Zika virus emergence and response

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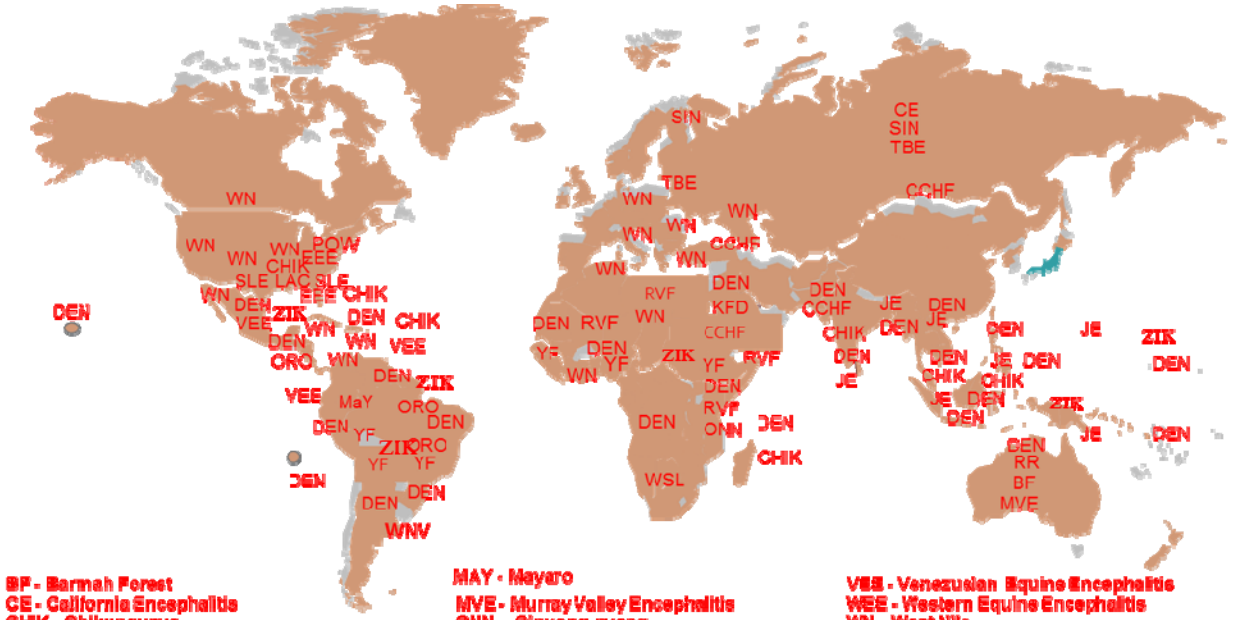
Wadsworth Center

NYS Dept. of Health

Global Distribution of Arboviruses

Arthropod-borne (arbo) viruses:

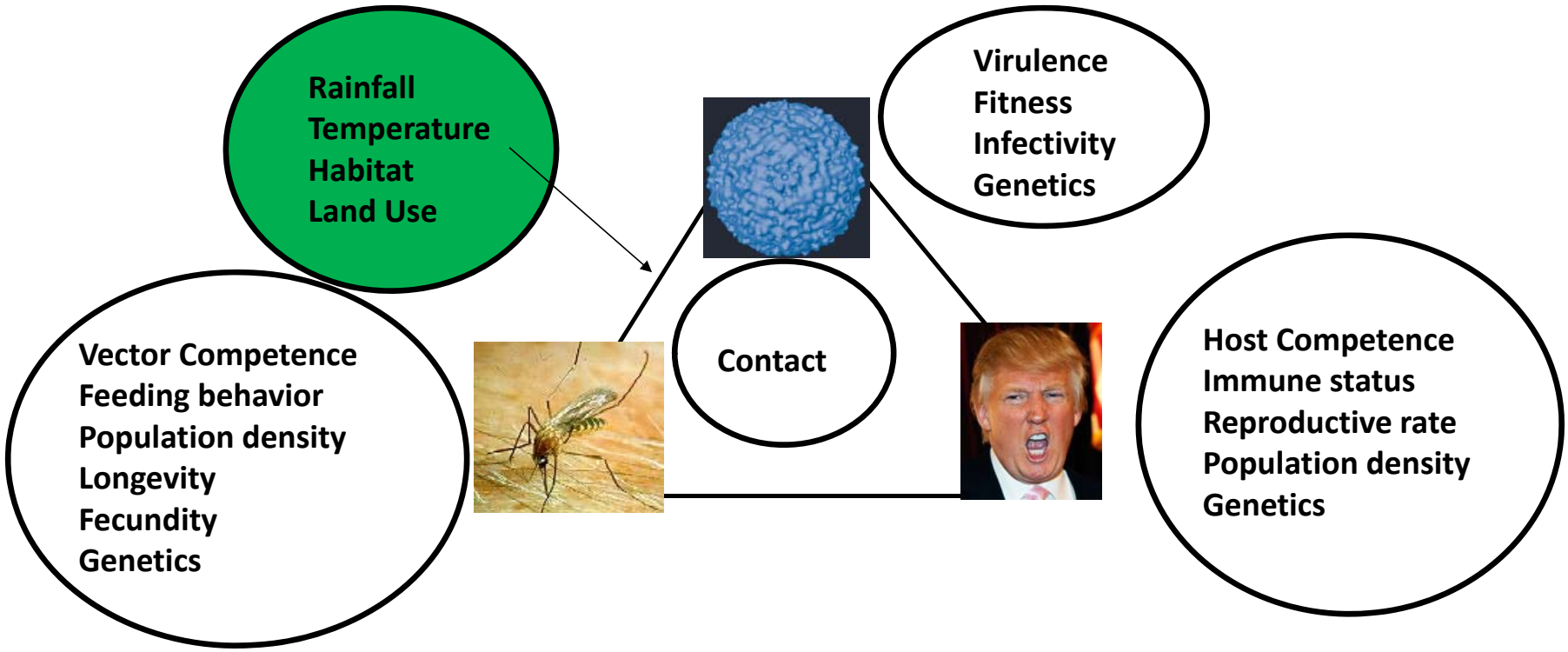
- **Almost exclusively RNA viruses**
- **Arthropod vectors include mosquitoes (most), ticks, sandflies and midges**
- **>120 associated with human disease**
- **Most in families Flaviviridae, Togaviridae, or Bunyaviridae**
- **Significant global expansion in recent decades**



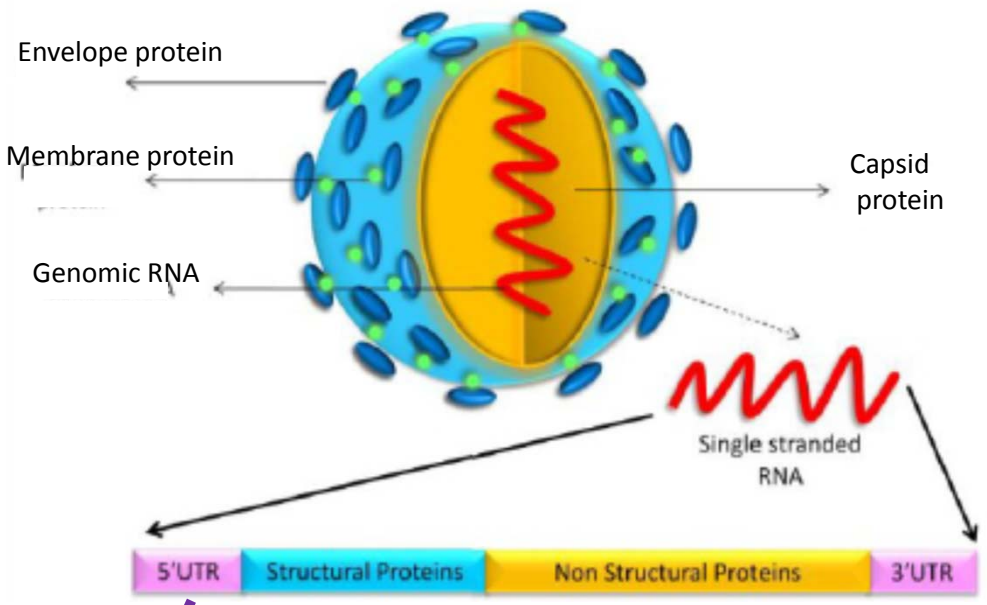
Known Arboviral Introductions to the United States

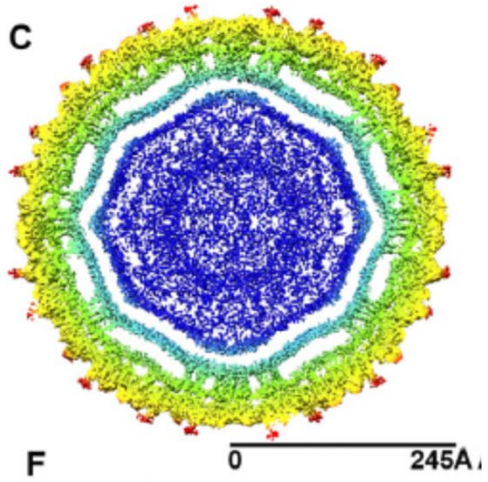
- Eastern equine encephalitis, South American genotype, LA (1966)
- Mayaro, LA (1967)
- Venezuelan equine encephalitis,TX (1971)
- Yellow fever, TN, CA, TX (1996, 1999, 2002)
- West Nile, NY (1999)
- Dengue HI, FL, TX, NY
- Chikungunya 2014- FL
- Zika-2016

Factors affecting arbovirus emergence and spread

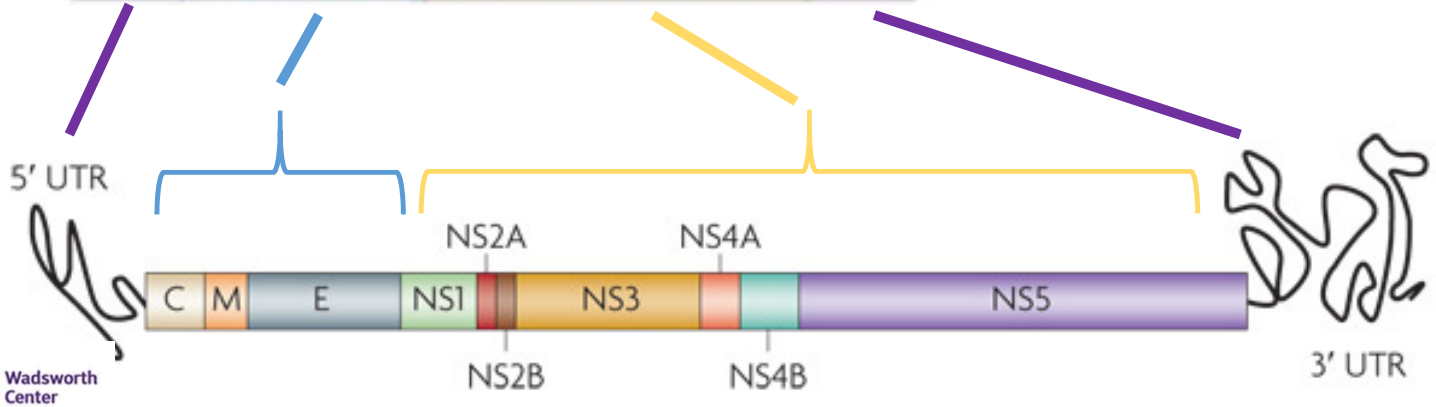


Virus structure and Flavivirus genome organization





Sirohi et al., Science 10.1126/science. aaf5316(2016).



Ashraf U et al. . 2015. Viruses 7:219

ZIKA

J Cohen Science 8 Feb 2016

ZIKV transmission cycle

Mosquito: West & Central Africa (sylvatic) and Asia and Americas (urban)

Non-human primates

TRANSITION

Forest *Aedes spp.*

A africanus
A luteocephalus
A furcifer-taylori

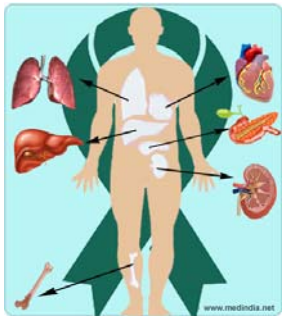
SYLVATIC CYCLE

Aedes aegypti *& Ae. albopictus*

URBAN CYCLE

Other modes of transmission

- **Maternal-fetal**
  - Intrauterine
  - Perinatal
- **Other**
  - Sexual Transmission
  - Blood transfusion
  - Organ or tissue transplantation
  - Tears?



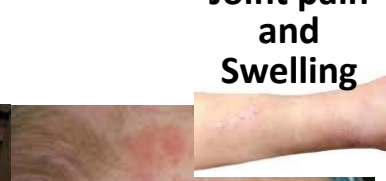
Modified from Gould EA and Higgs S 2009 Trans Royal Soc Trop Med Hyg

Yap Island ZIKV Outbreak , 2007

- **Serosurveys suggested 73% attack rate (>5000 cases)**
- **First outbreak outside of Africa and Asia**
- **Just 49 symptomatic patients had confirmed infections.**

Number of Patients (%)	Illness Sign or Symptom
28 (90 %)	Macular or papular rash
20 (65 %)	Fever
20 (65 %)	Arthritis or arthralgia
19 (55 %)	Conjunctivitis (red eyes)
15 (48 %)	Myalgia (body aches)
14 (45 %)	Headache
12 (39 %)	Pain behind eyes
6 (19 %)	Swelling of limbs
3 (10 %)	Vomiting

Mostly subclinical, illness mild and self limiting
Lasting 2 to 7 days



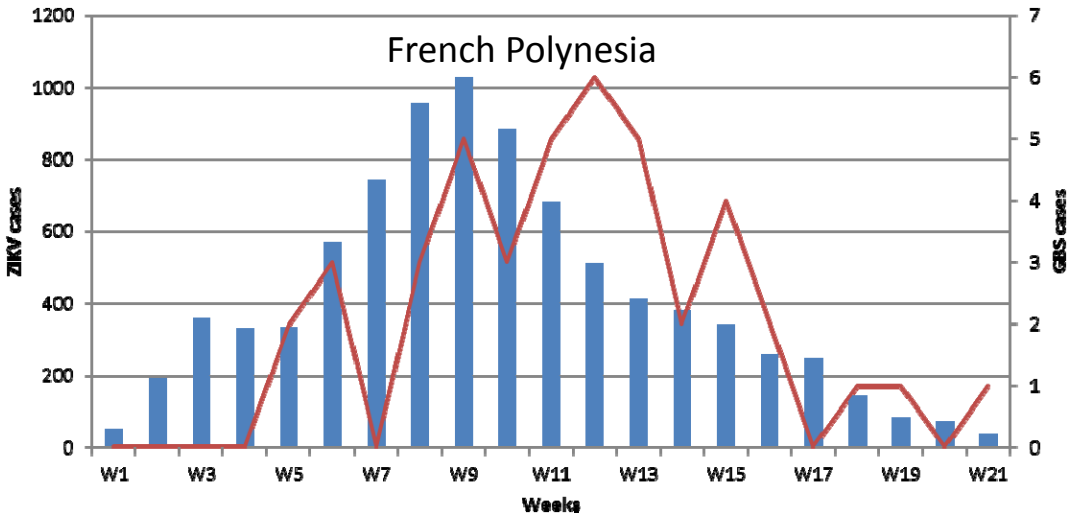
Joint pain and Swelling

Zika Rashes

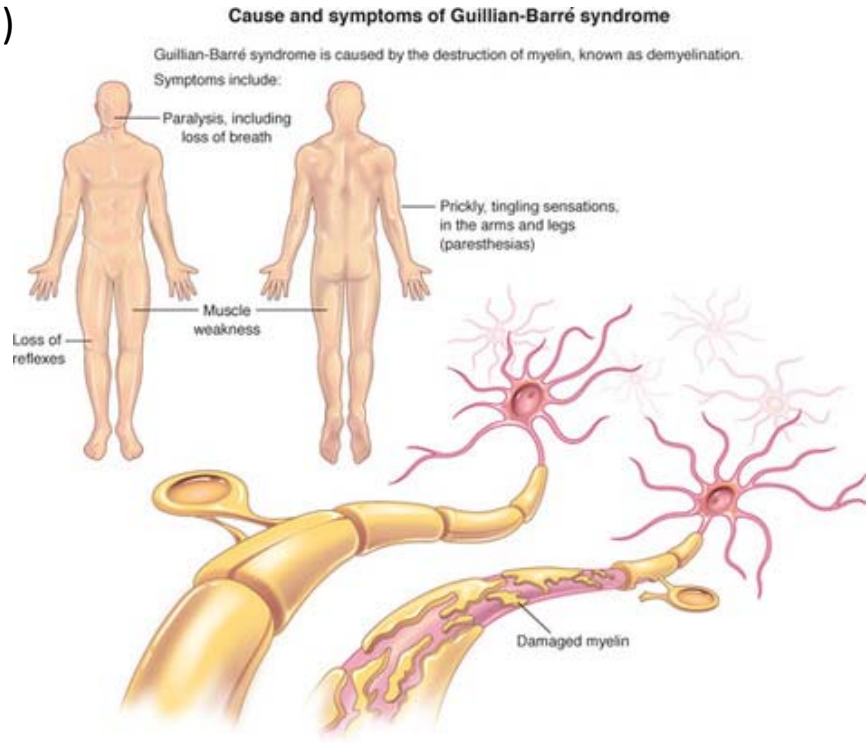
Conjunctivitis

ZIKV, French Polynesia and Guillain-Barré Syndrome, 2013-2014

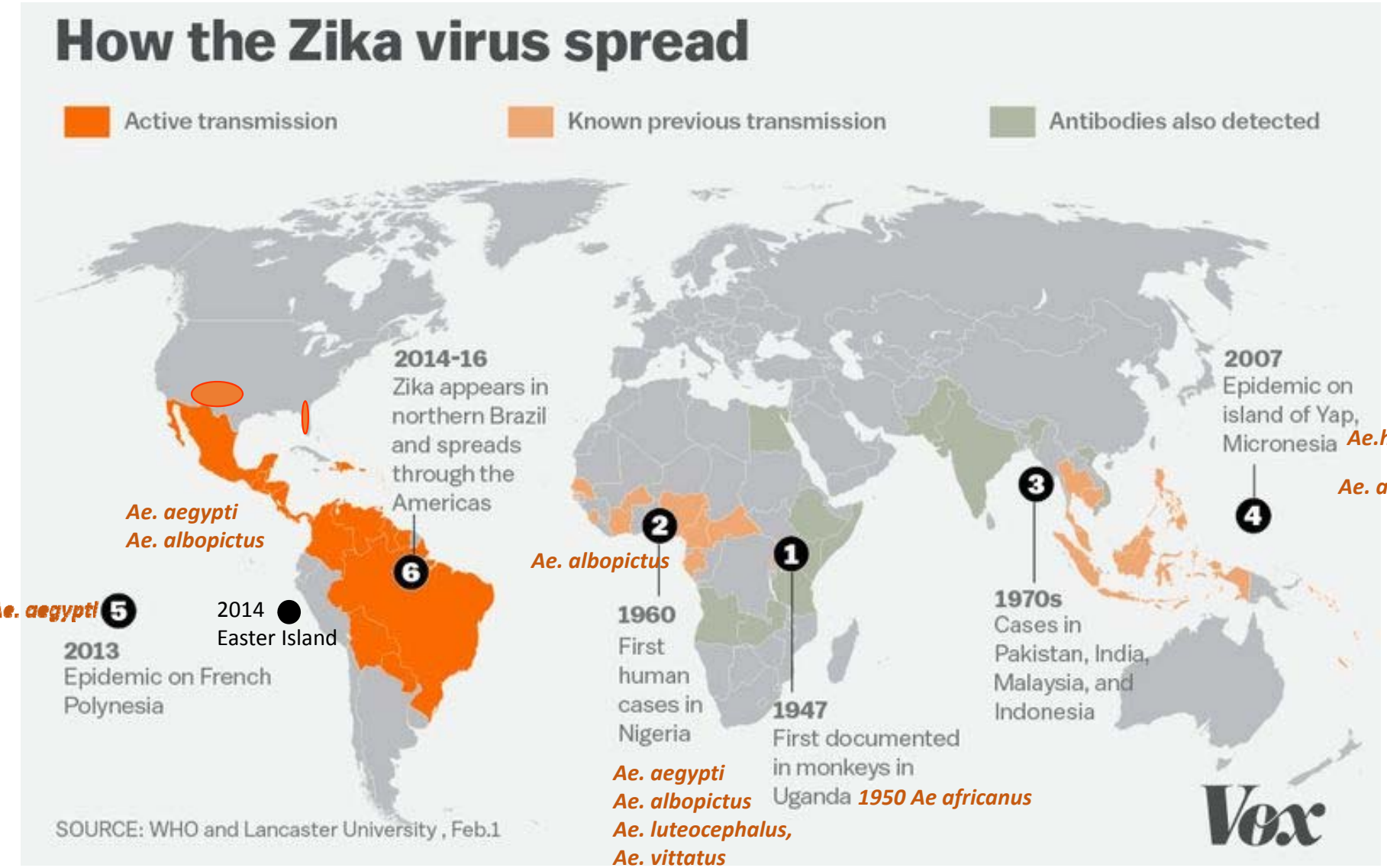
- Largest outbreak to date(~32,000 cases),
- An increase in Guillian-Barré Syndrome was observed in addition to other neurologic symptoms
- Past incidence was about 5 cases per year
- During the ZIKV outbreak, 42 cases in 6 months (20X increase)
- All had ZIKV neutralizing antibody
- 15 admitted to intensive care units
- Estimated .01% of cases



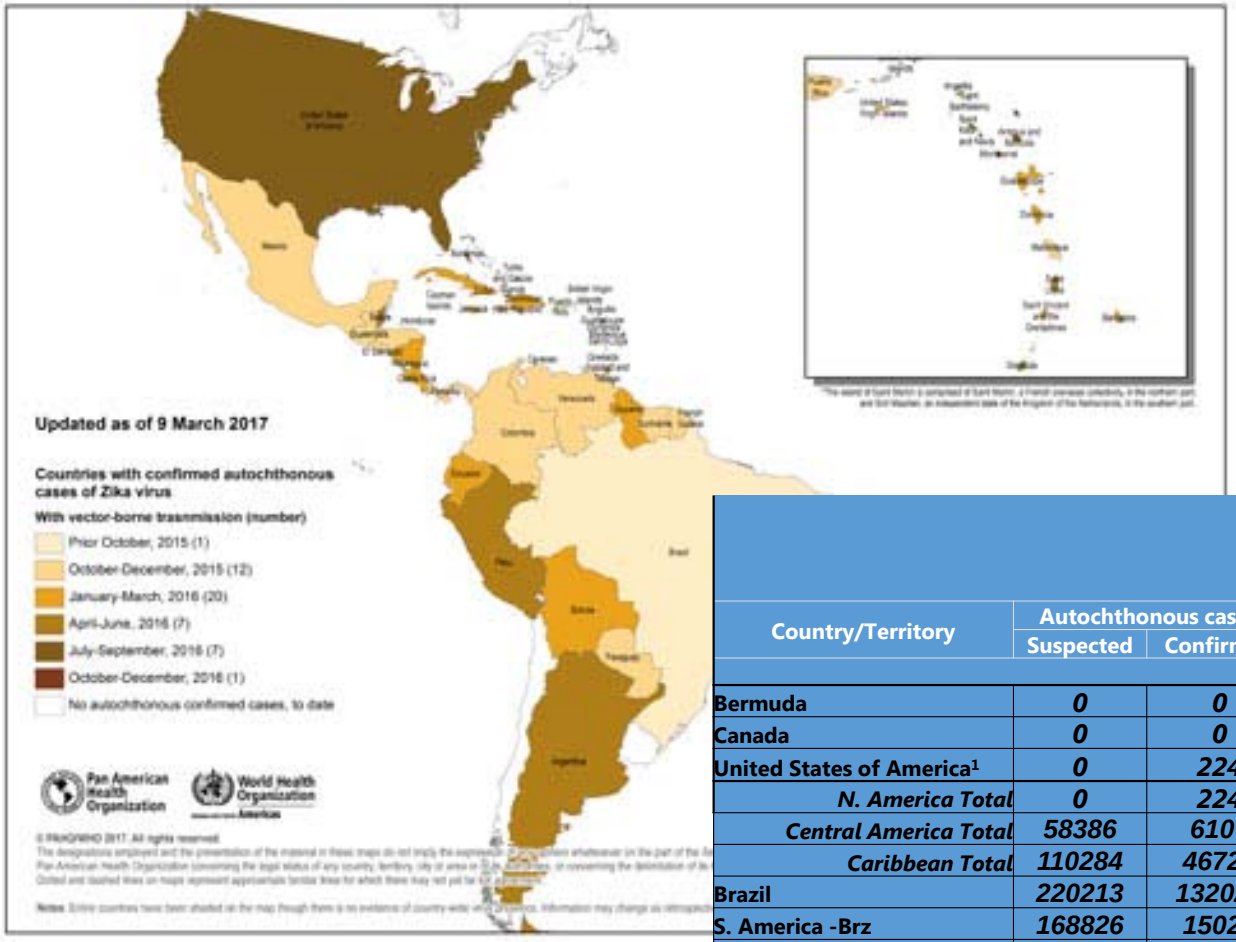
Courtesy of D. Musso



http://cpreplab.weebly.com/guillain-barreacute-syndrome.htm


How the Zika virus spread
Active transmission
Known previous transmission
Antibodies also detected
2014-16
Zika appears in northern Brazil and spreads through the Americas
Ae. aegypti
Ae. albopictus
6
Ae. aegypti
5
2013
Epidemic on French Polynesia
2014
Easter Island
Ae. albopictus
2
1960
First human cases in Nigeria
Ae. aegypti
Ae. albopictus
Ae. luteocephalus,
Ae. vittatus
1
1947
First documented in monkeys in Uganda 1950 Ae africanus
3
1970s
Cases in Pakistan, India, Malaysia, and Indonesia
2007
Epidemic on island of Yap, Micronesia
Ae.hensilii
Ae. aegypti
4
SOURCE: WHO and Lancaster University , Feb.1
Vox

ZIKV in the Americas-48 countries



Cumulative cases

Data as of 20 April 2017 2:00 PM EST

Country/Territory	Autochthonous cases[a]		Imported cases	Incidence Rate[b]	Deaths among Zika cases[c]	Confirmed congenital syndrome	Population X 1000[e,f]
	Suspected	Confirmed					
Bermuda	0	0	6	0.00	0	0	71
Canada	0	0	486	0.00	0	1	36284
United States of America[1]	0	224	4935	0.07	0	63	325296
N. America Total	0	224	5427	0.06	0	64	361651
Central America Total	58386	6107	77	138.9	0	77	46437
Caribbean Total	110284	46729	234	345.8	9	138	45269
Brazil	220213	132021	0	168.1	11	2621	209553
S. America -Brz	168826	15020	111	70.1	0	163	211975
S. America Total	389039	147041	111	130.0	11	2784	210764
TOTAL	**557709**	**208705**	**5864**	**76.4**	**20**	**3068**	**1003509**

Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth

By: Tang, HL (Tang, Hengli)[1]; Hammack, C (Hammack, Christy)[1]; Ogden, SC (Ogden, Sarah C.)[1]; Wen, ZX (Wen, Zhexing)[2,3]; Qian, XY (Qian, Xuyu)[2,4]; Li, YJ (Li, Yujing)[9]; Yao, B (Yao, Bing)[9]; Shin, J (Shin, Jaehoon)[2,5]; Zhang, FR (Zhang, Feiran)[9]; Lee, EM (Lee, Emily M.)[1] ...More

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CELL STEM CELL

Micro

Zika Virus Infection with Prolonged Maternal **emature death of the**

Virem

ses birth defects

Zika Virus RNA Re

Julu Bhatnagar, Demi B. Rab
B.C. Estetter, Tadaki Suzuki, Ja
Lambert, Robert Lanciotti, Titi
Saad, Wun-Ju Shieh, and Sheri

Author affiliations: Centers for
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Neiva, Colombia (F. Bolaños); Instituto Nacional de Salud, Bogota, Colombia (E.A. Parra S

Beatriz C. Freitas, João L. M.
, Graciela C. Pignatari, Sarah
Wesley N. Brandão, Cristiano
Garcez, Carlos A. Buchpigel,
A. Zanotto, Jean Pierre S.

ARTICLE

Received 5 Oct 2016 | Accepted 12 Jan 2017 | Published 21 Feb 2017

DOI: 10.1038/ncomms14575 OPEN

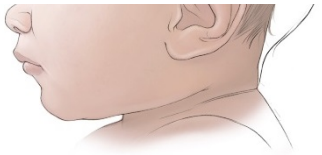
Intrauterine Zika virus infection of pregnant immunocompetent mice models transplacental transmission and adverse perinatal outcomes

Meghan S. Vermillion[1,2], Jun Lei[3], Yahya Shabi[3], Victoria K. Baxter[1,2], Nathan P. Crilly[1,†], Michael McLane[3], Diane E. Griffin[1], Andrew Pekosz[1], Sabra L. Klein[1] & Irina Burd[3]

Nature **534**, 267–271 (09 June 2016) | doi:10.1038/nature18296

Colombia- 113 ZCS out of >107,000 reported infections

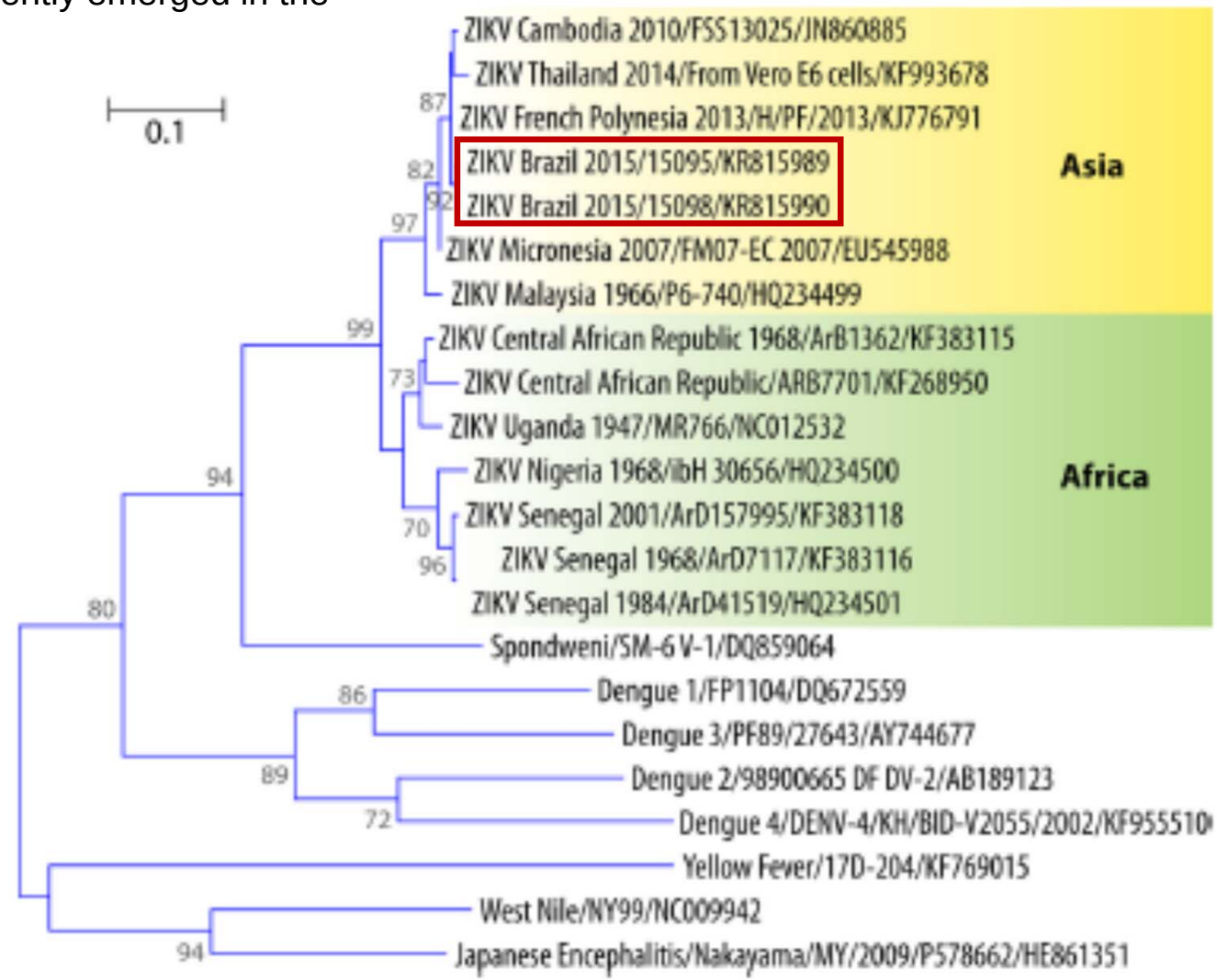
Puerto Rico- 12 ZCS out of 38,000 infections



Abclawcenters.com

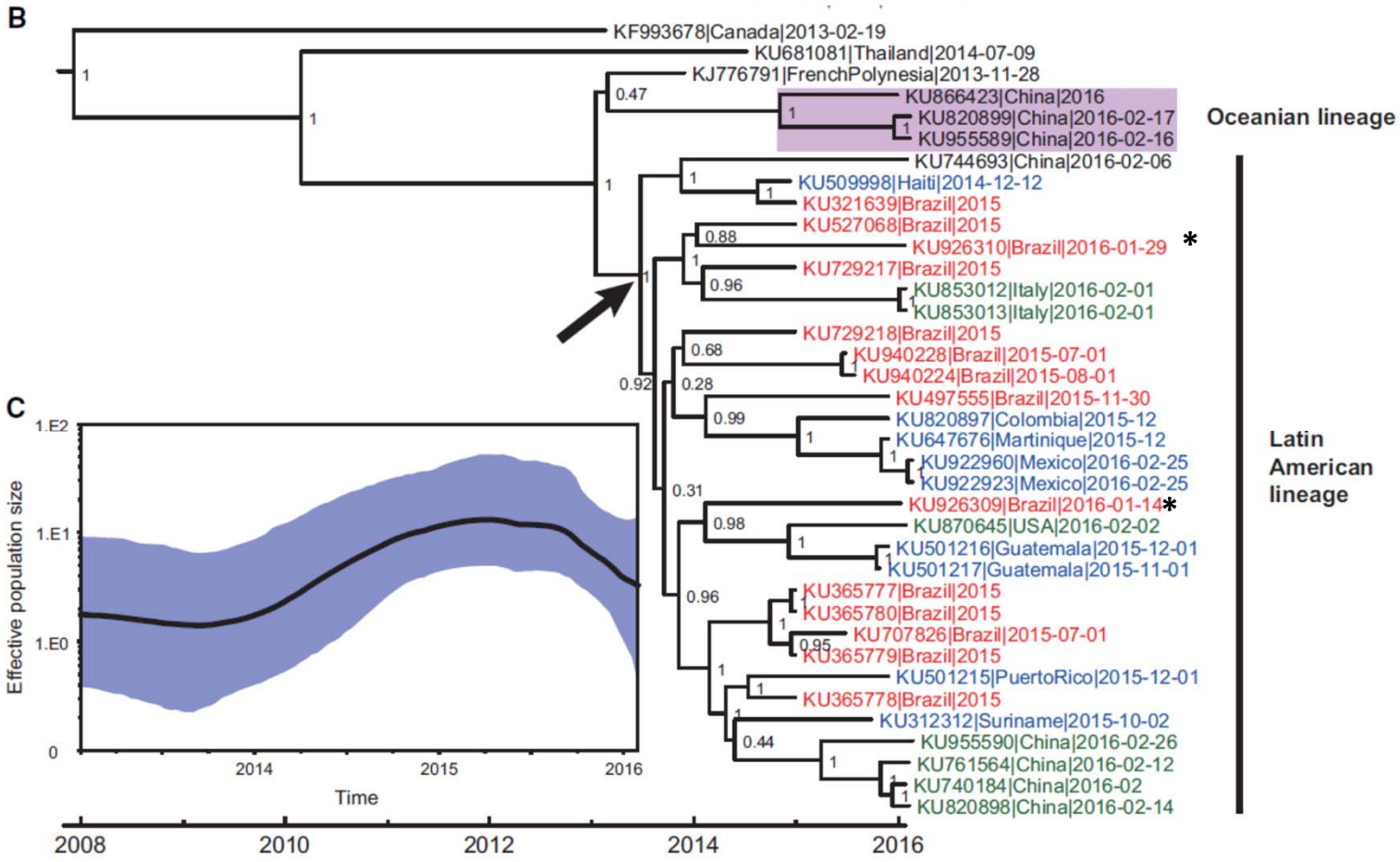
Normally, Brazil has 150 -200 births of babies with microcephaly /yr

Phylogenetic tree of ZIKV showing the African and Asian lineages, including the strains that recently emerged in the Pacific and Brazil.



D Musso & DJ Gubler. CMR. 2016

Phylogenetic tree, Zika 2016



Beast analysis using strict molecular clock

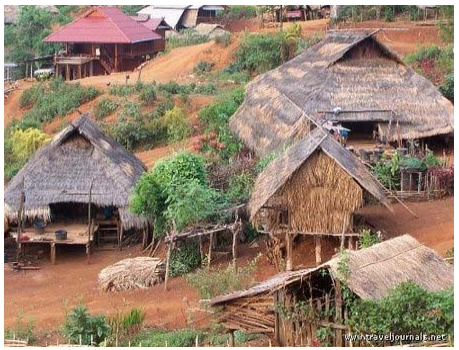
W Shi et al. ,Emerging Microbes & Infections (2016) **5**, e68

Why Zika, Why now?... Why not?

Aedes aegypti

What makes it such a good vector?

- **Originally native to forests in West Africa**
- **Transported in water storage containers on slave ships around the world**
- **Adapted to close association with humans**
  - **Eggs & larvae in containers**
  - **Adults rest inside houses**
  - **Feed frequently and (almost) exclusively on human blood**
- **Now – in tropical and subtropical climates worldwide**



Eradication of *Ae. aegypti?*

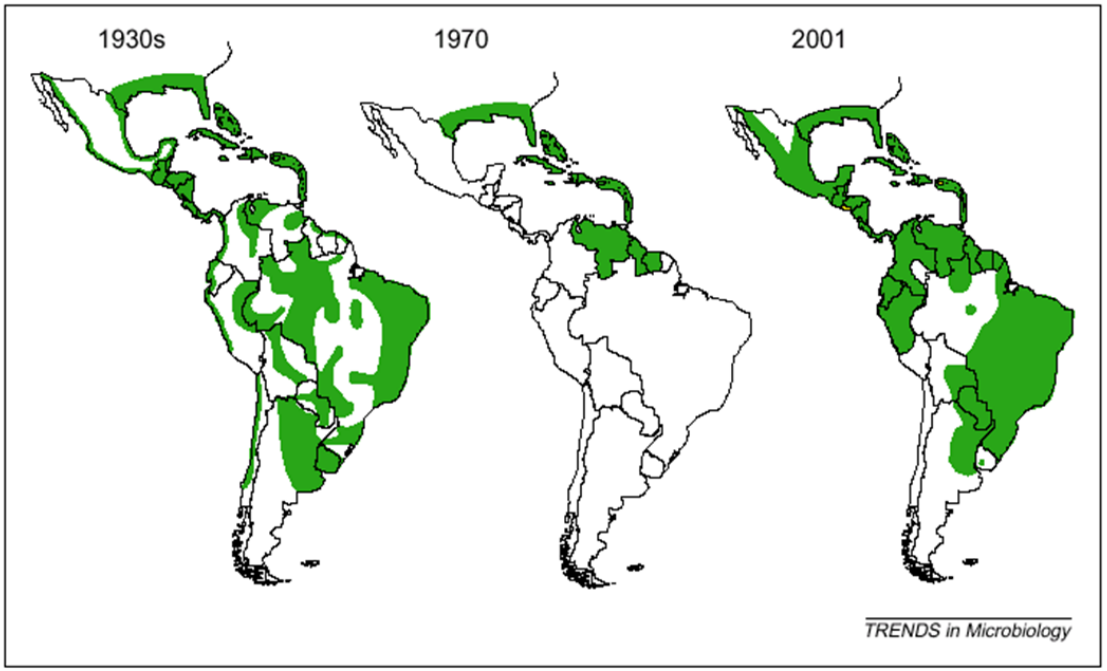
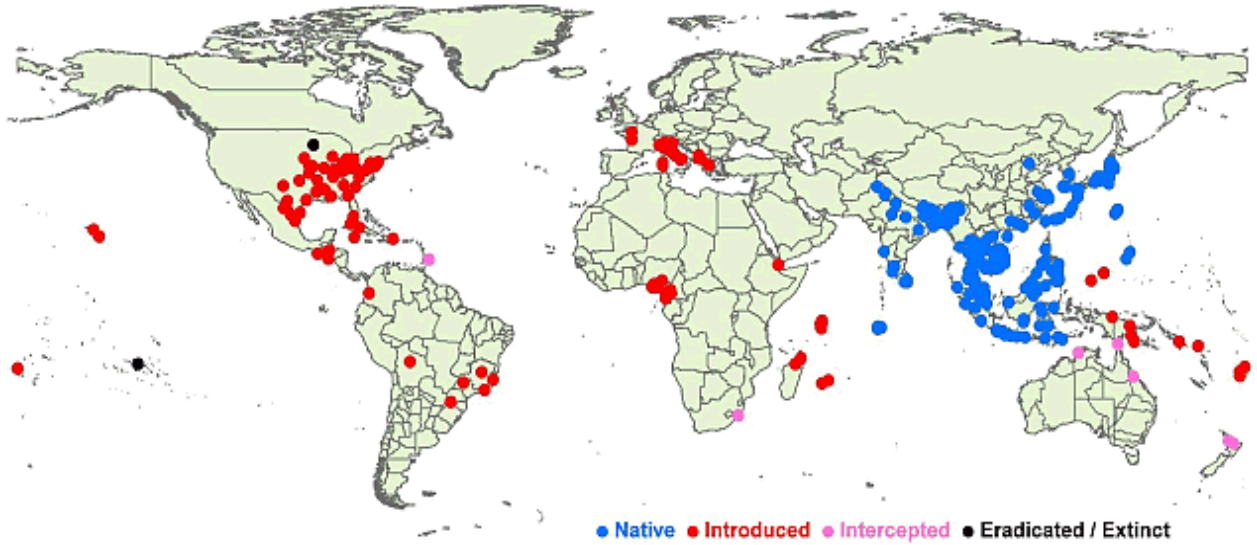


Fig. 1. *Aedes aegypti* distribution in the Americas: 1930s, 1970 and 2001.

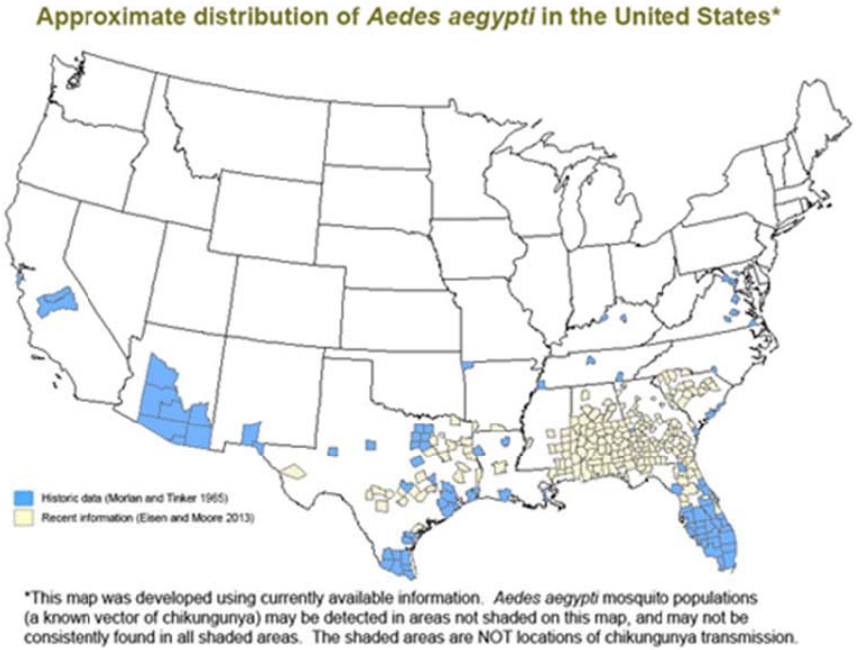
- *Ae. aegypti* eradication campaign 1940's – 1970's
- Since then *Ae. aegypti* has reestablished itself over former range

www.cdc.gov/ncidod/dvbid/dengue/map-ae-aegypti-distribution.htm

Aedes albopictus
(Asian Tiger Mosquito)



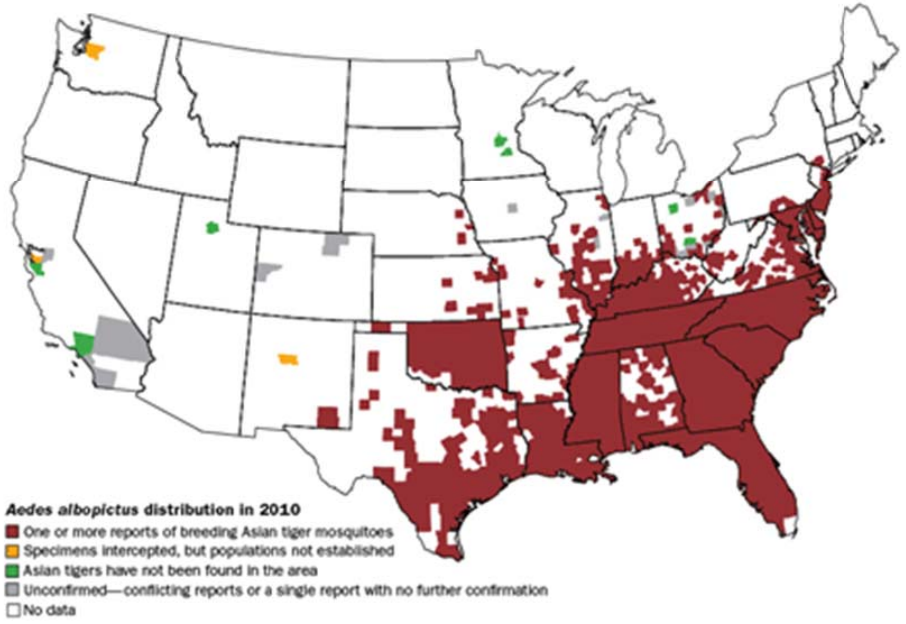
- Native to forests in Southeast Asia; gradually 'domesticated'
- Shipped around the world in used tires, 'lucky bamboo'
- Tolerates colder temps compared to *Ae. aegypti*
- Diverse habitats – urban, suburban
- Larval breeding sites – natural to artificial
- Prefers mammal blood but also reptiles, birds, amphibians
- Introduced to Texas in 1985



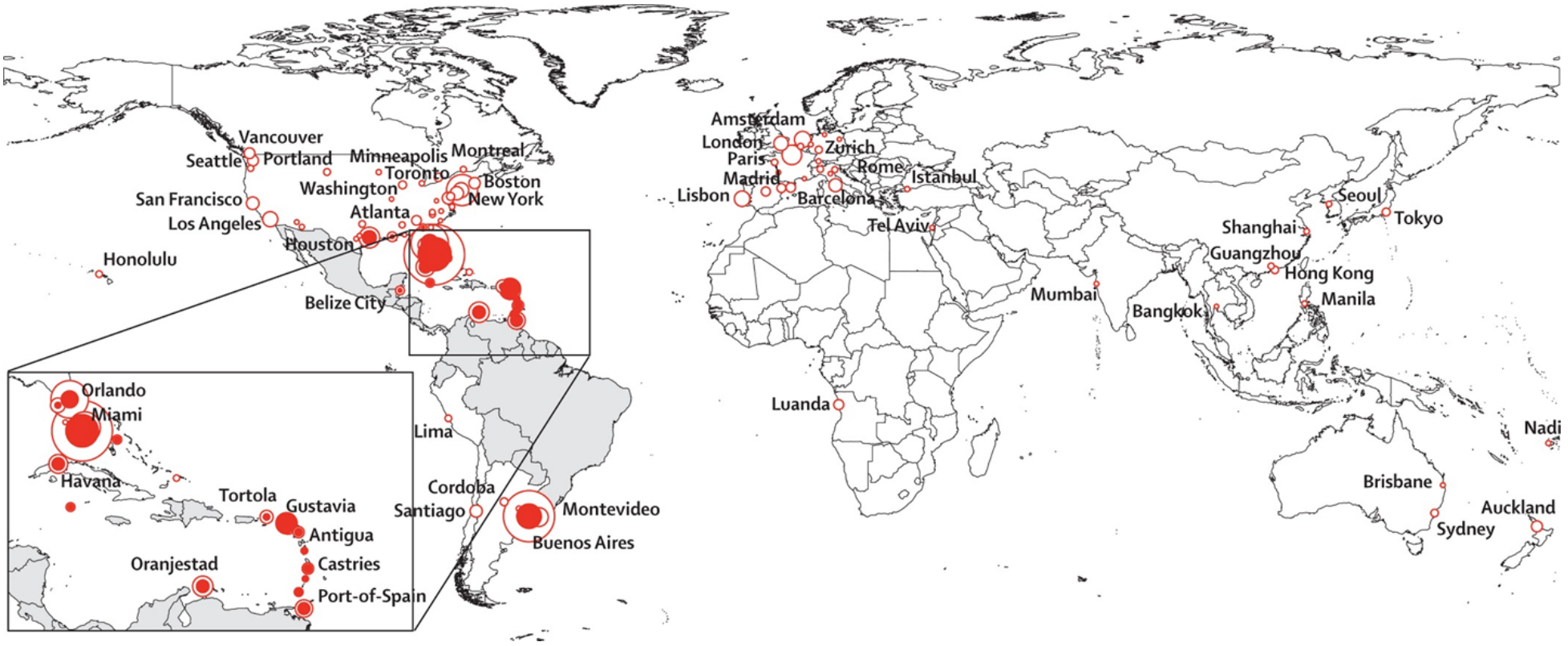
Ae aegypti

Ae albopictus

Cisr.ucr.edu

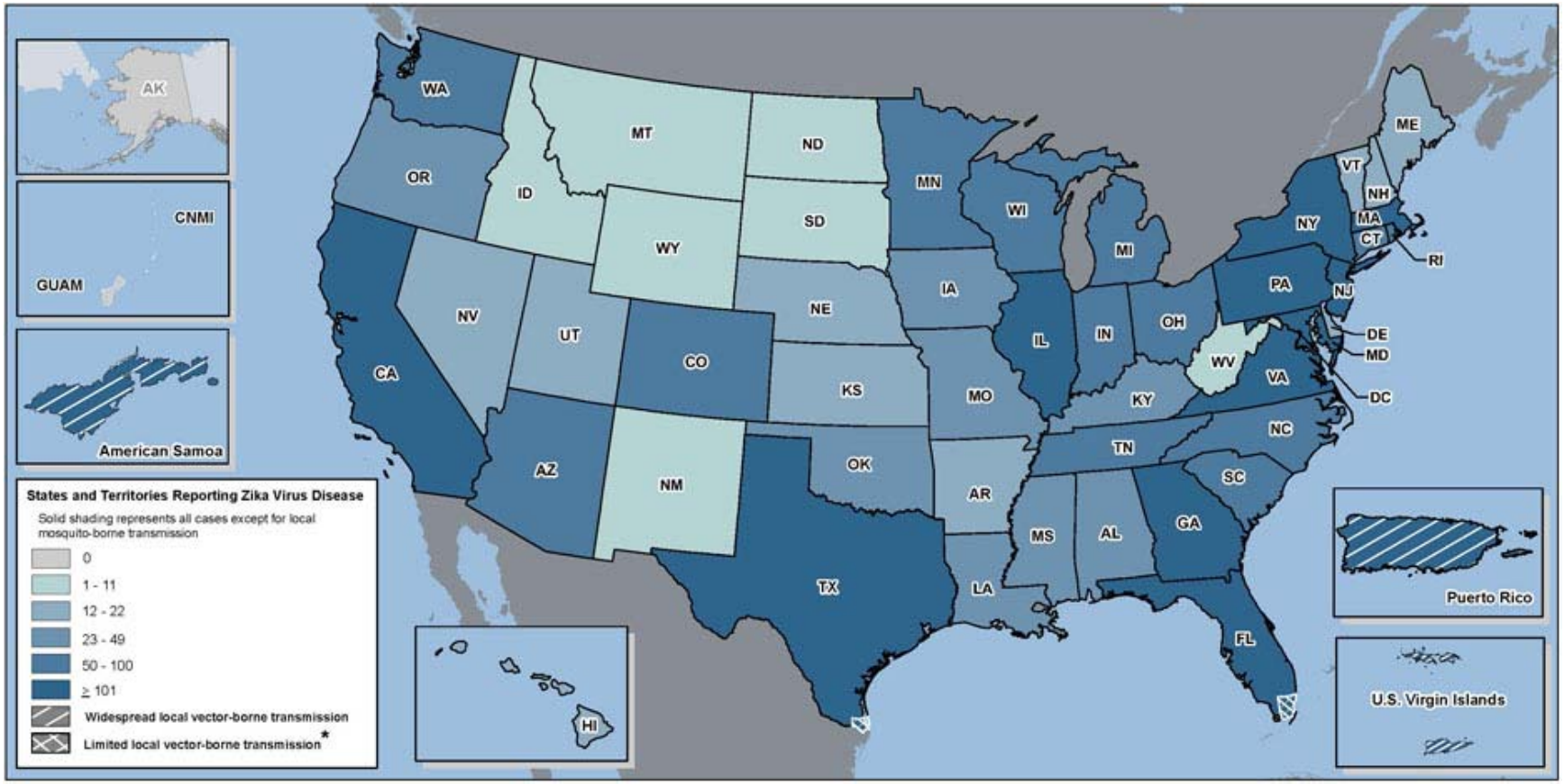


Risk map for spread of Zika virus



LM Gardner, N Chen, S Sarkar . The Lancet Infectious Diseases Mar 17 2016

ZIKV in the United States

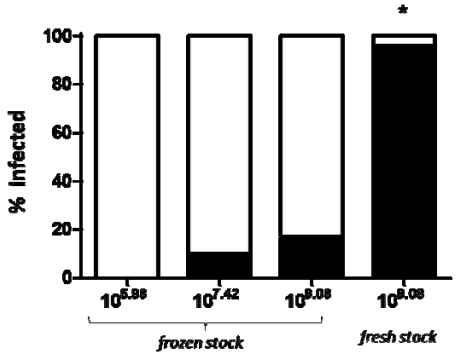


Lab confirmed Zika cases in NYS=1020 (all imported)

ZIKV- unique safety considerations?

Zika Uniqueness = Risk Group
-persistence in reproductive tissues/fluids
-documented sexual transmission
-unique tropism-cross placental/fetal infections
-well-documented teratogenic effects

- BSL-2 agent
- Minimal aerosol risk
- Specimen titers similar/below other flaviviruses
- Risk of laboratory infection minimal in the absence of inoculation—documented single case
- Difficult to culture from primary samples
- Particularly sensitive to freeze-thaw



Original Manuscript

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DOI: 10.1177/1535676017703383
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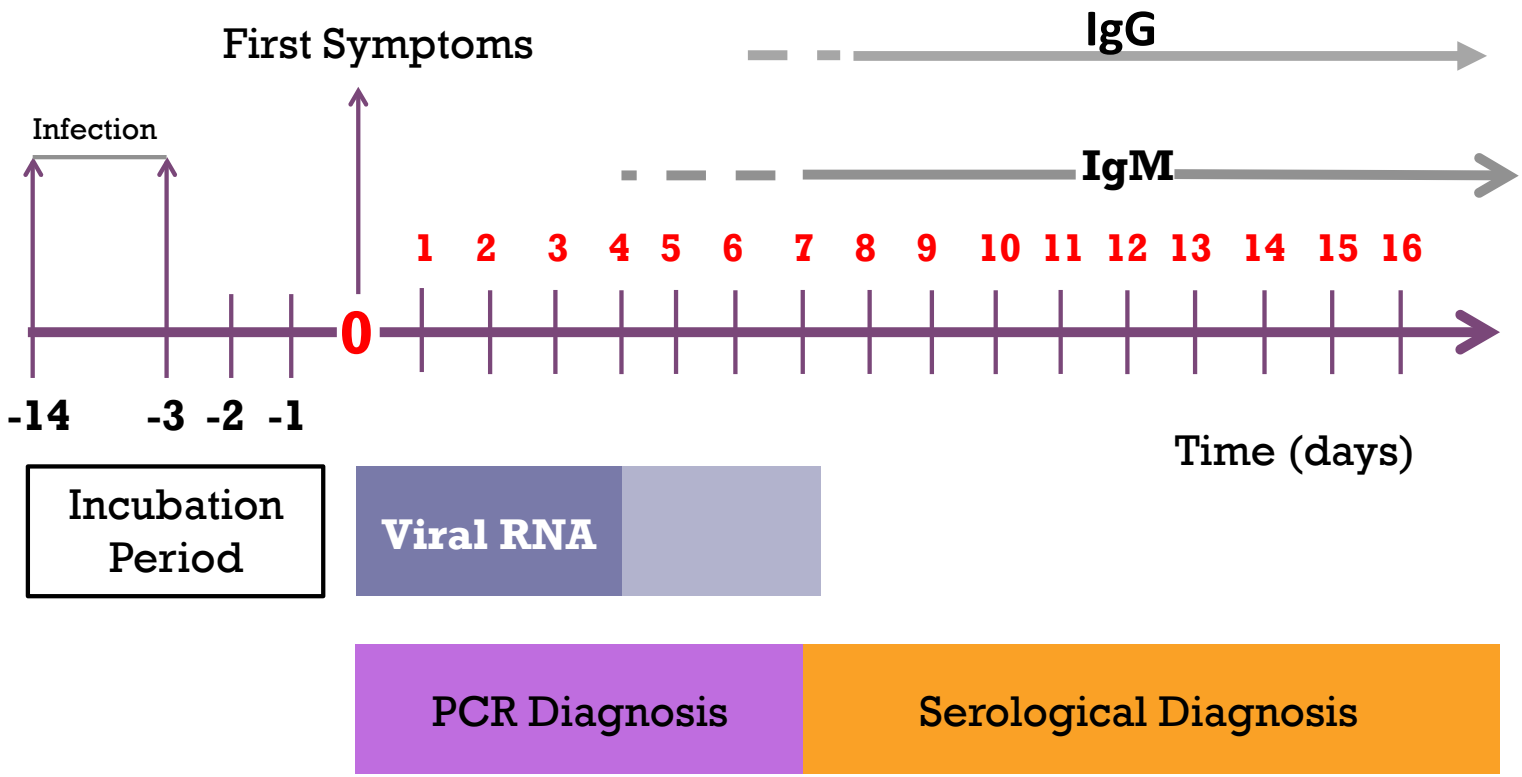
SAGE

Unreliable Inactivation of Viruses by Commonly Used Lysis Buffers

Kiet A. Ngo[1], Susan A. Jones[1], Theresa M. Church[2], Meghan E. Fuschino[2], Kirsten St. George[2], Joseph Maffei[1], Laura D. Kramer[1], and Alexander T. Ciota[1]

DIAGNOSTICS

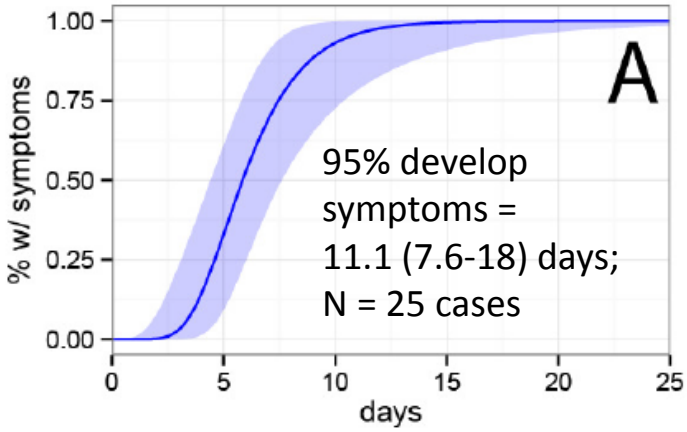
Zika Virus: Markers of Infection



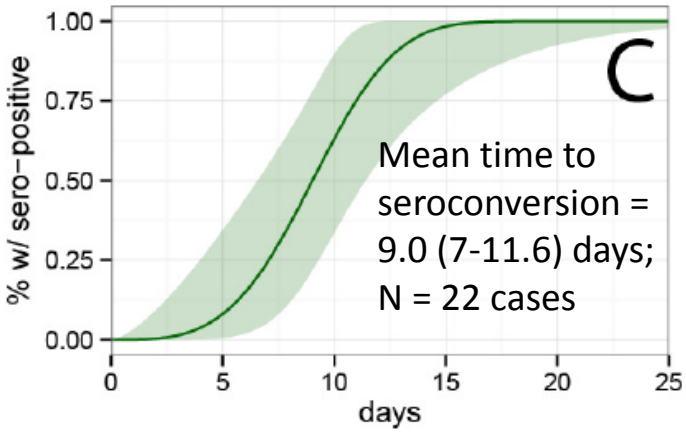
Slide courtesy of Desiree Lebeaud and Michele Barry

Time to key events in the course of Zika infection and their implications: a systematic review and pooled analysis

symptoms

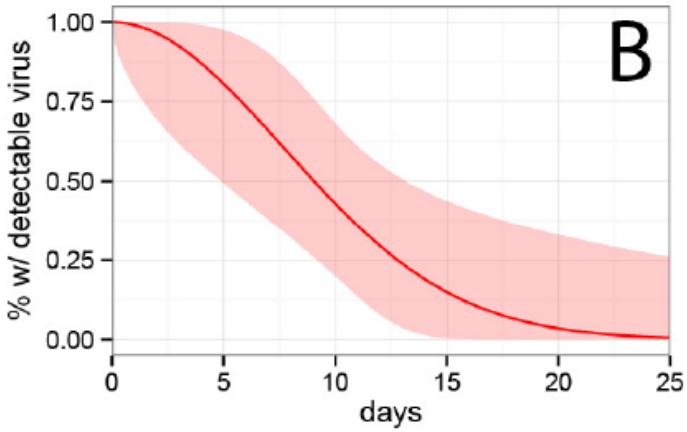


Incubation 5.9 (4.4-7.6) days



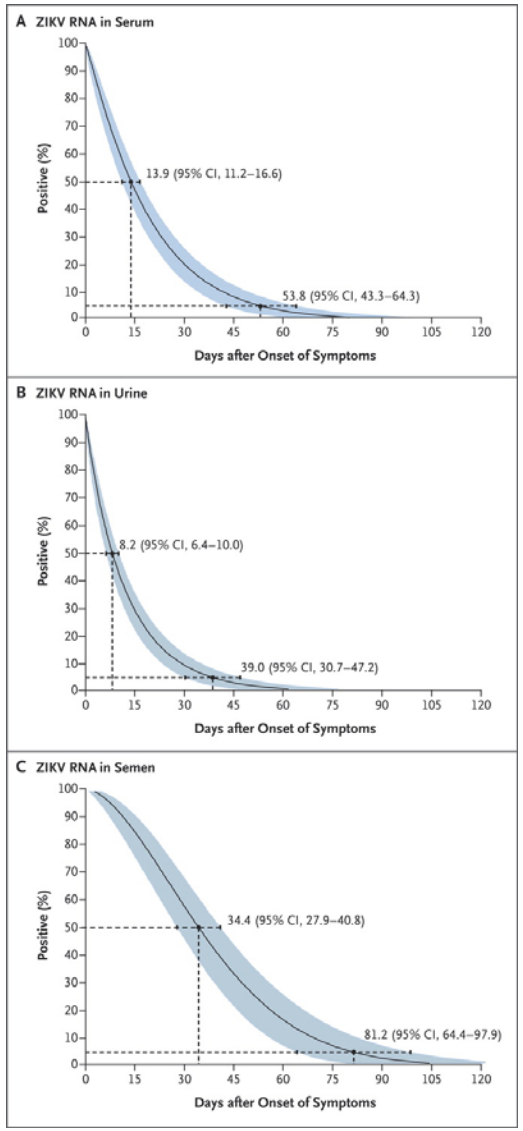
antibody

virus



Mean viremia duration = 9.9 (6.8-21.4) days; 5% > 18.9 (12.6-79.5) days; N = 22 cases

Lessler et al., Bull World Health Organ; 1 Apr 2016



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Persistence of Zika Virus in Body Fluids — Preliminary Report

Gabriela Paz-Bailey, M.D., Ph.D., Eli S. Rosenberg, Ph.D., Kate Doyle, M.P.H., Jorge Munoz-Jordan, Ph.D., Gilberto A. Santiago, Ph.D., Liore Klein, M.S.P.H., Janice Perez-Padilla, M.P.H., Freddy A. Medina, Ph.D., Stephen H. Waterman, M.D., M.P.H., Carlos Garcia Gubern, M.D., Luisa I. Alvarado, M.D., and Tyler M. Sharp, Ph.D.

February 14, 2017 | DOI: 10.1056/NEJMoa1613108

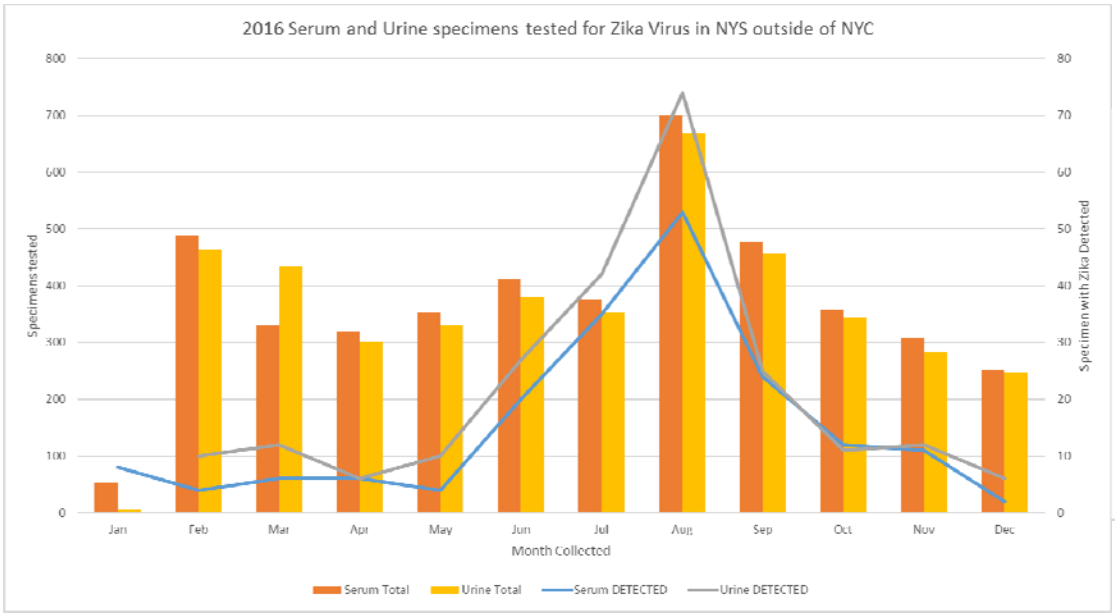
Max 188 days= RNA++ semen

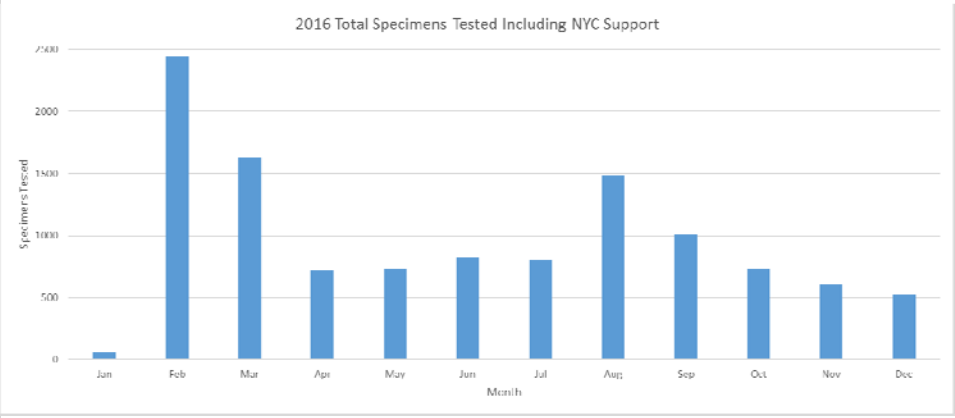
Samples types for Zika diagnostic testing

Sample type	Test of choice	Utility
Serum/plasma/whole blood*	rRT-PCR, ELISA, PRNT	Infection, surveillance
Urine	rRT-PCR	Infection, surveillance
Saliva	rRT-PCR	Infection but no added benefit
Semen	rRT-PCR	Infectivity ?
CSF	rRT-PCR, ELISA, PRNT	Neurological infection, GBS
Amniotic Fluid	rRT-PCR	Transmission to fetus
Placental tissue, umbilical cord, fetal tissues	rRT-PCR, pathology, IHC	Transmission to fetus

Slide courtesy of Kirsten St. George

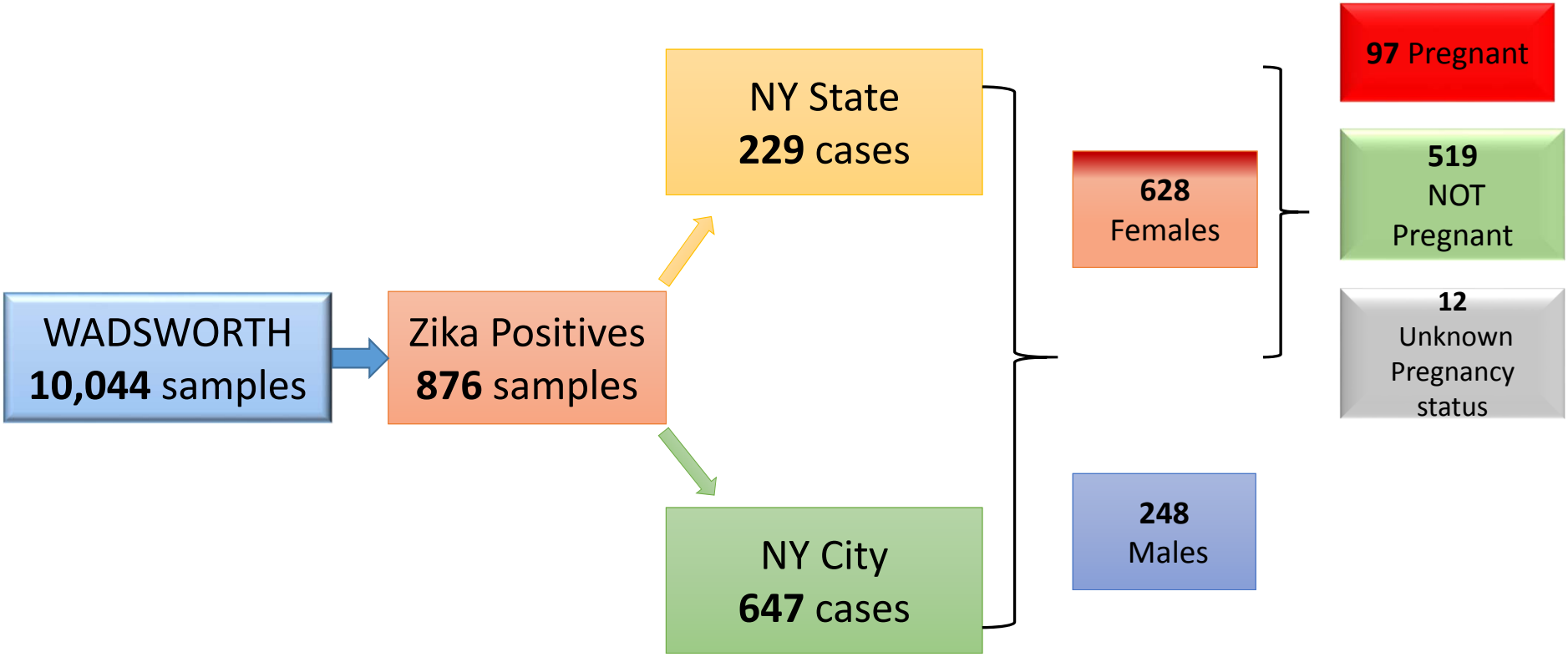
Wadsworth Laboratory ZIKV testing- Clinical Virology and Diagnostic Immunology Laboratories





Slide courtesy of A. Dean/St. George

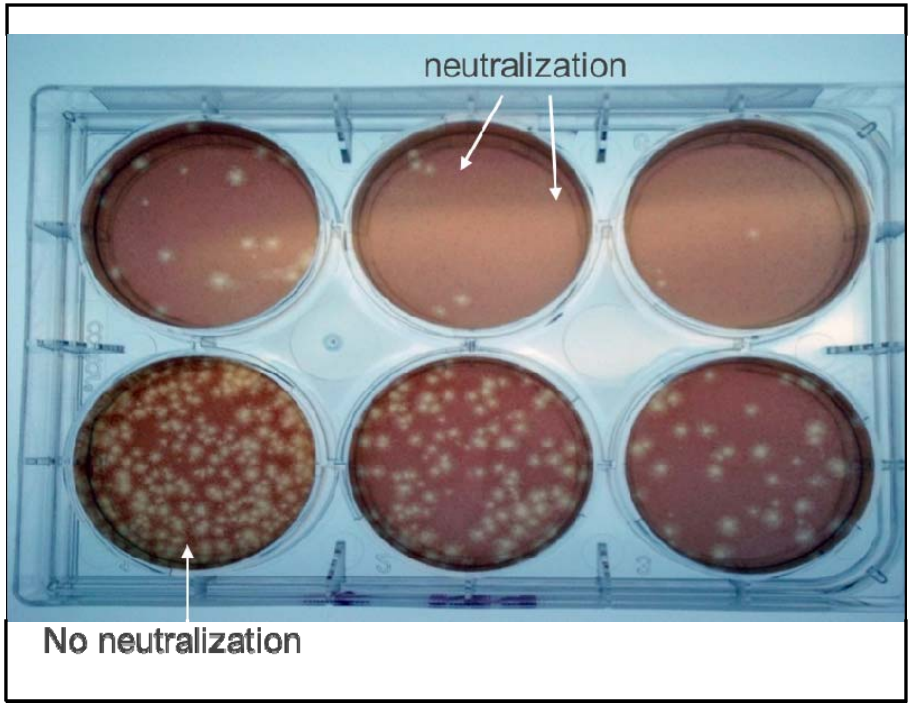
NYS Zika Data through 2016



Data provided by NYSDOH Division of Epidemiology

Plaque reduction neutralization test (PRNT)

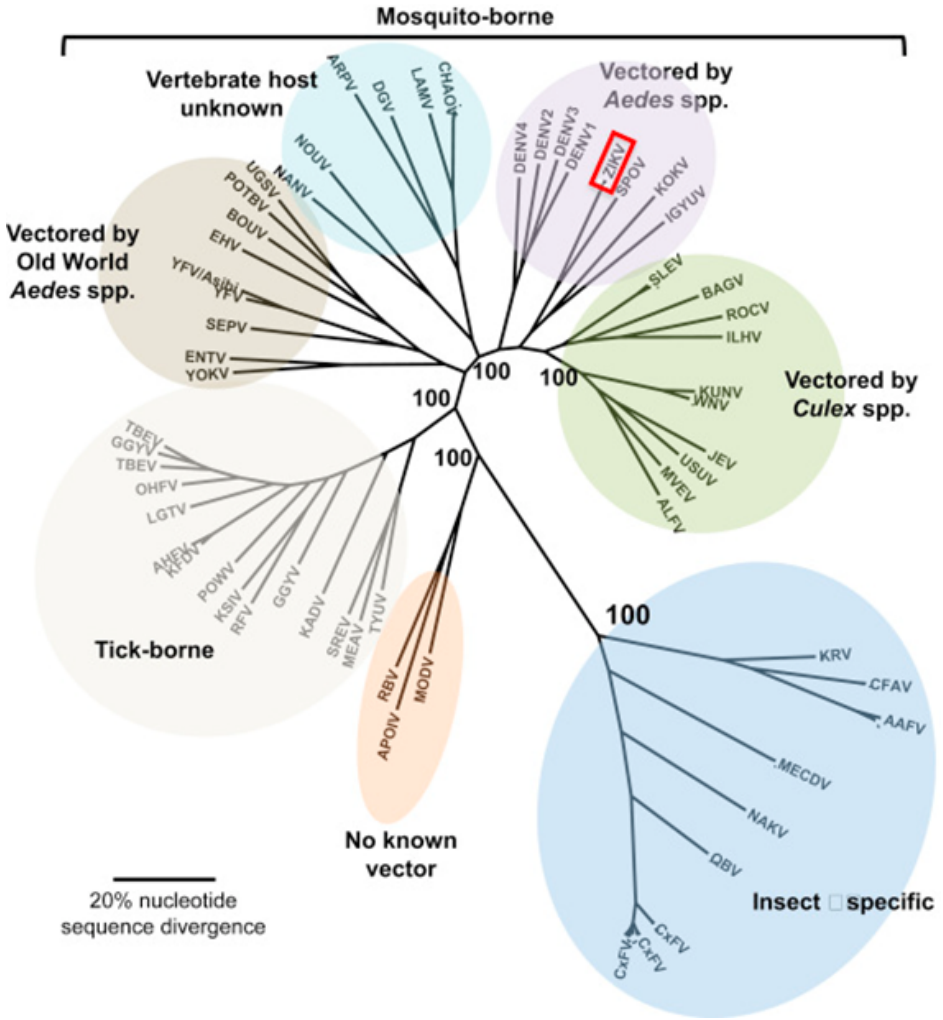
- Method to quantify the neutralizing antibody titer to a specific virus
- Serially diluted sera are mixed with a constant, known concentration of virus
- Perform on acute and convalescent sera
  - 90% reduction indicates infection with specificity
  - 4-fold rise in titer indicates current or recent infection



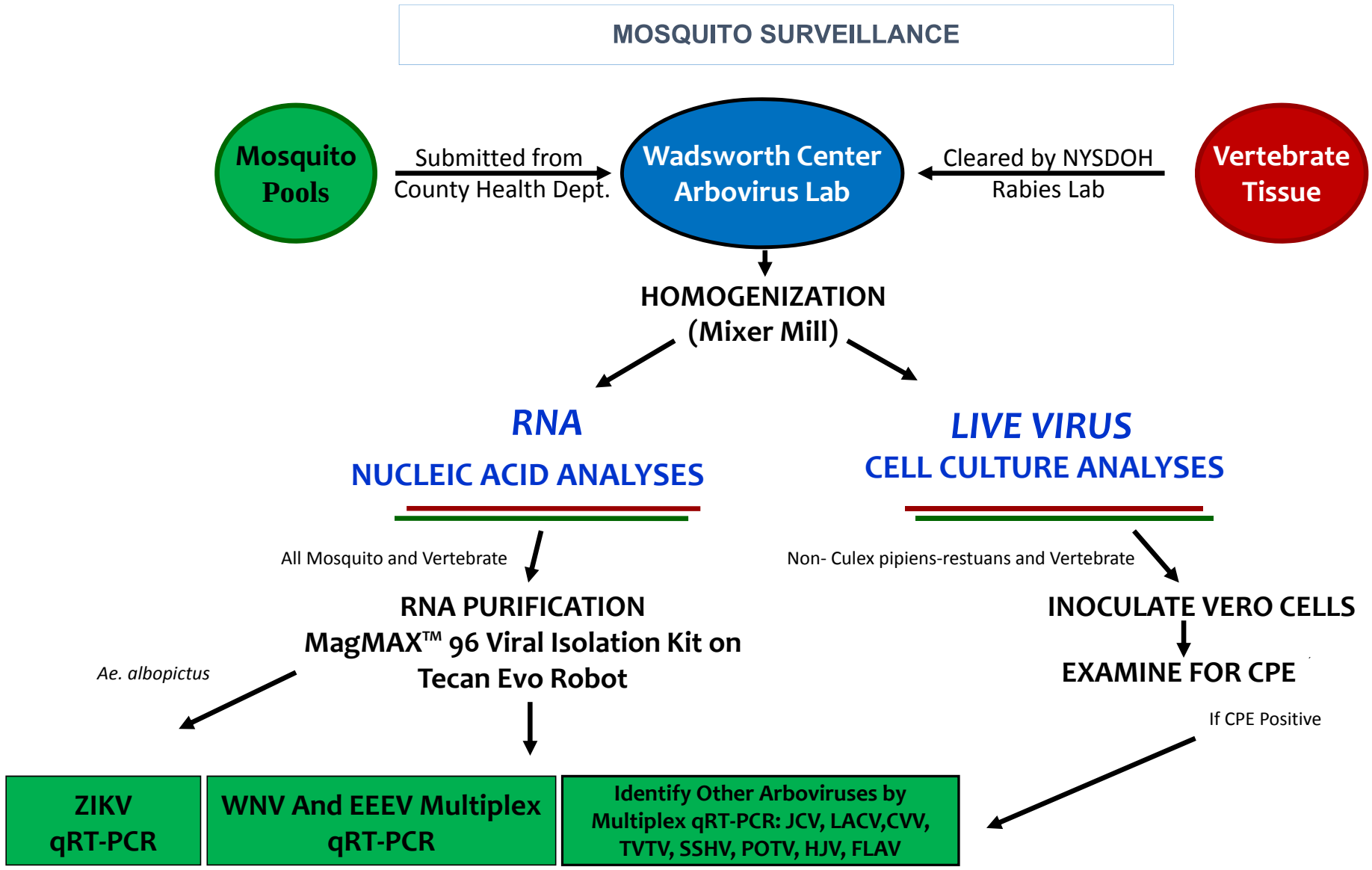
2001-2015= Arbovirus Laboratory performed ~200 PRNTs/year
Since Zika= ~200 PRNTs/month---cross neutralizations performed—moved to screening neuts

Flaviviruses with serologic cross reactivity to Zika virus

- Dengue virus serotypes 1-4
- Yellow fever virus
- West Nile virus
- St. Louis encephalitis virus
- Japanese encephalitis virus
- Powassan virus



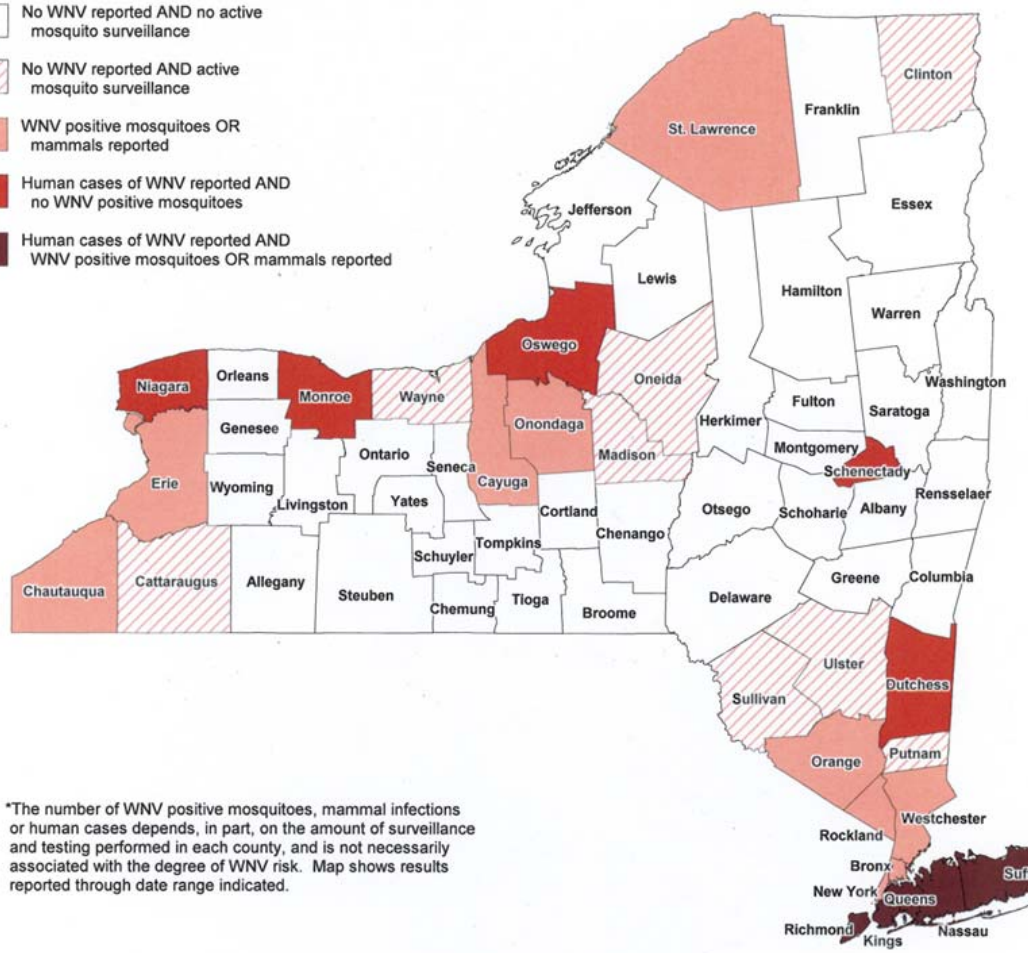
Slide courtesy of Kirsten St. George


MOSQUITO SURVEILLANCE
Mosquito Pools
Submitted from County Health Dept.
Wadsworth Center Arbovirus Lab
Cleared by NYSDOH Rabies Lab
Vertebrate Tissue
HOMOGENIZATION (Mixer Mill)
RNA
NUCLEIC ACID ANALYSES
LIVE VIRUS
CELL CULTURE ANALYSES
All Mosquito and Vertebrate
Non- Culex pipiens-restuans and Vertebrate
RNA PURIFICATION
MagMAX™ 96 Viral Isolation Kit on Tecan Evo Robot
INOCULATE VERO CELLS
EXAMINE FOR CPE
If CPE Positive
Ae. albopictus
ZIKV qRT-PCR
WNV And EEEV Multiplex qRT-PCR
Identify Other Arboviruses by Multiplex qRT-PCR: JCV, LACV,CVV, TVTV, SSHV, POTV, HJV, FLAV

2016 West Nile Virus Surveillance Summary

Positive Test Results* (1/1/2016 - 10/29/2016)

- No WNV reported AND no active mosquito surveillance
- No WNV reported AND active mosquito surveillance
- WNV positive mosquitoes OR mammals reported
- Human cases of WNV reported AND no WNV positive mosquitoes
- Human cases of WNV reported AND WNV positive mosquitoes OR mammals reported



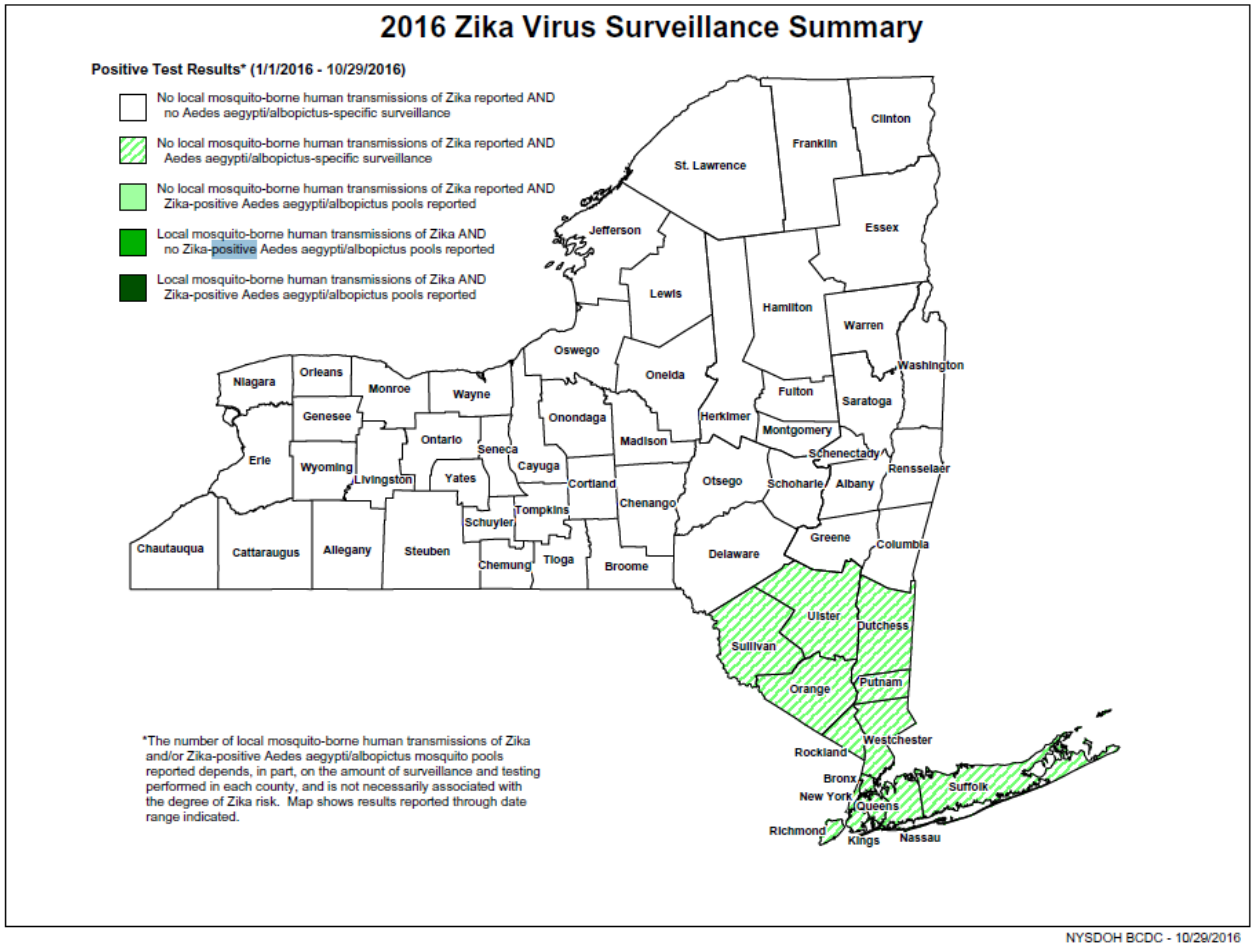
*The number of WNV positive mosquitoes, mammal infections or human cases depends, in part, on the amount of surveillance and testing performed in each county, and is not necessarily associated with the degree of WNV risk. Map shows results reported through date range indicated.

NYSDOH BCDC - 10/29/2016

NYS arbovirus (mosquito-borne) positives-2003-2013

Family	Virus	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	total
Flaviviridae	West Nile	200	53	266	178	92	154	60	514	273	694	341	2825
Togaviridae	Eastern Equine	10	17	5	68	20	21	59	66	45	1	53	365
	Highlands J	1	6	1	1	*	*	23	11	36	*	25	104
Bunyaviridae	South River	*	*	*	1	*	4	*	*	*	*	0	5
	Potosi	52	*	*	14	*	49	*	*	5	20	10	150
	Cache Valley	41	1	6	7	1	13	*	6	10	23	10	118
	Trivitatus	3	4	1	13	*	7	14	8	*	*	6	56
	Jamestown Canyon	4	6	2	1	2	12	10	8	*	*	4	49
	La Crosse	1	1	1	3	*	*	6	*	*	*	*	12
	California serogroup	*	*	*	*	*	*	*	*	*	12	16	28
Rhabdoviridae	Flanders	20	69	92	1	*	13	19	10	39	23	59	345
-	ALL	332	157	374	287	115	273	191	623	420	777	508	4057

Zika Virus Surveillance Map



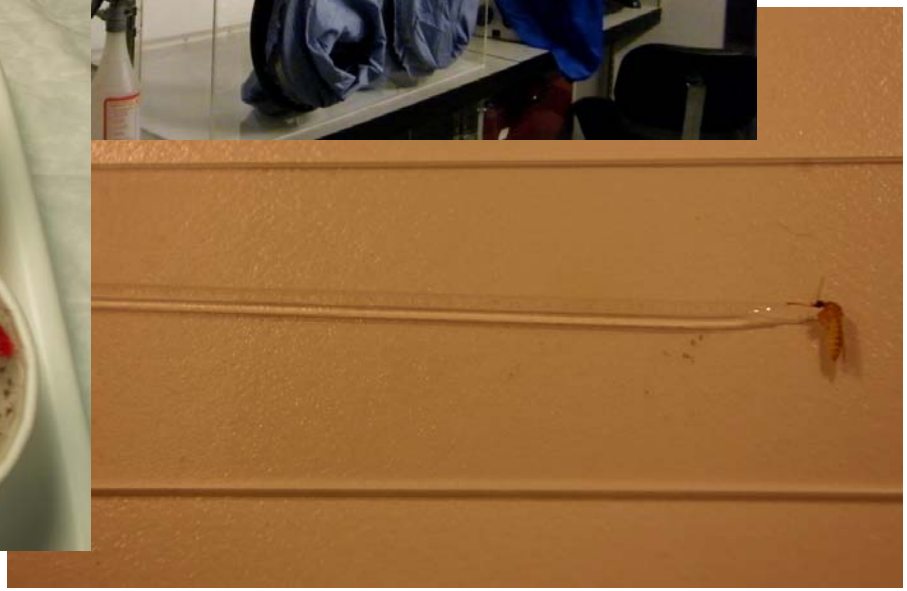
The BG-Sentinel mosquito trap:

- mimics convection currents created by a human body
- employs attractive visual cues
- releases artificial skin emanations through a large surface area
- can be used without CO2 to specifically capture selected mosquito species
- is an excellent general mosquito trap when used with CO2

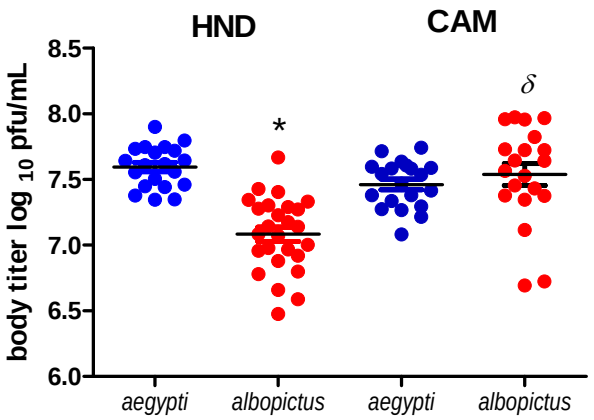
2016 Arbovirus Surveillance

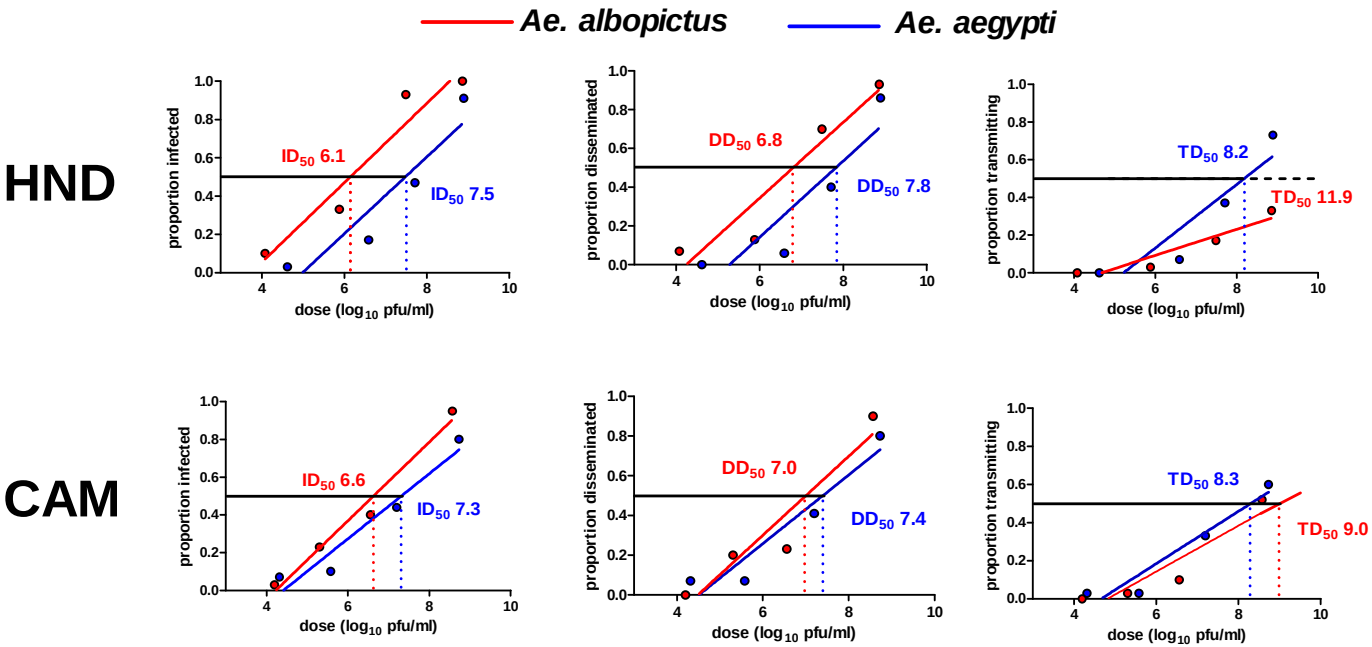
COUNTY	Pools Tested	WNV(+)	EEE(+)	AVE POOL SIZE	SUBMISSION DATES	ALB POOLS TESTED	AVE POOL SIZE	ZIKA (+)
Bronx	0	0	0	0	9/2-9/30/2016	13	24	0
Chautauqua	166	2	0	51	7/19-9/28/2016			
Clinton	51	0	0	26	6/21-8/30/2016			
Cattaraugus	60	0	0	44	6/21-7/26/2016			
Cattaraugus/Buffalo	287	0	0	37	7/19-9/28/2016			
Erie	348	36	0	26	7/25-9/26/2016			
Madison	130	0	0	35	6/3-9/9/2016			
Nassau	371	20	0	27	6/7-10/4/2016	166	26	0
Oneida	120	0	0	38	6/3-8/26/2016			
Onondaga	478	4	0	45	6/7-9/30/2016			
Orange	292	2	0	26	6/20-10/11/2016			
Oswego	478	0	5	40	6/3-9/15/2016			
Putnam	28	0	0	29	6/30-9/28/2016			
Rockland	574	51	0	49	6/14-9/27/2016	57	23	0
Suffolk	1400	142	0	39	6/7-10/12/2016	491	27	0
Ulster	15	0	0	16	9/14-9/16/2016			
Westchester	274	3	0	30	6/17-10/7/2016	62	17	0
Wayne	85	0	0	16	7/19-9/20/2016			
Total	5156	260	5			789		0

Mosquito experimentation- understanding transmission potential of ZIKV



16 aa differences





Volume 23, Number 7—July 2017

Research

Effects of Zika Virus Strain and *Aedes* Mosquito Species on Vector Competence

Alexander T. Ciota✉, Sean M. Bialosuknia, Steven D. Zink, Matthew Brecher, Dylan J. Ehrbar, Madeline N. Morrissette, and Laura D. Kramer

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Suggested citation for this article

On This Page

Viremia levels estimated to be <3.0 log10pfu/ml, much lower than dengue and chikungunya viruses

Potential role for vertical transmission of ZIKV in mosquitoes?

Vertical Transmission of Zika Virus by *Aedes aegypti* and *Ae. albopictus* Mosquitoes

Alexander T. Ciota, Sean M. Bialosuknia, Dylan J. Ehrbar, Laura D. Kramer

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To determine the potential role of vertical transmission in Zika virus expansion, we evaluated larval pools of perorally infected *Aedes aegypti* and *Ae. albopictus* adult female mosquitoes; ≈1/84 larvae tested were Zika virus–positive; and rates varied among mosquito populations. Thus, vertical transmission may play a role in Zika virus spread and maintenance.

- 1 in 84 larvae infected from infected female -significantly higher than what has historically been measured for other flaviviruses

What's next?

- High levels of ZIKV clinical testing are expected to continue for the immediate future- new assays with increased specificity
- ZIKV mosquito surveillance efforts will continue through 2017
- Continued sequencing and assessment of strain and population-specific transmissibility/evolutionary potential
- Development and utilization of ZIKA infectious clone for genetic/mechanistic studies


HEALTHY LIVING 04/03/2017 06:06 pm ET | Updated Apr 04, 2017

Scientists Start Second Phase Of Zika Vaccine Testing

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Dylan Ehrbar	Charlie Renker

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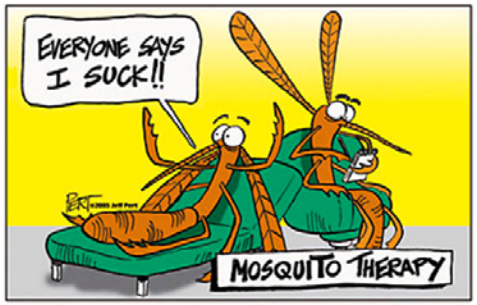
S. Wong- Diagnostic Immunology Lab

QUESTIONS?



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