

Zika Virus in the Americas

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Biosecurity Research Institute (BRI): Pat Roberts Hall:

Zika virus: Discovery

Rockefeller Foundation dedicated to arbovirus
discovery 1947 (sentinel macaque),
1948 (mosquito),
1954 (human)

Adapted from National Academy
presentation by R. Rosenberg

Zika virus: Discovery

Adapted from National Academy presentations by
R. Rosenberg (platform photo by S. Beard)
And presentation by Scott Weaver

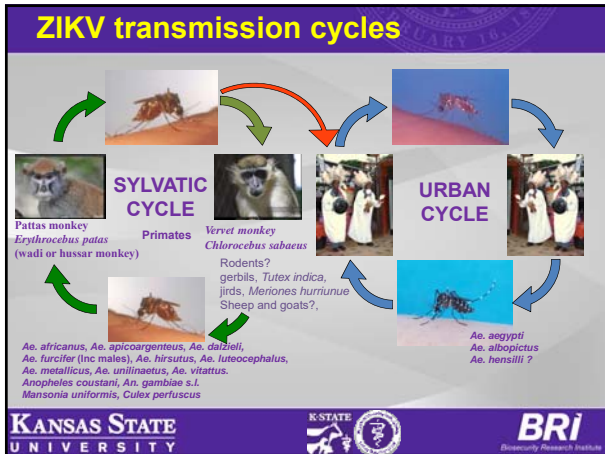
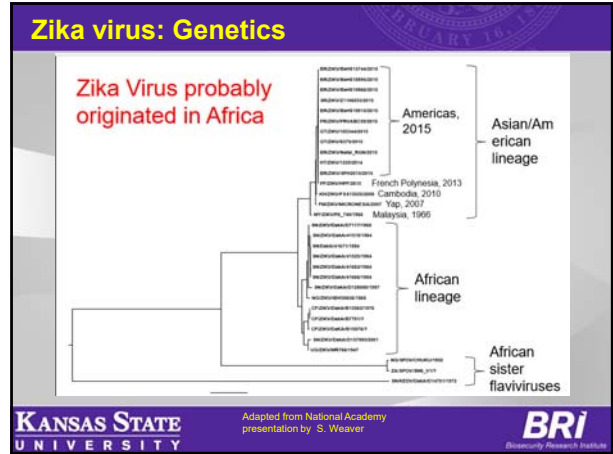
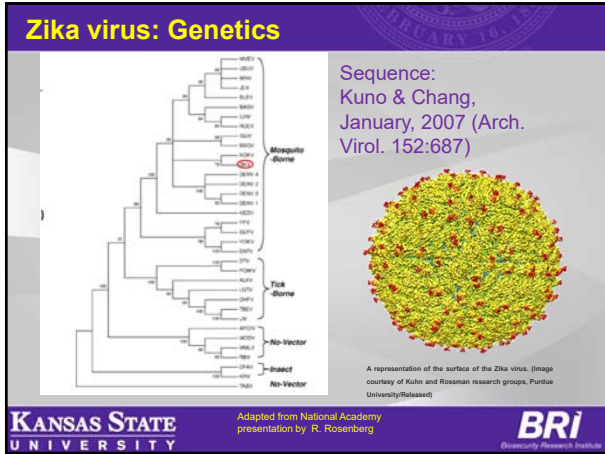
Zika virus: Genetics

A member of the Flaviviridae family of RNA viruses

Genome is single positive strand of RNA ~ 10,600
nt Transmitted by mosquitoes

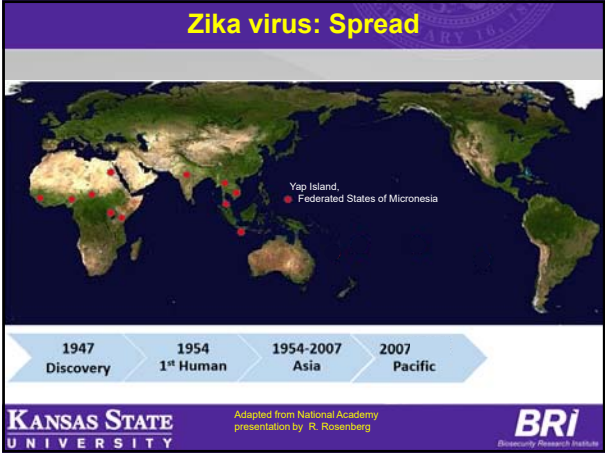
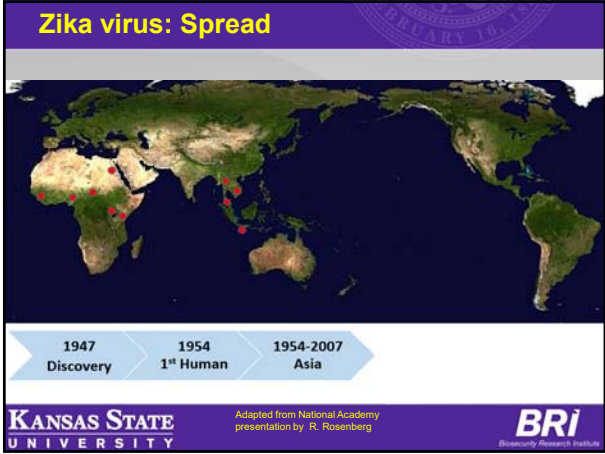
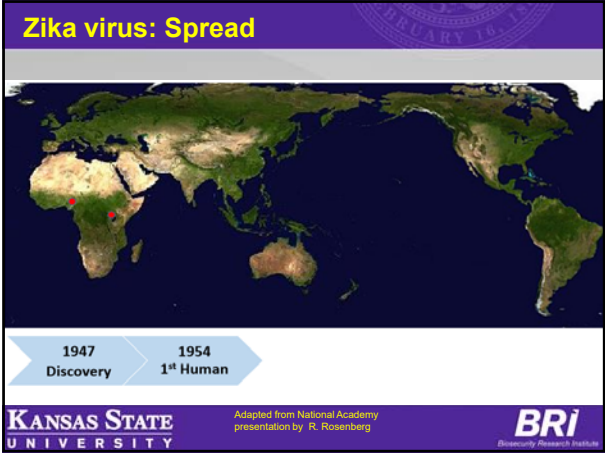
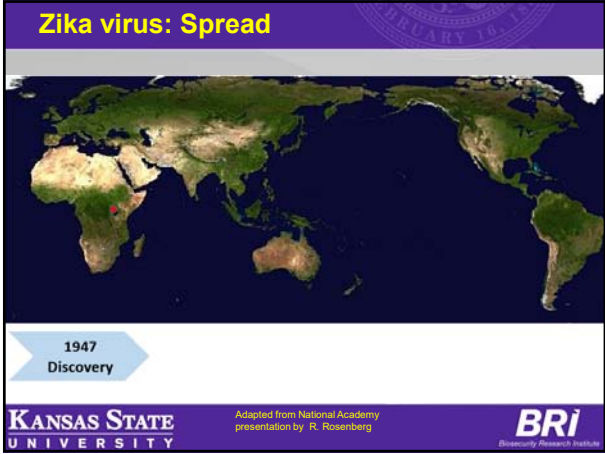
Virus particle contains a lipid bilayer, one genome
RNA, and three distinct types of viral proteins, : 1.
E - envelope protein 2. M - membrane protein/prM
– premembrane protein 3. C - capsid or core
protein

Adapted from National Academy
presentation by R. Kühn



ZIKV Getting to the Americas 1947 to 2014

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Zika virus: Yap Island

Adapted from National Academy presentation by R. Rosenberg

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Zika virus: Yap Island (2007)

THE NEW ENGLAND JOURNAL OF MEDICINE

ORIGINAL ARTICLE

Zika Virus Outbreak on Yap Island, Federated States of Micronesia

Mark R. Duffy, D.V.M., M.P.H., Tai-Ho Chen, M.D., W. Thane Hancock, M.D., M.P.H., Ann M. Powers, Ph.D., Jacob L. Kool, M.D., Ph.D., Robert S. Lanciotti, Ph.D., Moses Pretrick, B.S., Maria Marfel, B.S., Stacey Holzbauer, D.V.M., M.P.H., Christine Dubray, M.D., M.P.H., Laurent Guillaumot, M.S., Anne Griggs, M.P.H., Martin Bel, M.D., Amy J. Lambert, M.S., Janeen Laven, B.S., Olga Kosoy, M.S., Amanda Panella, M.P.H., Brad J. Biggerstaff, Ph.D., Marc Fischer, M.D., M.P.H., and Edward B. Hayes, M.D.

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Zika virus: Yap Island

49 confirmed and 59 probable cases of Zika virus disease. Most patients had mild illness.

Rash, fever, arthralgia, and conjunctivitis were common symptoms. No hospitalizations, hemorrhagic manifestations, or deaths due to Zika virus were reported.

Estimated that 73% of Yap residents 3 years of age or older were infected with Zika virus (more than 900 people in total).

The mosquito vector was not identified but *Aedes hensilli* was the predominant mosquito species identified.

Adapted from National Academy presentation by R. Rosenberg

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Zika virus: Yap Island

Aedes hensilli as a Potential Vector of Chikungunya and Zika Viruses

Abstract: An epidemic of Zika virus (ZIKV) illness that occurred in July 2007 on Yap Island in the Federated States of Micronesia prompted entomological studies to identify both the primary vector(s) involved in transmission and the ecological parameters contributing to the outbreak. Local and global surveys were performed to identify the major container-caring mosquito species for the study territory, which mosquitoes were also subjected to back-to-back epidemic, field, and genetic tests at select sites around the capital city. The predominant species found on the island was *Aedes (Stegomyia) hensilli*. An *Aedes* species was identified from the island that exhibited ecological, taxonomic, and genetic characteristics that were distinct from those of *Aedes (Stegomyia) albopictus* and *Aedes (Stegomyia) triseriatus*. Molecular analysis of the genome of *A. hensilli* was undertaken to determine the likelihood of this species serving as a vector for ZIKV and other arboviruses. Infective rates of up to 80%, 92%, and 100% and dissemination rates of 23%, 40%, and 17% for ZIKV, Chikungunya, and Dengue 2 virus, respectively, were found supporting the possibility that this species served as a vector during the Zika outbreak and that it could play a role in transmitting other mosquito-borne arboviruses.

VIRUS	INFECTION RATE	DISSEMINATION RATE
ZIKA	86%	23%
CHIKUNGUNYA	62%	80%
DENGUE	20%	17%

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Contents lists available at ScienceDirect

Journal of Clinical Virology

Journal homepage: www.elsevier.com/locate/jcv

Short communication

Detection of Zika virus in saliva

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ABSTRACT

Background: During the largest Zika virus (ZIKV) outbreak ever reported that occurred from October 2013 to March 2014 in French Polynesia, we observed that several patients presenting the symptoms of acute phase ZIKV fever were tested negative in blood by ZIKV real time PCR (RT-PCR).

Objective: As we have previously detected ZIKV RNA in the saliva of a young child, we investigated the use of saliva as an alternative sample for routine ZIKV RNA detection.

Study design: Over a month period, 1487 samples collected from 452 patients presenting symptoms of Zika fever (saliva only, blood only or both samples) were tested using a specific ZIKV RT-PCR. A medical questionnaire was available for most of the patients.

Results: ZIKV was more frequently detected in saliva compared to blood. For the 182 patients with both samples collected, tests were positive for 21 (11.6%) in saliva while negative in blood and tests were positive for 14 (24.8%) in blood while negative in saliva; the difference in mean days after symptoms onset and the percentage of the main symptoms of Zika fever for patients only positive in saliva or in blood was not significant.

Conclusion: This use of saliva sample increased the rate of molecular detection of ZIKV at the acute phase of the disease but did not modify the number of detections of ZIKV RNA. Saliva was of particular interest when blood was difficult to collect (children and seniors especially).

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ZIKV 2015 to Present

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Zika virus: Spread

1947 Discovery

1954 1st Human

1954-2007 Asia

2007-2014 Pacific

2015 Americas

KANSAS STATE UNIVERSITY Adapted from National Academy presentation by R. Rosenberg **BRI** *Bioscience Research Institute*

Zika Virus in Brazil, May 2015

NOVA

Brazil has confirmed cases of Zika virus infection

FOLHA DE S. PAULO

Ministry of Health Confirms 16 Cases of Zika Virus in Brazil

BRASIL

BRASIL

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Zika Case Distribution: February 3, 2016



Where Zika Virus Is In The Americas

Countries and territories with CDC travel alerts related to the Zika virus

Zika virus is transmitted:

- By mosquitoes of the Aedes genus**
The same mosquito that spread dengue and chikungunya viruses.
- From mother to child**
It's rare, but a woman infected with Zika can pass the virus to her child during birth, or possibly to the fetus during pregnancy.
- Possibly through blood or sexual contact**
This link is not confirmed.

Source: WHO, CDC

Zika virus: Human infections

Potential Sexual Transmission of Zika Virus

Oliver Messer, Claudine Roche, Emille Rubin, Tuzhan Nhas, Anita Telesker, Van Mai Cao-Lemmas

In December 2013, during a Zika virus (ZIKV) outbreak in French Polynesia, a patient in Tahiti sought treatment for hematuria, and ZIKV was isolated from his semen. ZIKV transmission by sexual intercourse has been previously reported. This observation supports the possibility that ZIKV could be transmitted sexually.

Zika virus (ZIKV) is a mosquito-borne arbovirus in the *Flaviviridae* family, genus *Flavivirus*. It was first isolated in 1947 from a rhesus monkey in the Zika forest of Uganda (1). Sporadic human cases were reported from the 1950s in Asia and Africa. The first reported large outbreak occurred in 2007 on Yap Island, Federated States of Micronesia (2). The largest known ZIKV outbreak reported occurred in October 2013 in French Polynesia, South Pacific (3), a territory of France comprising 17 inhabited islands, an estimated 28,000 persons (11% of the population) sought medical care for the illness (4). The most common symptoms of Zika are sore eyes, fever, rash, and conjunctivitis. Most of the patients had mild disease, but rare neurologic complications have been described in other patients in French Polynesia (5).

The Study
In early December 2013, during the ZIKV outbreak, a 40-year-old man in Tahiti had symptoms of ZIKV infection, without a high fever (temperature from 37.7°C to 38.7°C) and rash. Symptoms lasted 3 days. Eight weeks later, he described a second episode of conjunctivitis compatible with ZIKV infection (temperature from 37.7°C to 38°C, without fever, and rash) (6).

We extracted RNA using the NuSieve nextGEM system (BioLabs, Beverly, MA, USA). From 200 µL of blood and from 50 µL of semen and urine, both were eluted by 10 µL of elution buffer. We used 5 µL of cDNA extracted for amplification. We tested blood and semen RNA extracts using real-time reverse transcription PCR (RT-PCR) as described using 2 primer-pair amplification sets specific for ZIKV (7). The RT-PCR results were positive for ZIKV in semen and negative in blood, and confirmed by sequencing of the primer-probe E1E1-E1E2 encompassing the 481-511 protein coding region of ZIKV. The generated sequence (GenBank accession no. JQ415110) was identical to the previously reported at the beginning of the ZIKV outbreak (8). Three days later, we collected a semen sample, then a second set of blood and semen samples. Urine and semen from the second collection were not found to contain traces of blood by both direct and microscopic examinations. RT-PCR detected ZIKV RNA in the semen and urine, but not in the blood sample.

We quantified ZIKV RNA loads using an RNA virus-titric microarray method that covers the region targeted by the 2 primer-probe sets. RNA loads were: 2.9×10^7 copies/mL and 1.1×10^7 copies/mL in the first and second semen samples, respectively, and 3.8×10^7 copies/mL in the urine sample.

We cultured semen and urine as described for detection of ZIKV in cell culture (9). Briefly, 200 µL of each sample diluted in 200 µL of 1% fetal calf serum (FCS) minimum essential medium (MEM) were inoculated into Vero cells (ATCC CCL-21) (10).

2011 Zika virus: Human infections

DOI: 10.3201/eid1705.101939
Suggested citation for this article: Foy BD, Kobylinski KC, Foy JLC, Blitvich BJ, Travassos da Rosa A, Haddock AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis. 2011 May; [Epub ahead of print]

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

Brian D. Foy, Kevin C. Kobylinski, Joy L. Chilson Foy, Bradley J. Blitvich, Amelia Travassos da Rosa, Andrew D. Haddock, Robert S. Lanciotti, and Robert B. Tesh

ZIKV 2016 in US

Zika virus; First case in US January 11, 2016

First Zika Virus Case in Continental United States Confirmed in Texas
January 11, 2016

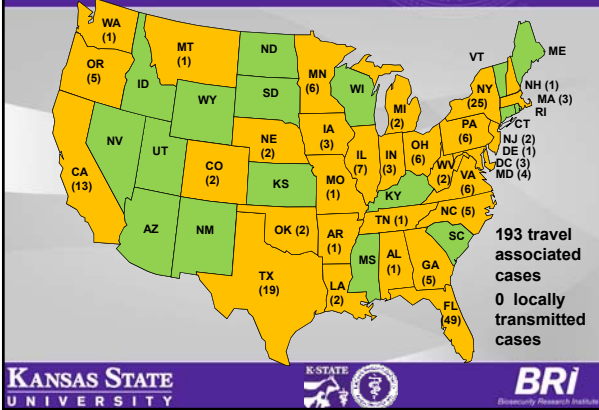
Traveler from El Salvador to Harris County, Houston, Texas

First sexually transmitted case in US February 2, 2016

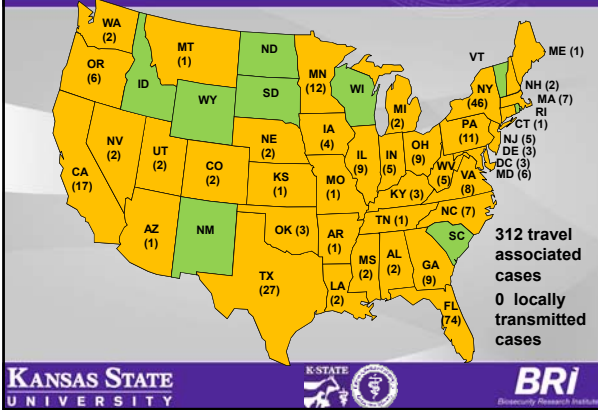
Texas reports first case in the USA of sexually transmitted Zika virus
Lisa Truitt, USA TODAY | 12:09 a.m. EST February 2, 2016

TWO CASES OF ZIKA VIRUS IN DALLAS COUNTY

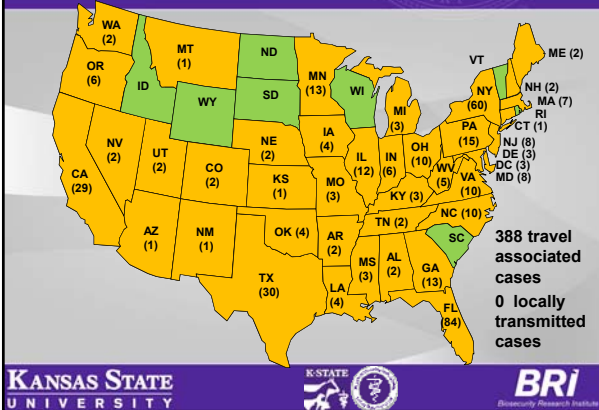
Zika Case Distribution by Week: March 9, 2016



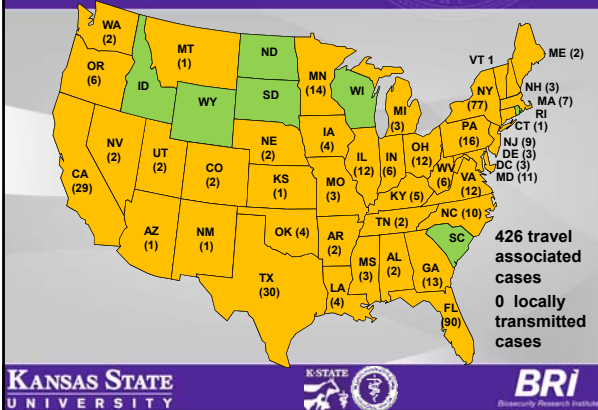
Zika Case Distribution by Week: March 30, 2016



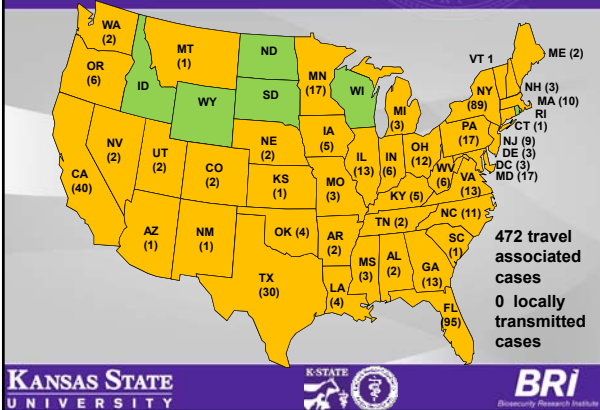
Zika Case Distribution by Week: April 20, 2016



Zika Case Distribution by Week: April 27, 2016



Zika Case Distribution by Week: May 4, 2016



15 July 2016

New York reports first female-to-male Zika transmission via sex

Published July 15, 2016 · Reuters · Facebook · Twitter · LinkedIn · Email · Print



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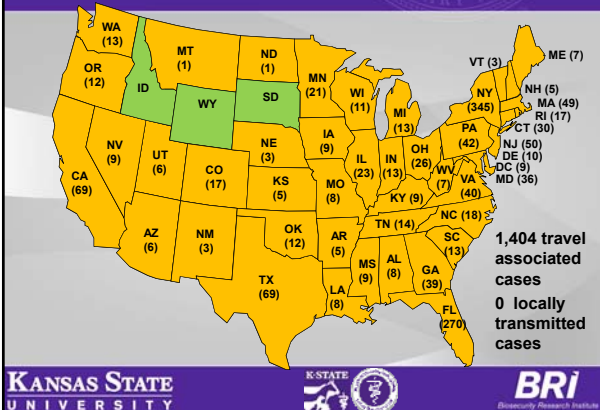
1 Tip of a flat belly:

redirectyourcarbs.com
Cut down a bit of your belly every day by using this 1 weird old tip.

Golden Corral

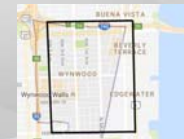
New York City's health department on Friday reported the first female-to-male transmission of the Zika virus, which is most typically spread by the bite of an infected mosquito.

Zika Case Distribution by Week: July 20, 2016



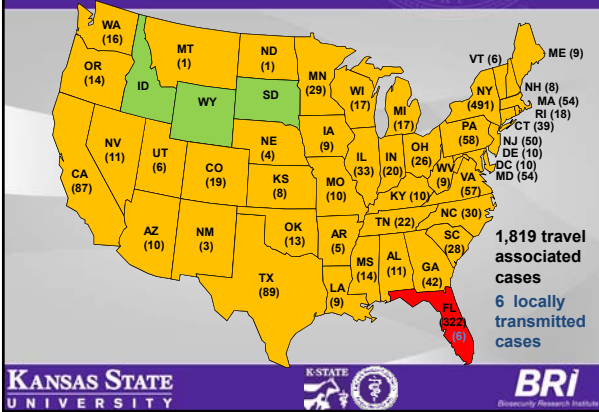
August 3, 2016 Local transmission of Zika in Miami

Last week, the Centers for Disease Control and Prevention reported something it had been anticipating for months:

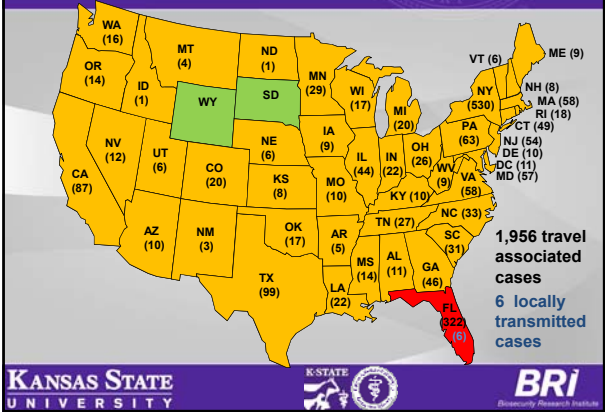


Carlos Varas, a Miami-Dade County mosquito inspector, sprays around homes in the Wynwood area of Miami on Tuesday. Emily Michot/Miami Herald/TNS via Getty Images

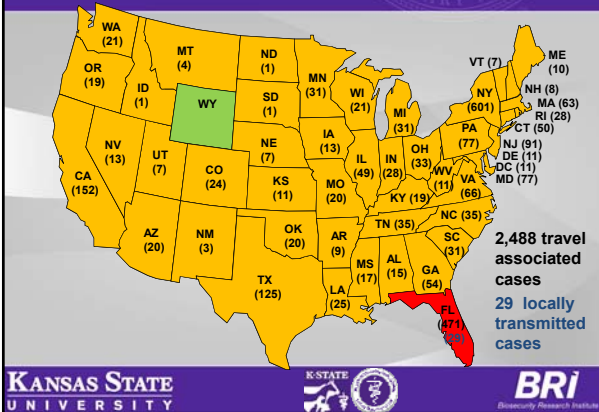
Zika Case Distribution by Week: August 3, 2016



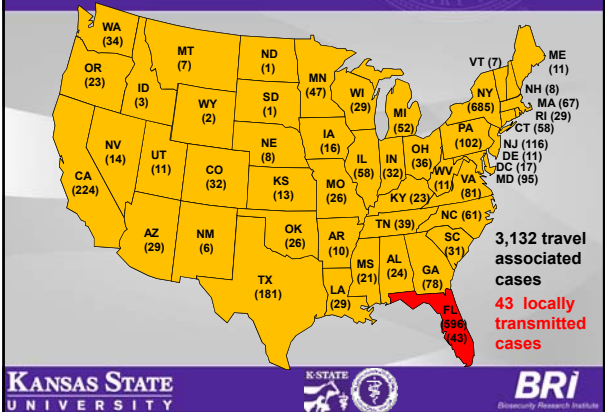
Zika Case Distribution by Week: August 10, 2016

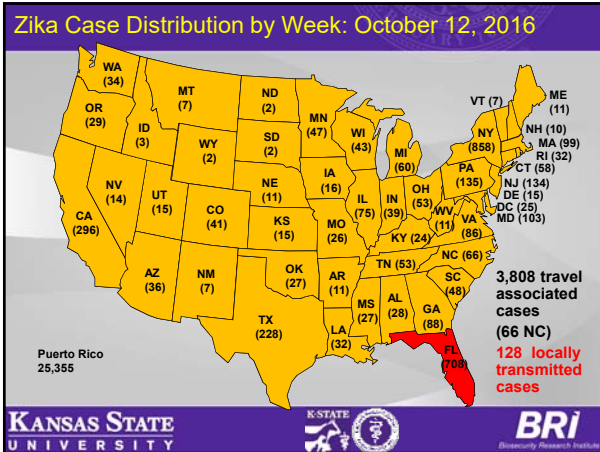


Zika Case Distribution by Week: August 24, 2016



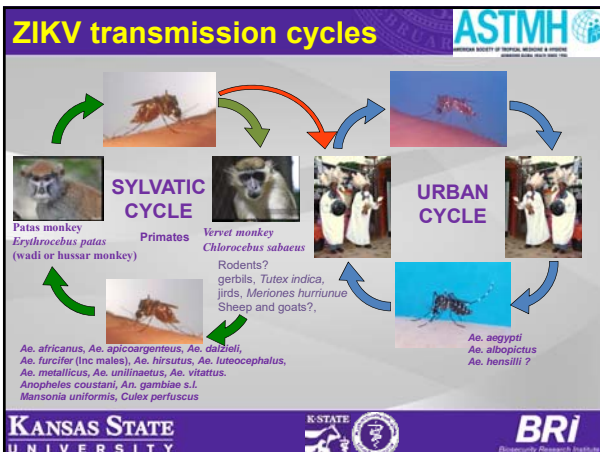
Zika Case Distribution by Week: September 14, 2016





MOSQUITO INFECTIONS

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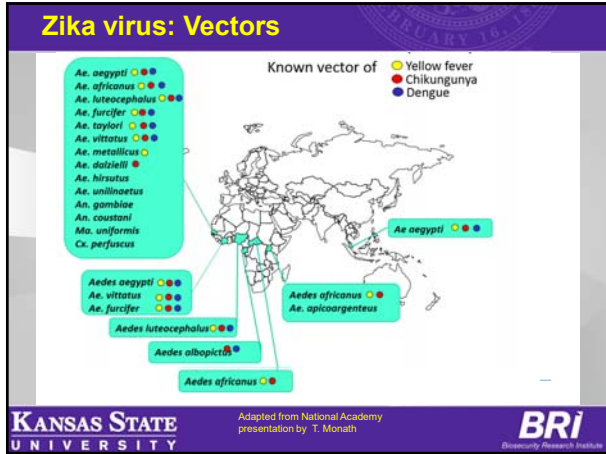
Zika virus: Vectors

The first isolation of Zika virus from mosquito samples was made in 1948 from *Aedes africanus*.

Lately, many other *Aedes* species have been surveyed for the detection of Zika virus, and thus far, Zika virus has been detected by RT-PCR or isolated from many mosquito species, human beings, and non-human primates.

Adapted from National Academy presentation by T. Monath

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Zika virus: Vectors

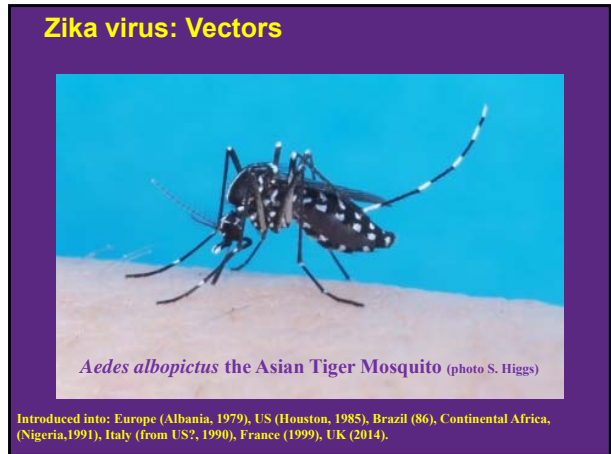
OPEN ACCESS | Freely available online | PLOS ONE

Zika Virus in Gabon (Central Africa) – 2007: A New Threat from *Aedes albopictus*?



Gilda Grand^{1,2}, Malania Caron^{1,2}, Illich Manfred Momba^{1,2}, Dieudonné Nkangha^{1,2}, Staniens Mbowi Ondo¹, Davy Jalla^{1,2}, Didier Fontenille¹, Christophe Faugny^{1,2}, Eric Maurice Leroy^{1,2}

Abstract
 Background: Chikungunya and dengue viruses emerged in Gabon in 2007, with large outbreaks primarily affecting the capital Libreville and several northern towns. Both viruses subsequently spread to the south-east of the country, with new outbreaks occurring in 2010. The mosquito species *Aedes albopictus*, that was known as a secondary vector for both viruses, recently invaded the country and was the primary vector involved in the Gabonese outbreaks. We conducted a retrospective study of human sera and mosquitoes collected in Gabon from 2007 to 2010, in order to identify other circulating arboviruses.
 Methodology/Principal Findings: Sample collections, including 4312 sera from patients presenting with painful febrile disease, and 6652 mosquitoes belonging to 9 species, split into 247 pools (including 137 pools of *Aedes albopictus*), were screened with molecular biology methods. Five human sera and two *Aedes albopictus* pools, all sampled in an urban setting during the 2007 outbreak, were positive for the Ruvubu Zika (ZIKV). The ratio of *Aedes albopictus* pools positive for ZIKV was similar to that positive for dengue virus during the concurrent dengue outbreak, supporting similar mosquito infection rates and, presumably, underlying a human ZIKV outbreak. ZIKV sequences from the mosquito and NS3 genes were amplified from a human serum sample. Phylogenetic analysis placed the Gabonese ZIKV at a basal position in the African lineage, pointing to recent genetic diversification and spread.
 Conclusions/Significance: We provide the first direct evidence of human ZIKV infections in Gabon, and its first occurrence in the Asian tiger mosquito, *Aedes albopictus*. These data reveal an unusual natural life cycle for this virus, occurring in an urban environment, and potentially representing a new emerging threat due to this novel association with a highly invasive vector whose geographic range is still expanding across the globe.

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What about *Culex* species?

October 10, 2016

EMERGING INFECTIOUS DISEASES*

Volume 22, Number 10—October 2016

Letter

Culex pipiens and *Aedes triseriatus* Mosquito Susceptibility to Zika Virus

Matthew T. Ahnatz, Stephen A. Pinhalo, Jorge E. Couros, and Lynn C. Barbakow
Author affiliations: University of Wisconsin, Madison, Wisconsin, USA

Table




Competence of mosquitoes, by species, as Zika virus vectors, 14 days after peroral infection, United States†

No virus particles/flies tested (N)

Biological replicates 1, mean 4.02 1log₁₀ PFU/ml, n 102-647

Biological replicates 2, mean 4.74 1log₁₀ PFU/ml, n 102-636

Biological replicates 3, mean 4.83 1log₁₀ PFU/ml, n 102-645


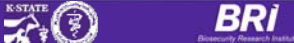
Competence of mosquitoes, by species, as Zika virus vectors, 14 days after peroral infection, United States†

No virus particles/flies tested (N)

Mosquito species	Biological replicates 1, mean 4.02 1log ₁₀ PFU/ml, n 102-647			Biological replicates 2, mean 4.74 1log ₁₀ PFU/ml, n 102-636			Biological replicates 3, mean 4.83 1log ₁₀ PFU/ml, n 102-645		
	I	D	T	I	D	T	I	D	T
<i>Culex pipiens</i>	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)
<i>Aedes triseriatus</i>	ND	ND	ND	0/20 (0)	0/20 (0)	0/20 (0)	4/13 (31)	0/4 (0)	0/4 (0)
<i>Ae. albopictus</i>	4/9 (44)	3/9 (33)	1/4 (25)	1/4 (25)	0/1 (0)	0/1 (0)	ND	ND	ND
<i>Ae. japonicus</i>	ND	ND	ND	ND	ND	ND	17/17 (100)	12/17 (71)	4/17 (24)

*Zika virus strain PRVABC59 (GenBank accession no. KU012123) was originally isolated from a traveler to Puerto Rico in December 2013. It is referred to, abbreviated, as ZIKV in this article. †Transmission. ‡Originated from eggs collected in Texas in 2002 and collected at the Iowa State University Medical Entomology Laboratory. §Originated from eggs collected in Texas in 2002 and 2003 and collected at the Iowa State University Medical Entomology Laboratory. ¶Originated from eggs collected in Missouri in 2002 and collected at the Illinois Natural History Survey. ††Black head (compared to gray).

MAILING LIST: The authors, their institutions, and their contributions to this journal are listed on the inside cover of this issue. The U.S. Department of Health and Human Services, the Public Health Service, the Centers for Disease Control and Prevention, and the authors' affiliated institutions. Use of trade names for identification and does not imply endorsement by any of the groups named above.





October 27, 2016


ORIGINAL ARTICLE

Culex Species Mosquitoes and Zika Virus

Matthew T. Ahnatz, Stephen A. Pinhalo, Jorge E. Couros, and Lynn C. Barbakow
Author affiliations: University of Wisconsin, Madison, Wisconsin, USA



Culex pipiens quinquefasciatus



Vero beach is 140 miles from Miami beach

Culex p. quinquefasciatus (Vero Beach, Florida), collected August 2015, 7th generation.

Fed with blood meal containing 6.96log₁₀TCID₅₀/mL of ZIKV strain PRVABC59, Asian lineage (human isolate from Puerto Rico). Approximately 10 million virus particles per mL.



None became infected

Also tested: *Cx. pipiens* from Mercer County, NJ, F₁ and *Cx. pipiens* from Anderson, CA, F₁. None became infected

Dr. D. Velandingham
CVII

Dr. S. Huang
CVII

Vector Borne and Zoonotic Diseases

September 1, 2016

Eurosurveillance

Vol. 21 | Weekly issue 36 | 1 September 2016

RAPID COMMUNICATION
Culex mosquitoes are experimentally unable to transmit Zika virus

Ferreira J, et al. ...

Correspondence: Ana Rita Ferreira ...

We report that two laboratory colonies of Culex quinquefasciatus and Culex pipiens quinquefasciatus were experimentally unable to transmit ZIKV either as an index mosquito or as a vector to a naive mosquito.

Outbreaks due to Zika virus (ZIKV) are expanding and affecting most tropical regions [2]. The rapid spread may be related to the efficiency of human-biting vector species and Aedes albopictus mosquitoes, which are ZIKV vectors. However, with increasing spread, other mosquito species may be important to transmit the ZIKV as observed in laboratory studies [2].

Background
Culex quinquefasciatus is a common mosquito species in Brazil. It is a potential vector for ZIKV as observed in laboratory studies [2].

Sept 2, 2016

Zika virus replication in the mosquito Culex quinquefasciatus in Brazil

D. R. D. Guedes, M. H. S. Paiva, M. M. A. Donato, P. F. Barbosa, L. Kozlovsky, S. W. dos S. Rocha, K. I. A. Santos, M. M. Crope, R. M. R. Barbosa, C. M. F. Oliveira, M. A. S. Melo-Santos, L. Paul, M. T. Cardoso, R. F. de O. Fraga, A. L. S. de Oliveira, W. S. Louf, C. A. Petroni, C. F. J. Ayres

Affiliations:
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2 Universidade Federal de Pernambuco, Centro Acadêmico do Agreste - Rodovia BR-104, km 59 - Nova Cantina, Caruaru - PE - Brazil, CEP: 55002-970.

3 Laboratory of Virology and Experimental Therapy (LAVITE), Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz-Permanente, Av. Moraes Rego, s/n campus da UFPE, Cidade Universitária, Recife-PE, Brazil, CEP: 50670-420.

4 Center for Statistics and Geoprocessing, Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz-Permanente, Av. Moraes Rego, s/n campus da UFPE, Cidade Universitária, Recife-PE, Brazil, CEP: 50670-420.

5 Department of Molecular and Cellular Biology, University of California Davis, Davis, CA, 95616 USA.

September 6, 2016

Culex quinquefasciatus from Rio de Janeiro Is Not Competent to Transmit the Local Zika Virus

Marcelo Suzuki Peres, et al. ...

Abstract
Culex quinquefasciatus is a common mosquito species in Brazil. It is a potential vector for ZIKV as observed in laboratory studies [2].

Background
Culex quinquefasciatus is a common mosquito species in Brazil. It is a potential vector for ZIKV as observed in laboratory studies [2].

Methods
Culex quinquefasciatus from Rio de Janeiro was tested for its ability to transmit ZIKV in a laboratory setting.

Results
Culex quinquefasciatus from Rio de Janeiro was found to be incompetent to transmit ZIKV.

Conclusions
Culex quinquefasciatus from Rio de Janeiro is not a competent vector for ZIKV.

September 7, 2016

Culex pipiens quinquefasciatus: a potential vector to transmit Zika virus

Xiao-xia Guo, et al. ...

Abstract
Zika virus (ZIKV) has become a threat to global health since the outbreak in Brazil in 2015. Although ZIKV is generally considered an Aedes-transmitted pathogen, new evidence has shown that parts of the virus chain reaction, Culex-transmitted viruses. Therefore, it is important to evaluate the competence of Culex species for ZIKV to understand their potential as vectors.

Background
Culex pipiens quinquefasciatus is a common mosquito species in Brazil. It is a potential vector for ZIKV as observed in laboratory studies [2].

Methods
Culex pipiens quinquefasciatus from Rio de Janeiro was tested for its ability to transmit ZIKV in a laboratory setting.

Results
Culex pipiens quinquefasciatus from Rio de Janeiro was found to be competent to transmit ZIKV.

Conclusions
Culex pipiens quinquefasciatus from Rio de Janeiro is a competent vector for ZIKV.

ZIKV: WHAT IS LEFT TO DO?

Surveillance
Predictive models

New vectors in the Americas
New control (RIDL, *Wolbachia*)
Insecticides
Repellants

Reagents
Detection
Diagnostics (differential)

Viral genetics:
Asian vs African
infectivity
pathogenicity

Disease
Guillain-Barre
microcephaly
sexual transmission
persistence

Treatments
Vaccines

New Vertebrates
(NW primates, rodents,
livestock)

Animal Models

ZIKV transmission cycles in the Americas?

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New World Primates (135 spp.)

FAMILY	COMMON NAME	NUMBER of SPECIES
Family Callitrichidae	Marmosets and Tamarins	20 marmosets 22 tamarins
Family Cebidae	Capuchins and Squirrel monkeys	9 capuchins 5 squirrel
Family Aotidae	Night/Owl monkeys	11
Family Pitheciidae	Titis, Sakis and Uakaris	29 titis 10 sakis 5 uakari
Family Atelidae	Howler, Spider, Woolly spider & Woolly	10 howler 7 spider 2 woolly spider 5 woolly

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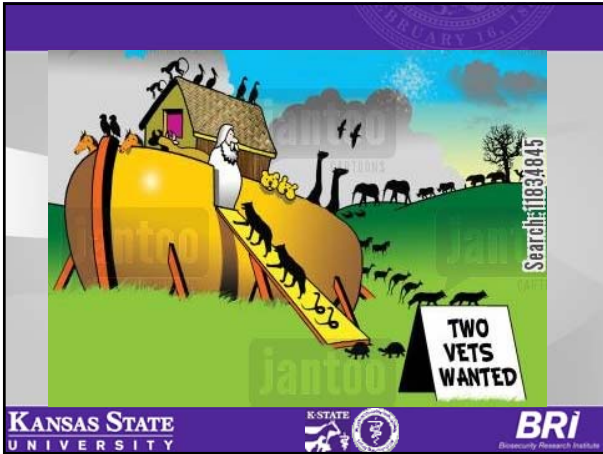
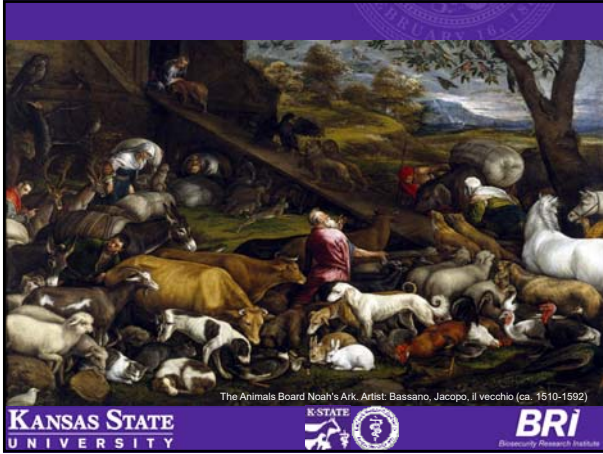
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INFECTIONS of "OTHER" VERTEBRATES

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Zika virus in animals: 1983

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Transactions of the Royal Society of Tropical Medicine and Hygiene, Vol. 77, No. 4, 462-465 (1983)

A sero-epidemiological survey for certain arboviruses (Togaviridae) in Pakistan

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Summary
Complement fixation test reactions to eight viruses of the family Togaviridae were studied in 372 serum samples (137 rodents, 174 domestic animals, 43 humans) from Pakistan. Antibodies to each virus were detected. The highest overall prevalence rates were for West Nile (WN) (7.5%), Japanese encephalitis (JE) (3.2%), and Zika (ZKA) (2.4%) viruses, followed by Sindbis (SIN), Chikungunya (CHK), Uganda 9 (UG9) and Ross River (RR) viruses (1.6 to 1.9%). One human serum (male, age 38 years) reacted with Dengue 1 (DENV) virus antigen titre 1:32. Antibodies to each virus except RR were detected in human sera; antibodies to RR virus were detected only in rodent and domestic animal sera. The roles of rodents in the epidemiology of WN, JE and ZKA viruses should be investigated. At least six of these eight viruses cause fevers in humans (seven of unknown origin comprise about one third of the febrile episodes recorded in Pakistan).

372 sera:
157 rodents, 172 domestic animals, 43 humans
Complement fixation tests for 8 viruses

Results Overall prevalence rates were highest for WN, DENV and JE viruses (3.2% to 7.5%) followed by ZKA (2.4%) and the other four viruses (Table 1). CF titres were high (1:16 and/or 1:32) for certain samples of each virus except CHK (Table 1). All ZKA and RF titres were 1:8 or higher. Between 60% and 70% higher of the WN, JE, SIN, and USS titres were 1:8. In rodent sera, 72% (18 of 25) reacted to one virus and 28% (7 of 25) reacted to two or more (mostly two) viruses. High CF titres (1:16 and/or 1:32) were obtained with WN, JE, SIN, ZKA, and RF viruses (Table 1).

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Zika virus antibodies in animals: 1983

M. A. DARWISH *et al.* 443

Table I—Antibodies against certain *Togaviridae* in sera from Pakistan

Sera	No. tested	SIN					CHIK					WN					JE					DE					ZIKA					UGS					KP				
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)				
Rodents (Total)	157	4	(2.5)	4	(2.5)	7	(4.5)	7	(4.5)	2	(1.3)	6	(3.8)	1	(0.6)	2	(1.3)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
<i>Tatera indica</i>	47	1		1		2		4		1		3		0		0		0		0		0		0		0		0		0		0		0		0		0			
<i>Meriones hurrianae</i>	33	0		1		2		2		1		2		0		0		0		0		0		0		0		0		0		0		0		0		0			
<i>Rattus norvegicus</i>	54	1		1		0		1		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0			
<i>Rattus norvegicus</i>	9	0		1		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0			
<i>Rattus (Banded) indica</i>	2	0		0		1		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0			
<i>Neotoma indica</i>	7	0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0			
<i>Bandicota bengalensis</i>	2	0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0			
<i>Colubus elater</i>	1	0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0			
<i>Felis tigris</i>	0	0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0			
Domestic animals (Total)	172	1	(0.6)	0		17	(9.9)	2	(1.2)	7	(4.1)	2	(1.2)	10	(5.8)	3	(1.7)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Cow	44	0		1		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0			
Buffalo	33	0		0		5		1		2		0		0		0		0		0		0		0		0		0		0		0		0		0		0			
Sheep	46	1		0		11		1		5		1		0		0		0		0		0		0		0		0		0		0		0		0		0			
Goat	48	0		0		0		0		0		1		0		0		0		0		0		0		0		0		0		0		0		0		0			
Man (Total)	43	1	(2.3)	1	(2.3)	5	(11.6)	3	(7.0)	4	(9.3)	1	(2.3)	0		0		0		0		0		0		0		0		0		0		0		0					
Grand Total	372	6	(1.6)	5	(1.3)	39	(10.5)	12	(3.2)	13	(3.5)	9	(2.4)	13	(3.5)	3	(0.8)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				

RESULTS

- 3.8% rodents positive (n=6/157)
 - 3/47 *Tatera indica*, 2/33 *Meriones hurrianae*, 1/2 *Bandicota bengalensis*
- 1.2% domestic animals (n=2/172)
 - 1/46 sheep, 1/48 goat
- 2.3% humans (n = 1/43)

Zika virus antibodies in animals: 1983

Table II—CF titres in sera tested

Virus	No. sera	Reciprocal of serum end-point dilution				
		4	8	16	32	64
West Nile	29	9	11	9	0	0
Dengue 1	12	8	4	0	0	0
Dengue encephalitis	12	8	1	1	1	4
Zika	5	0	0	1	1	4
Uganda S	5	2	2	1	0	0
Chikungunya	5	4	1	0	0	0
Royal Free	1	0	0	1	1	2

Table III—CF titres* in rodent sera

Rodent species (No.)	Virus (no. each titre)							
	WNV	DENV	JE	SIN	CHIK	ZIKA	UGS	KP
<i>Tatera indica</i> (47)	16 (33)	16 (33)	4 (8)	8 (17)	8 (17)	4 (8)	4 (8)	12 (25)
<i>Meriones hurrianae</i> (33)	14 (42)	4 (12)	0	4 (12)	12 (36)	8 (24)	0	0
<i>Rattus norvegicus</i> (54)	0	12 (22)	12 (22)	4 (8)	4 (8)	0	4 (8)	0 (0)
<i>Rattus norvegicus</i> (9)	16 (18)	0	0	4 (5)	4 (5)	0	0	0 (0)
<i>Rattus norvegicus</i> (2)	0 (0)	0	0	0	0	0	0	0 (0)
<i>Bandicota bengalensis</i> (2)	0 (0)	0	0	0	0	0	0	0 (0)
<i>Neotoma indica</i> (7)	0 (0)	0	0	0	0	0	0	0 (0)
<i>Bandicota bengalensis</i> (2)	0 (0)	0	0	0	0	0	0	0 (0)

*Reciprocal of serum end-point dilution.

The reaction of all positive sera with JE antigen was monospecific except that one rodent (*T. indica*) serum was simultaneously positive for another Flavivirus (Zika virus). All other positive sera reacted monospecifically with the respective Flavivirus except from one rodent (*T. indica*) and one human (40-year-old female); these two sera reacted with both Zika and Uganda S viruses

Zika virus in animals: 1983 Conclusions

Antibodies against ZIKA virus were detected in sera from rodents, sheep, goats and one person (without antibodies to other flaviviruses).

These data are the first indication of ZIKA virus infection in rodents and domestic animals.

The natural history of this agent should be investigated in more detail

Gerbils, *Tatera indica*, and jirds, *Meriones hurrianae*, may be reservoirs of JE, WN and ZIKA viruses.

Rattus rattus and *R. norvegicus* are also potential sources of human infection by these viruses, especially in rural areas with dense mosquito populations

Zika virus in animals: 2016 (unpublished)



Dr. Izabela Ragan, Dr. Richard Bowen and PhD student Emily Blizzard



Zika virus in animals: 2016 (unpublished)

- Zika Virus strains
 - PRVA BC59: Puerto Rico December 2015
 - FSS13025: Cambodia 2010
- Inoculation dose: 10⁵ PFU/mL subcutaneous and intradermal
- The study
 - Challenged at day 0 and maintained animals for 28 days
 - Recorded clinical signs, temperatures
 - Collected sera at days post infection 0-5, 7,14,21,28
 - Collected tissues at necropsy
- Serology
 - Plaque reduction neutralization assay
 - PRNT 90
- Virus Isolation
 - Plaque assay on sera and tissues
 - Real Time RT-PCR on sera
- Histopathology and Immunohistochemistry

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Zika virus in animals: 2016 (unpublished)

- Results:
 - No clinical signs or febrile state detected
 - Viremia detected in frogs
 - Real time RT-PCR on sera
 - In progress
 - Detection of RNA in frogs and an armadillo

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No antibodies in

Armadillo, Pigeon, Chicken, Snake, Hamster, Mink, Ground Hog, Calf, Deer, Mouse, Sparrow

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Antibodies detected in:

Antibodies detected in goats, cottontail rabbits, ducklings, pigs, and frogs



Results of baby rabbits, raccoons, and frogs pending

Zika virus in animals: 2016 (unpublished)

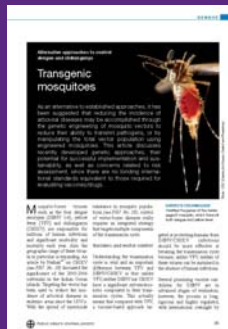
- Puerto Rican strain elicited stronger immune response than Cambodian strain
- No difference in susceptibility between young and adult animals
- No birth defects noted in newborns during study
 - Rabbits, deer mice



Zika virus in animals: 2016 (unpublished)

- Future work:
 - Additional species
 - Horses, dogs, cats, guinea pigs, pregnant sows, domestic mice, bats,
 - Immunohistochemistry and histopathology
 - Real time RT-PCR
 - Developing pregnancy model in animals

“Alternative Approaches to Control Dengue and Chikungunya”



Release of Insects carrying a Dominant Lethal gene (RIDL)

Wolbachia spp – infected mosquitoes. Intracellular bacteria that infects multiple tissues including ovaries and salivary glands.

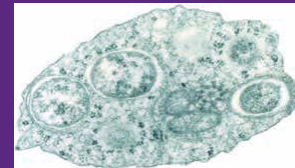


Photo: © Public Library of Science / Scott O'Neill Higgs, 2013

Acknowledgements

Many of the slides in this presentation were obtained from open source material and PowerPoints from the National Academies website. Slides were from presentations by Albert Ko, Richard Kuhn, Tom Monath, Ron Rosenberg (some original pictures by Ben Beard), and Scott Weaver. Refer to website for original images.

Many journals have provided open access to articles about Zika virus, for example Lancet, and Trans. R.S.T.M.H.

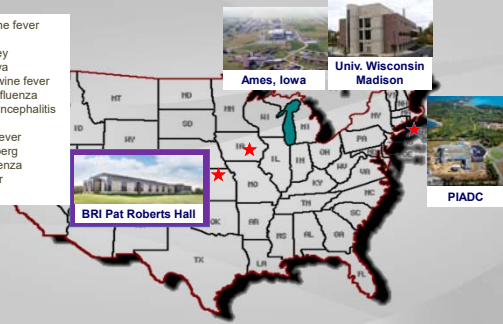
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BSL-3Ag Livestock Research Space

- African swine fever
- Akabane
- Cache Valley
- Chikungunya
- Classical swine fever
- HP avian influenza
- Japanese encephalitis
- PRRS
- Rift Valley fever
- Schmallenberg
- Swine influenza
- Yellow fever
- Zika



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THANK YOU



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