



2015 Science Writers Symposium

Developing Tools to Keep Dengue and Chikungunya Out of Our Blood Supply

Maria Rios, Ph.D.

Principal Investigator

FDA Center for Biologics Evaluation and Research

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Dengue Virus and Chikungunya Virus

Different viruses, similar threats

DENV is a Flavivirus (4 serotypes) and CHIKV is an Alphavirus

- Both cause intense outbreaks.
- Symptoms are indistinguishable at early phase of infection.
- DENV infection can progress to severe cases with bleeding, circulatory failure, shock—and may result in death.
- CHIKV infection can result severe and disabling symptoms, even death especially certain groups.
- Treatment: symptom management and supportive care—no specific drug or vaccine available.

CHIKV and DENV Transmission

Transmitted to humans by mosquitoes of the genus *Aedes* (Ae)

Ae. aegypti



&

Ae. albopictus



Both viruses can simultaneously co-infect humans and mosquitoes.

Ae. aegypti and Ae. albopictus Distribution in the U.S.

Risk of outbreak: the presence of transmitting vector and human cases

Approximate distribution of *Aedes aegypti* in the United States*



Approximate distribution of *Aedes albopictus* in the United States*



*Maps were developed using currently available information. Mosquito populations may be detected in areas not shaded on this map, and may not be consistently found in all shaded areas.

<http://www.cdc.gov/ncezid/dvbd>



Threats to Blood Safety

- Infections can be asymptomatic, and disease cases have a pre-symptomatic period.
 - Individuals feel well enough to donate blood

- Both viruses have been found in blood donated for transfusion, could be transfusion transmitted (TT)

TT-DENV has been documented TT-CHIKV is plausible

- No blood screening assays available for DENV or CHIKV.



Dengue in the Continental U.S.

Local transmission (outbreak) and imported cases

- Most cases reported in the 48 continental states are imported by travelers or immigrants <http://www.cdc.gov/dengue/epidemiology/>

Recent outbreaks (local transmission) of Dengue

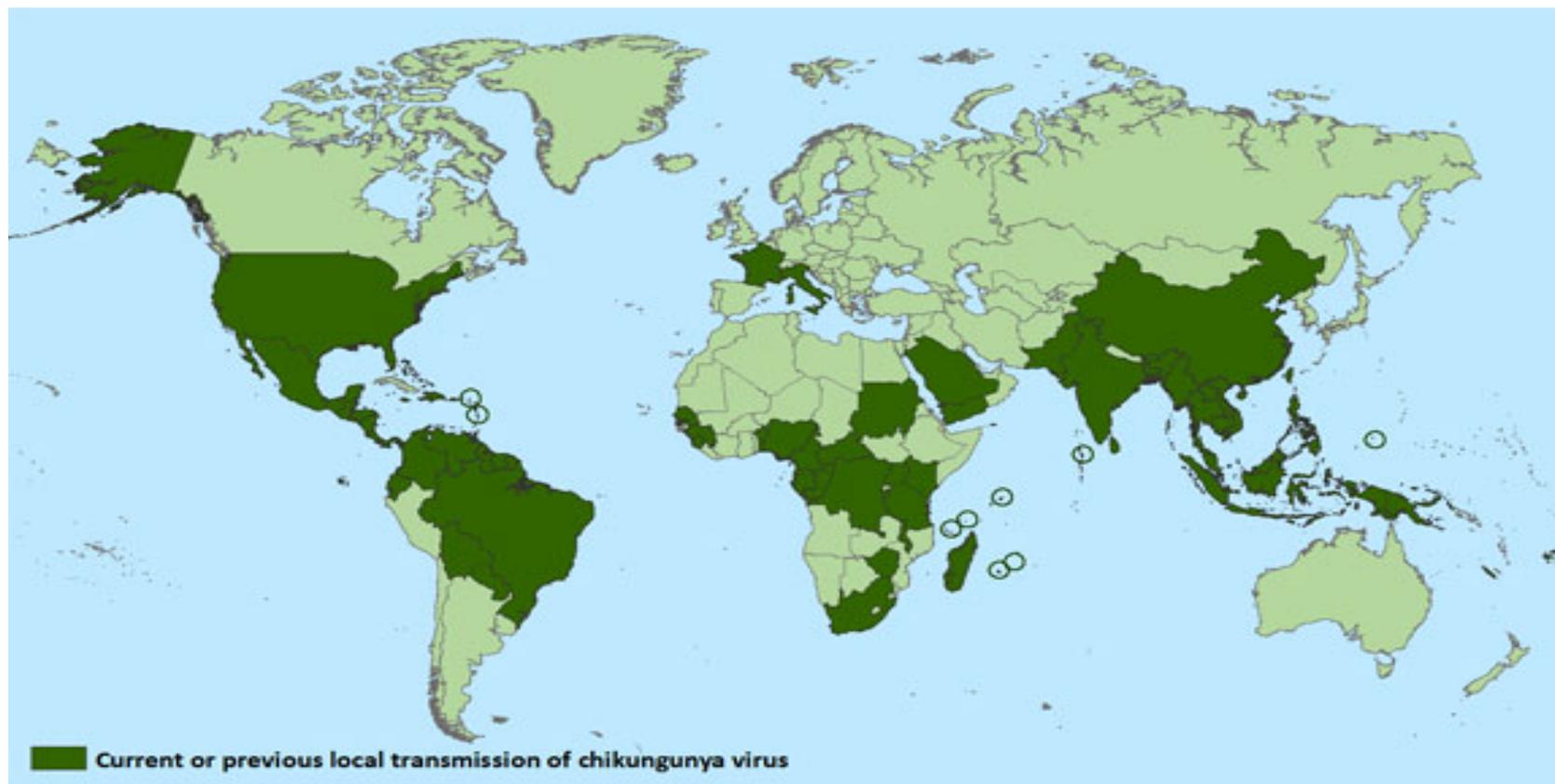
- South TX (2005) <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5631a1.htm>
 - 25 cases of DENV-2
- Key West, FL (2009–2010)
 - 2009: 28 cases DENV-1; 2010: 56 cases DENV-1, -2, -3 and -4
- DENV is endemic in Puerto Rico; circulation of the 4 serotypes
 - Major epidemic in 2010: 21,298 cases DENV-1, -2 and -4
- We performed genetic studies of DENV from these outbreaks and reported results (*Añez et al. Am J Trop Med Hyg 2012; 87:548-553*)

Muñoz Jordán et al EID 2013; 19: 652-4, DOI: <http://dx.doi.org/10.3201/eid1904.121295>



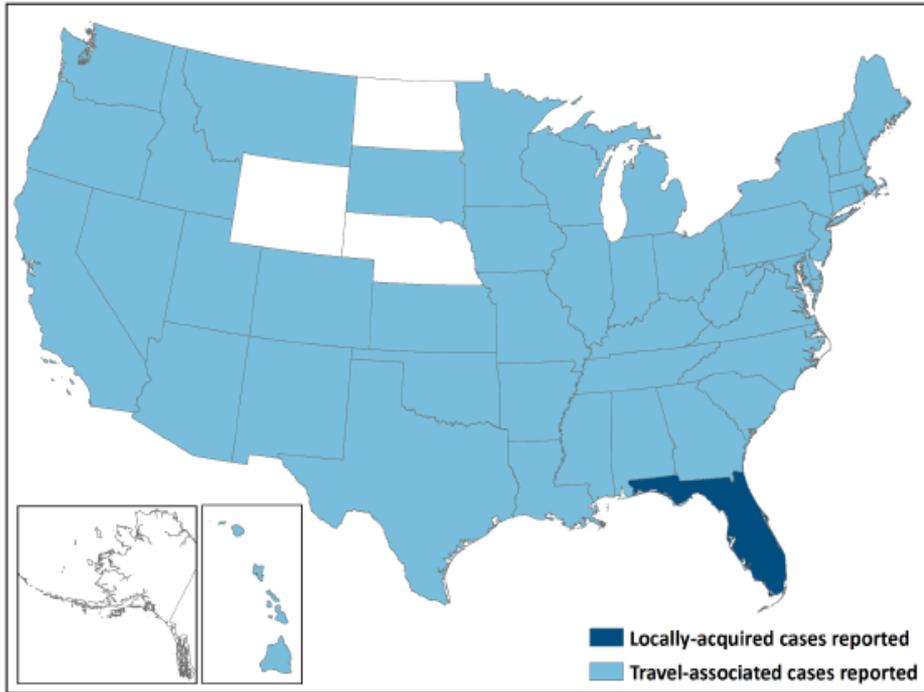
CHIKV Outbreaks Intensified since 2004

Africa, Asia, Europe, and the Indian and Pacific Ocean countries until 2013

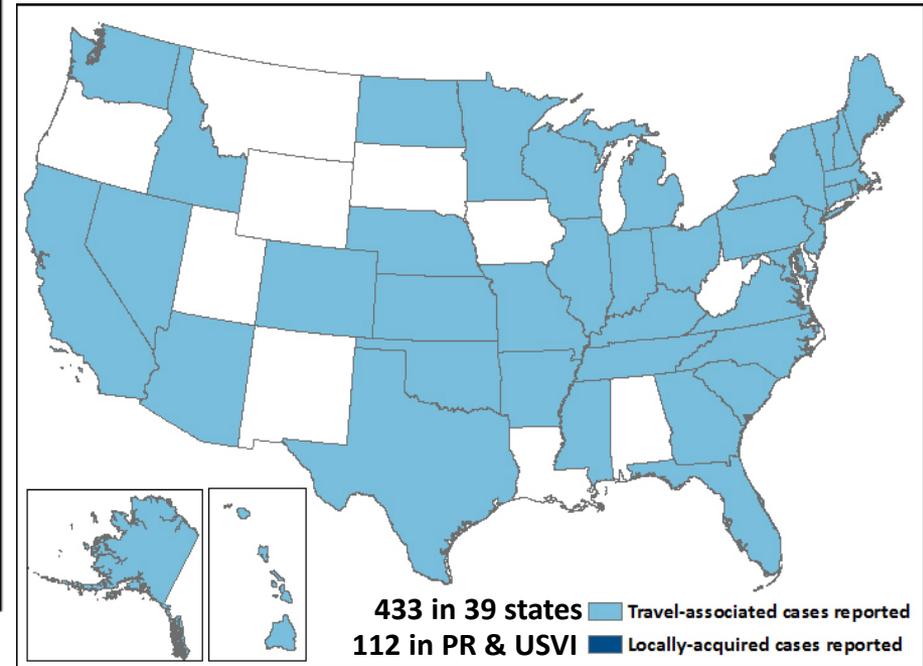


<http://www.cdc.gov/chikungunya/geo/index.html>

CHIKV Cases in the U.S. Reported to the CDC ArboNet



As of September 15, 2015



- 4,702 cases reported in Puerto Rico (including 4 fatalities), the U.S. Virgin Islands, & American Samoa
- WHO/PAHO reported >21,000 U.S. & territories (including imported, tested and suspected)

<http://www.cdc.gov/chikungunya/geo/united-states.html>



“What's Past Is Prologue”

Lessons and strategies from West Nile virus (WNV)

- Imported into U.S. in 1999, WNV is now endemic, causing 17 annual epidemics in the U.S.
 - ***Transmission by transfusion has been documented .***
 - 4–6 million infections
 - >18,000 cases neuroinvasive cases
 - 1,700 fatalities
- We developed critical viral reagents used for:
 - Assist assays development and evaluation of their analytical sensitivity (results are included in the package insert of the product)
 - Fulfill regularity requirements for evaluation of assays performance prior to: licensure and release new lot to market
- Since NAT implementation, >4,000 WNV-positive units have been removed from the blood supply and discarded



FDA's Actions to Manage DENV & CHIKV Threat

- **Held** public workshop to raise awareness of the risk of insect-borne viruses to blood safety and to discuss protective measures (2009)
- **Developed** non-infectious DENV and CHIKV to serve as reference standards (known as reference reagents)
 - to facilitate development of nucleic acid testing (genetic tests to detect virus in blood supply)
 - to evaluate how well these assays perform
 - ultimately for the pre-market evaluation of products

Añez et al Vox Sang 2015DOI: 10.1111/vox.12297;

Añez et al. Genome Announc. 2(4):e00587–14



Development CHIKV RNA Reference Reagent

- ➔ CHIKV strain (R91064) was used to produce viral stock in culture
- ➔ Biological and genetic characterization of viral stock
 - Infectivity titer (FFU) 2.10E+07



Complete Coding Region Sequence of a Chikungunya Virus Strain Used for Formulation of CBER/FDA RNA Reference Reagents for Nucleic Acid Testing

© Germán Añez, Daniel A. Heisey, Maria Rios

Laboratory of Emerging Pathogens, Division of Emerging and Transfusion Transmitted Diseases, Office of Blood Research and Review, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Bethesda, Maryland, USA

We have sequenced the complete coding region of a chikungunya virus isolate (R91064) used in the formulation of CBER/FDA RNA reference reagents for nucleic acid technology testing.

- ➔ Heat inactivation (HI) by incubation at 60° C for 90 minutes
 - Complete inactivation was verified by negative in Vero cells
- ➔ Concentration of HI-CHIKV stock was determined in collaboration with 7 laboratories in the US

CHIKV RNA Reference Reagent

Reference Reagent was formulated and tested in collaborative studies, results were published.

Stability and accelerating degradation studies

VoxSanguinis

The International Journal of Transfusion Medicine

ISBT International Society of Blood Transfusion

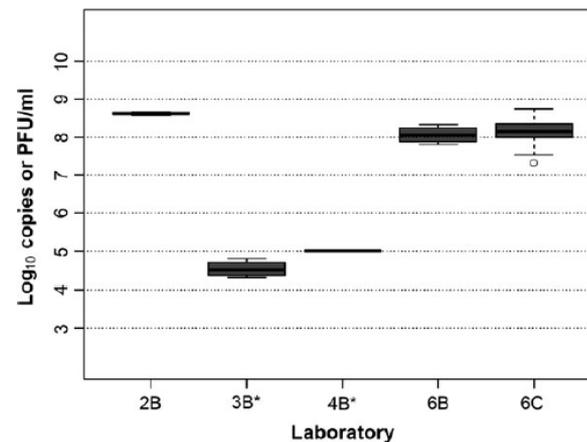
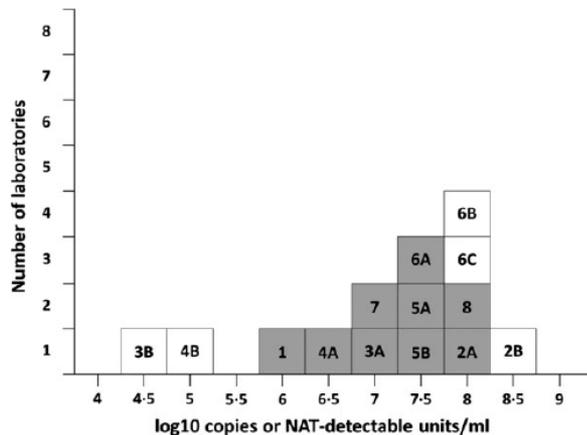
Vox Sanguinis (2015)

ORIGINAL PAPER

Published 2015. This article is a U.S. Government work and is in the public domain in the USA
DOI: 10.1111/vox.12297

Collaborative study for the characterization of a chikungunya virus RNA reference reagent for use in nucleic acid testing

G. Añez,^{1,†} Z. Jiang,² D. A. R. Heisey,^{1,††} S. Kerby,¹ M. Rios¹ & The Chikungunya virus Collaborative Study Group*





Actions Taken by FDA (continued)

- **Approved** clinical study protocols to evaluate suitability of assays for blood screening
 - DENV assays for the detection of viral antigen and nucleic acid following 2009–2010 outbreaks in Florida and Puerto Rico
- FDA is anticipating clinical study protocols for NAT assay that detects both DENV and CHIKV following 2013-2015 outbreaks in the Americas



How We Are Working to Keep the Blood Supply Safe

- **Monitoring** epidemics
 - **Participating** in monthly conference calls with U.S. public health service (PHS) agencies and Pan American Health Organization
- **Studying** the biology of infections and duration of viremia
- **Evaluating** potential strategies to avoid transmission by transfusion
 - **Importing** blood donations from low risk regions and enhancing donor deferrals or post-donations call-back
 - **Using** NAT screening and viral inactivation technology (Pathogen Reduction) when feasible: concern over affordability
 - **Deferring** travelers from donating blood for a period to cover duration of viremia