautions — United States, 2016

Christine K. Olson, MD[1]; Martha Iwamoto, MD[2]; Kiran M. Perkins, MD[3]; Kara N.D. Polen, MPH[4]; Jeffrey Hageman, MHS[3]; Dana Meaney-Delman, MD[5]; Irogue I. Igbinosa, MD[6]; Sumaiya Khan, MPH[7]; Margaret A. Honein, PhD[4]; Michael Bell, MD[3]; Sonja A. Rasmussen, MD[8]; Denise J. Jamieson, MD[1]

Efficacy of a Tetravalent Dengue Vaccine in Children in Latin America

Luis Villar, M.D., Gustavo Horacio Dayan, M.D., José Luis Arredondo-García, M.D., Doris Maribel Rivera, M.D., Rivaldo Cunha, M.D., Carmen Deseda, M.D., Humberto Reynales, M.D., Maria Selma Costa, M.D., Javier Osvaldo Morales-Ramírez, M.D., Gabriel Carrasquilla, M.D., Luis Carlos Rey, M.D., Reynaldo Dietze, M.D Kleber Luz, M.D., Enrique Rivas, M.D., Maria Consuelo Miranda Montoya, M.D., Margarita Cortés Supelano, M.I Betzana Zambrano, M.D., Edith Langevin, M.Sc., Mark Boaz, Ph.D., Nadia Tornieporth, M.D., Melanie Saville, M.B., B.S., and Fernando Noriega, M.D., for the CYD15 Study Group*

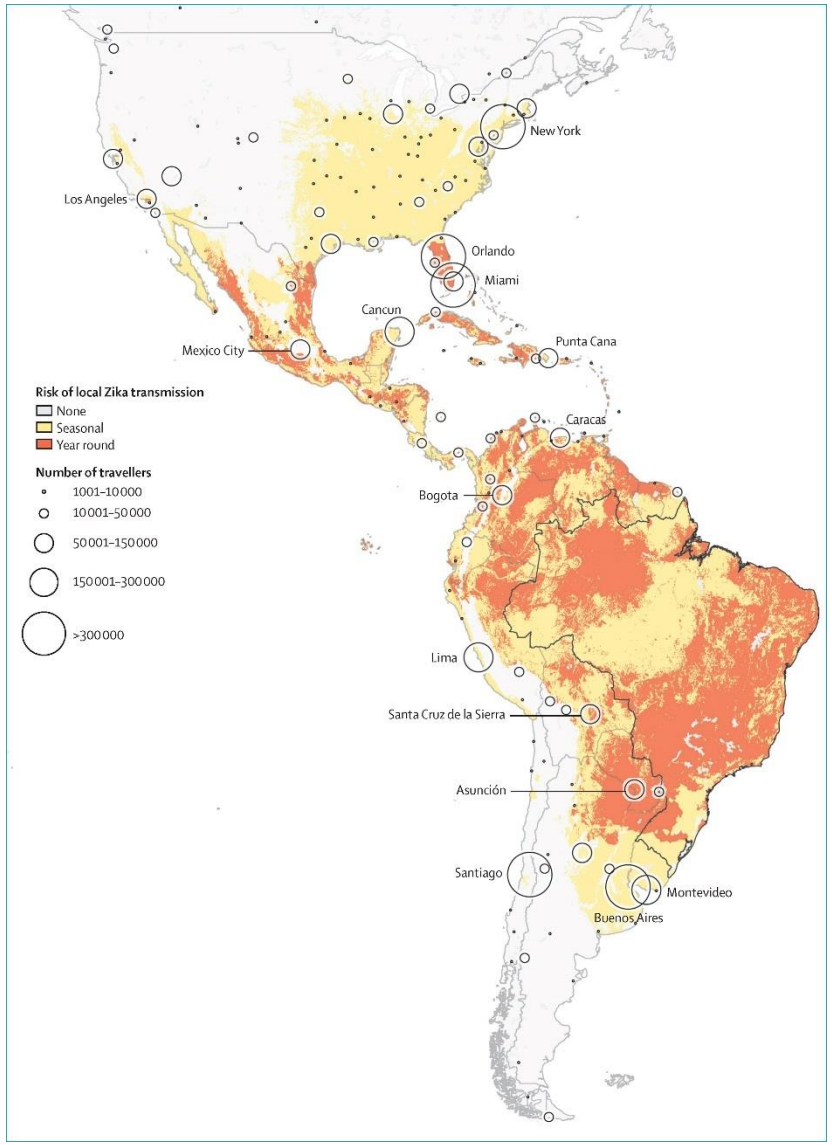
Table 2. Vaccine Efficacy against Any Serotype of Dengue.

Analysis	Vaccine Group			Control Group			Vaccine Efficacy (95% CI)
	Cases/ Events*	Person-Yr at Risk†	Incidence Density (95% CI)‡	Cases/ Events*	Person-Yr at Risk†	Incidence Density (95% CI)‡	
	no.		*no./100 person-yr*	*no.*		*no./100 person-yr*	%
Per-protocol analysis	176/176	11,793	1.5 (1.3–1.7)	221/221	5,809	3.8 (3.3–4.3)	60.8 (52.0–68.0)
Intention-to-treat analysis	277/280§	26,883	1.0 (0.9–1.2)	385/388§	13,204	2.9 (2.6–3.2)	64.7 (58.7–69.8)

Table 1. Annual Incidence of Hospitalization for Virologically Confirmed Dengue, According to Trial, Age Group, and Study Period.*

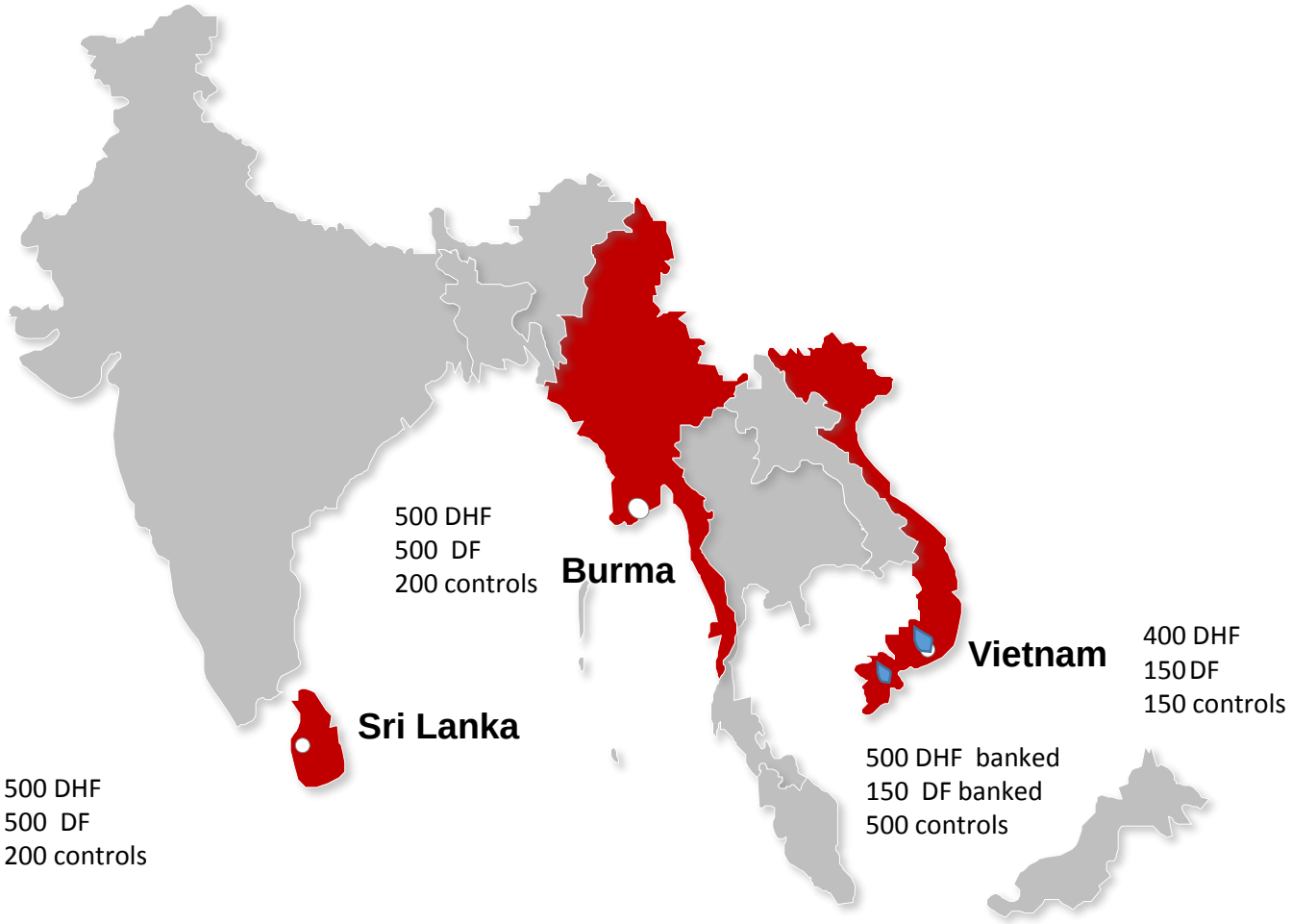
Trial, Age Group, and Study Period	Vaccine Group			Control Group			Relative Risk (95% CI)
	Cases of Dengue	Total Participants†	Annual Incidence Rate‡	Cases of Dengue	Total Participants†	Annual Incidence Rate‡	
		no.	*% (95% CI)*		*no.*	*% (95% CI)*	
CYD14							
All participants§	27	6,778	0.4 (0.3–0.6)	13	3387	0.4 (0.2–0.7)	1.04 (0.52–2.19)
2–5 yr	15	1,636	1.0 (0.6–1.6)	1	813	0.1 (0.0–0.7)	7.45 (1.15–313.80)
6–11 yr	10	3,598	0.3 (0.1–0.6)	8	1806	0.5 (0.2–1.0)	0.63 (0.22–1.83)
12–14 yr	2	1,544	0.1 (0.0; 0.5)	4	768	0.6 (0.2–1.4)	0.25 (0.02–1.74)
<9 yr	19	3,493	0.6 (0.4–0.9)	6	1741	0.4 (0.1–0.8)	1.58 (0.61–4.83)
≥9 yr	8	3,285	0.3 (0.1–0.5)	7	1646	0.5 (0.2–1.0)	0.57 (0.18–1.86)
CYD15							
All participants¶	16	13,268	0.1 (0.1–0.2)	15	6630	0.2 (0.1–0.4)	0.53 (0.25–1.16)
9–11 yr	10	6,029	0.2 (0.1–0.3)	9	3005	0.3 (0.1–0.6)	0.55 (0.20–1.54)
12–16 yr	6	7,239	<0.1 (0.0–0.2)	6	3625	0.2 (0.1–0.4)	0.50 (0.13–1.87)
CYD57							
All participants							
Year 3‖	22	2,131	1.1 (0.7–1.7)	11	1072	1.1 (0.6–2.0)	1.01 (0.47–2.30)
Year 4‖	16	2,131	0.8 (0.4–1.2)	17	1072	1.6 (0.9–2.5)	0.47 (0.22–1.00)
4 or 5 yr							
Year 3	5	393	1.4 (0.5–3.2)	1	192	0.6 (0.0–3.1)	2.44 (0.27–115.54)
Year 4	5	393	1.3 (0.4–2.9)	3	192	1.6 (0.3–4.5)	0.81 (0.16–5.24)
6–11 yr							
Year 3	17	1,738	1.1 (0.6–1.7)	10	880	1.2 (0.6–2.3)	0.86 (0.37–2.10)
Year 4	11	1,738	0.6 (0.3–1.1)	14	880	1.6 (0.9–2.7)	0.40 (0.16–0.94)
<9 yr							
Year 3	19	1,338	1.5 (0.9–2.4)	6	665	1.0 (0.4–2.1)	1.57 (0.60–4.80)
Year 4	13	1,338	1.0 (0.5–1.7)	12	665	1.8 (0.9–3.1)	0.54 (0.23–1.29)
≥9 yr							
Year 3	3	793	0.4 (0.1–1.2)	5	407	1.3 (0.4–3.1)	0.31 (0.05–1.58)
Year 4	3	793	0.4 (0.1–1.1)	5	407	1.2 (0.4–2.8)	0.31 (0.05–1.58)

Anticipating the international spread of Zika virus from Brazil

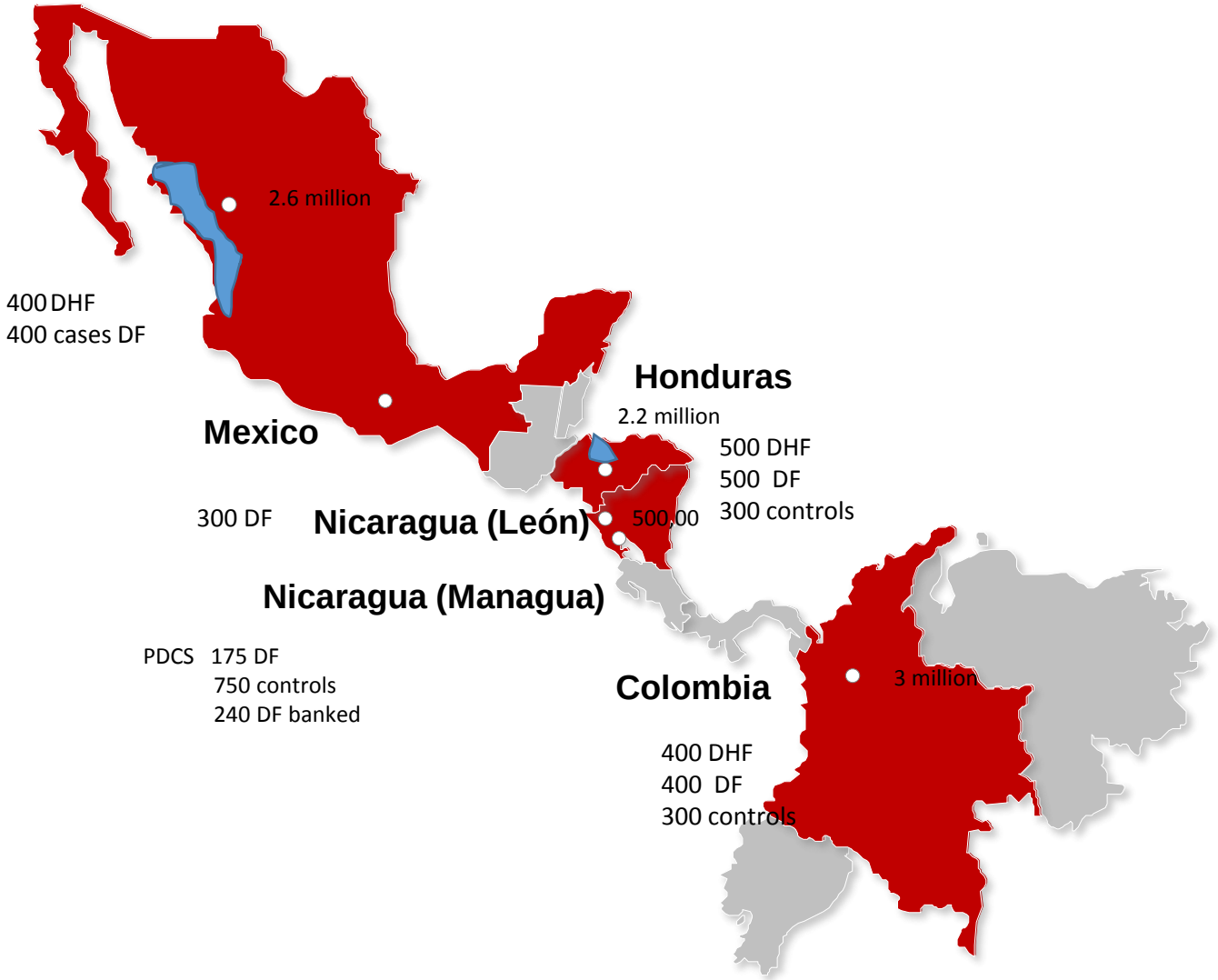


Bogach I et al. Lancet 2016

Study Populations - Asia



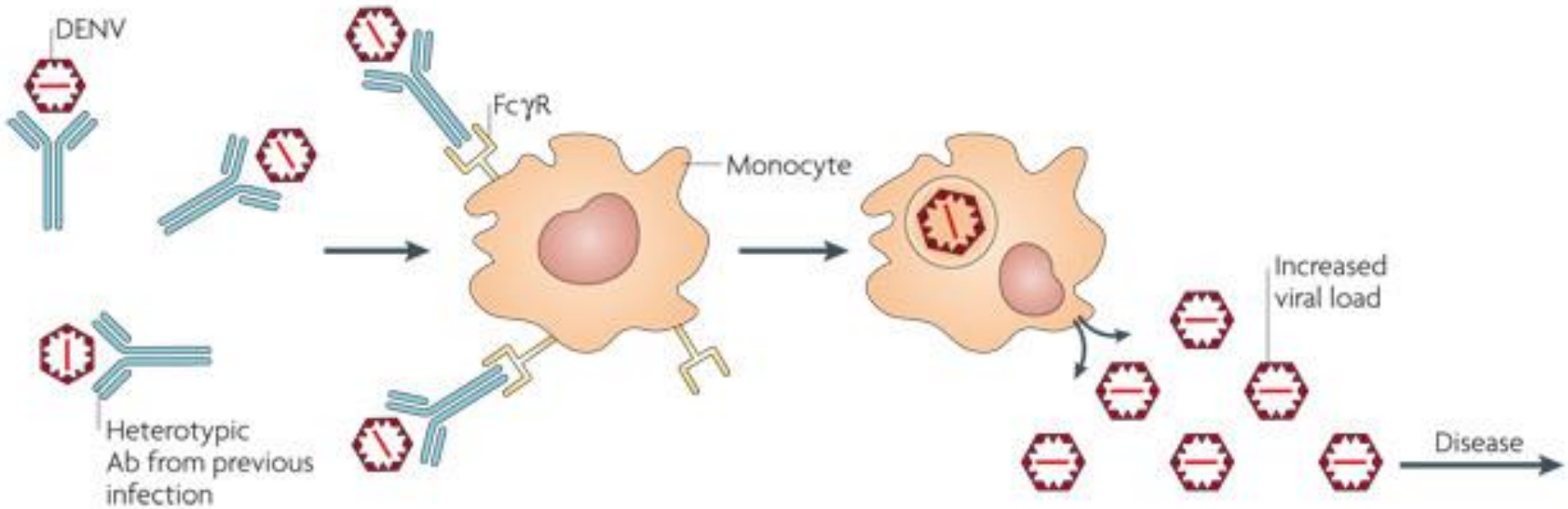
Study Populations - Americas



Pilot Studies

- Cohort of 200 persons in Bucaramanga, Colombia
- Goal is to assess Zika viral shedding in infected persons – blood, saliva, urine, semen and using quantitative PCR will be establish longitudinal curves of viral shedding and potentially of predictors of longer duration and peak viral load
- Similar study in Leon, Nicaragua
- A third study will be to establish a biobank of placentas in Colombia

What is the Role of Antibody Dependent Enhancement?



Summary

- Pregnany complications of Zika virus were an “unknown unknown”
- Emerging evidence demonstrating evidence for causality but not complete
- Major knowledge gaps and these lead to challenges in preventing transmission