Emerging Arboviral Threats: Dengue, Chikungunya, and Zika

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Talk Outline

- Describe epidemiology, clinical manifestations, and diagnosis of dengue, chikungunya, and Zika
- Analyze factors responsible for recent outbreaks of chikungunya and Zika
- Options for prevention including vaccines
Global Dengue Risk

Suitability for Dengue Transmission
- High suitability
- Low suitability
- Unsuitable or nonendemic

WHO Dengue Estimates

3.6 billion people at risk worldwide for dengue infection

120 endemic countries

390 million infections per year
About 100 million DF/DHF cases annually

~500,000 DHF/DSS cases per year require hospitalization
~5% die
Factors Contributing to Dengue Emergence

Lack of effective mosquito vector control

Distribution of *Aedes aegypti*

1970

(end of global eradication program)

2002
**DENGUE ± WARNING SIGNS**

**CRITERIA FOR DENGUE ± WARNING SIGNS**

**Probable dengue**
- live in / travel to dengue endemic area.
- Fever and 2 of the following criteria:
  - Nausea, vomiting
  - Rash
  - Aches and pains
  - Tourniquet test positive
  - Leukopenia
  - Any warning sign

**Laboratory-confirmed dengue**
(important when no sign of plasma leakage)

**Warning signs***
- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargement >2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count

* (requiring strict observation and medical intervention)
Dengue Hemorrhagic Manifestations

Dengue Hemorrhagic Manifestations
SEVERE DENGUE

1. Severe plasma leakage
2. Severe haemorrhage
3. Severe organ impairment

CRITERIA FOR SEVERE DENGUE

Severe plasma leakage
leading to:
• Shock (DSS)
• Fluid accumulation with respiratory distress

Severe bleeding
as evaluated by clinician

Severe organ involvement
• Liver: AST or ALT $\geq 1000$
• CNS: Impaired consciousness
• Heart and other organs
Immune Response to Dengue Infection

Chikungunya Virus

- Genus *Alphavirus*, family *Togaviridae*: ssRNA virus
- Identified in the 1950s in Africa (Southern Province, Tanganyika)
- Disease given a Kimakonde name: chikungunya
  - According to an educated local chief, term derived from a root verb—kungunyala—meaning to dry up or become contorted
    - Lumsden WHR. TRSTM 1955
Chikungunya Virus
Epidemiology

Maintained in sylvatic cycle involving wild primates and forest-dwelling mosquitoes

Increasing prevalence in Asian countries where it established an urban cycle 2005:
>5,000 cases Comoros Islands then
  – Explosive outbreak in Reunion during Southern Hemisphere summer
  – Estimated 266,000 residents affected (pop. 770K)
  – 248 possible deaths due to CHIKV
  – *Ae. albopictus* implicated as local vector
Modes of Transmission and Reservoirs

- Primarily spread by bite of infected *Aedes* spp. mosquitoes

- Mother-to-child transmission documented in context of intrapartum maternal viremia
  - 49% of women with peripartum viremia had vertical transmission
  - Overall 2.5% of exposed neonates became infected


- Reservoirs = humans during epidemics and monkeys, rodents, and birds during non-epidemic periods
Clinical Manifestations

- Mean incubation period of 2-4 d (range 1-12 d)
- Abrupt onset of symptoms including high fever (up to 40°C), HA, back pain, myalgias, and arthralgias
- Joint pains can be intense, affecting mainly extremities (ankles, wrists, phalanges) and large joints (knees, hips)
- Rash present in 40-50% of cases

Skin Manifestations

Facial and abdominal rash with edema of the face and hand

Arthralgias and Arthritis

- Relapsing, incapacitating joint pains = hallmark of chikungunya
- Often have initial severe febrile polyarthritis followed by disabling peripheral rheumatism lasting for months
- Arthralgias or arthritis can persist for 4 mo in 33% of patients, 20 mo in 15% and 3-5 years in 10%
- May cause decreased ability to ambulate and carry out activities of daily life
Diagnosis of CHIKV Infection

Two main diagnostic methods:
- RT-PCR: useful during initial viremic phase
- Serology (IgM, IgG)

Virus isolation - inoculation of mosquito cell cultures, mosquitoes, mammalian cell culture, or mice

Symptoms
- Fever, usually lasts about 1 week (90% of patients)
- Myalgia, usually lasts 7–10 days (90% of patients)
- Polyarthritis, polyarthritis, or both, can last weeks to months (95% of patients)
- Rash, lasts about 1 week (40–50% of patients)

Infection
- 2–6 days Incubation period
- Approximately 1 week
- Weeks to months
- Years
- Viremia, usually lasts 5–7 days
- IgM detectable 3–8 days after symptom onset, usually persists for 1–3 months
- IgG detectable 4–10 days after symptom onset, persists for years

Weaver & Lecuit NEJM 2015
NUEVO VIRUS!

ZIKA

ZIKA VIRUS
Zika Virus

- Single stranded RNA virus of *Flavivirus* genus
- Closely related to dengue, West Nile, yellow fever, and Japanese encephalitis viruses
- Primarily vector is *Ae. aegypti* but several other *Aedes* spp. capable of transmission
Two Distinct Zika Lineages – Only One Serotype

- African and Asian
- 3 genotypes: West African, East African, and Asian
  - Baud D et al. Lancet 2017
- All strains same serotype
  - Identical surface antigens
  - Antibodies elicited after infection with Asian lineage potently inhibit both lineages in vitro
  - Dowd K et al. Cell Reports 2016
Asian Viral Lineage

Enfissi A et al EID 2006

Figure: Phylogenetic relations between the envelope gene sequences of Suriname ZIKV and other ZIKV
Zika Virus Transmission Cycles

Sylvatic (jungle) cycle

Epidemic (urban) cycle

Rabe I & Meaney-Delman D. CDC Zika presentation January 2016
Non-Vector Transmission

Proven:
- Sexual
  - Male to female; male to male; female to male
- Transfusion
  - Documented in Brazil, Martinique, French Polynesia

Theoretically possible:
- Breast milk
  - 2+ reports of moderate to high VL in breast milk
    (cultured in one case but no MTCT)
- Saliva (?)
- Tears (?)
Sexual Transmission

- Preliminary semen carriage studies:
  - Up to 188 days by PCR
  - Replication competent Zika in semen for 69 days
- High viral load in semen (and urine)
- Rarely hematospermia or microhematospermia
- Viral shedding in vaginal secretions to 14 days and in cervical mucus to day 11 post-symptom onset
- Time from sexual contact to symptom onset 8-21 days

Hamer DH et al. Curr Infect Dis Rep 2017
Transmission – Transfusion

- Martinique January to June 2016
  - Screened 4129 blood donations
  - 1.84% positive by nucleic acid testing
- Contacted donors to determine whether they were or became symptomatic
  - Mean log_{10} RNA higher if symptomatic (P = .0013)
Epidemiology

First human case diagnosed 1962 in Uganda

Serosurveys – neutralizing antibodies in East and West Africa, India, and SE Asia
  – Late 1940s to late 1990s

Outbreaks in Yap, Micronesia in 2007; French Polynesia 2013, Easter Island 2014

Brazil early 2015 then spread in the Americas

39 countries and territories have reported local vector-borne Zika cases.
PAHO/WHO Data for the Americas 2015-2017
Estimated Incubation Period

- Median = 6.2 days
- Estimated range 3-14 days
- Krow-Lucal ER et al. EID 2017
Clinical Manifestations

- Estimated 80% asymptomatic
  - More recent data suggest 40-60% asymptomatic

Typical presentation:
- Fever (65%)
- Maculopapular rash (duration 2-14 d; median 6 d)
- Arthralgias (duration 1-14 d; median 3.5 d)
- Conjunctivitis
- Less commonly, myalgias, headache, retro-orbital pain, joint swelling, vertigo, vomiting, paraesthesias, dysguesia, subcutaneous hematomas
ZIKV Clinical Features

Brasil P et al. NEJM 2016
Chen LH. Zika virus infection in a Massachusetts resident after travel to Costa Rica: a case report. Ann Int Med 2016

Rash post travel to Haiti
<table>
<thead>
<tr>
<th>Feature</th>
<th>Zika</th>
<th>Dengue</th>
<th>Chikungunya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Rash</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Arthralgia/ arthritis</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Myalgia</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Headache</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>Rare</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Shock</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
## Assay Results 346 Patients Nicaragua

Waggoner JJ et al. CID 2016

<table>
<thead>
<tr>
<th>ZCD Assay Result</th>
<th>Number, n (%) of all Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>263 (76.0)</td>
</tr>
<tr>
<td><em>Monoinfections</em></td>
<td></td>
</tr>
<tr>
<td>ZIKV</td>
<td>47 (13.6)</td>
</tr>
<tr>
<td>CHIKV</td>
<td>91 (26.3)</td>
</tr>
<tr>
<td>DENV&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54 (15.6)</td>
</tr>
<tr>
<td><em>Coinfections</em></td>
<td>71 (20.5)</td>
</tr>
<tr>
<td>ZIKV-CHIKV</td>
<td>16 (4.6)</td>
</tr>
<tr>
<td>ZIKV-DENV&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 (1.7)</td>
</tr>
<tr>
<td>CHIKV-DENV&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43 (12.4)</td>
</tr>
<tr>
<td>ZIKV-CHIKV-DENV&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 (1.7)</td>
</tr>
<tr>
<td>Negative</td>
<td>83 (24.0)</td>
</tr>
</tbody>
</table>

Abbreviations: CHIKV, chikungunya virus; DENV, dengue virus; ZCD, multiplex real-time reverse-transcription polymerase chain reaction for the detection and differentiation of ZIKV, CHIKV, and DENV; ZIKV, Zika virus.

<sup>a</sup> Serotypes of 109 DENV-positive samples: DENV-2, 107; DENV-1, 1; DENV-4, 1.
Complications of Zika
Zika Neurological Complications

- Congenital Zika syndrome
- Guillain-Barré syndrome (GBS)
- Meningoencephalitis
- Acute myelitis
- Hearing loss
- Posterior uveitis
Weekly cases of suspected ZIKV infections and Guillain-Barre syndrome in French Polynesia between October 2013 and April 2014

Cao-Lormeau, Lancet 2016
ZIKV-Associated Guillain-Barre Syndrome

42 patients – median age 42 y; 74% men; 100% with neutralizing antibodies to Zika

Estimated 0.24 cases per 1000 ZIKV infections

Rapid progression to nadir (median 6 d from onset neurological symptoms)

Clinical presentation: generalized muscle weakness (74%), inability to walk (44%), and facial palsy (64%)

Cao-Lormeau VM et al Lancet 2016
Distribution suspected and confirmed Zika and GBS cases by EW Americas 2015-2017
Congenital Zika Syndrome

Courtesy of NBC News
Notified cases of microcephaly up to 3 Feb 2016

Vasco Aragao MF et al. BMJ 2016
Epidemic curve of microcephaly cases among at-term newborns and preceding ZIKV circulation

Pernambuco State, Brazil, 2015

Outbreak rash & fever DEN test (-)  
ZIKV lab confirmed
Zika Virus and Microcephaly in Brazil

De Oliveira WK et al. Lancet 2017
Fetal Brain Disruption Sequence

- Extreme microcephaly
- Overlapping sutures
- Prominent occipital bone
- Scalp rugae*
- Marked neurological impairment

Congenital Zika Syndrome

- Early miscarriage
- Brain injury related problems
  - Eye abnormalities
  - Hearing impairment
  - Seizures
  - Swallowing impairment
  - Hydrocephalus
  - Limb abnormalities
  - Severe irritability
  - Developmental delay
ZIKV Diagnostics

Illness in Days

-2 -1 0 1 2 3 4 5 6 7 8 9 1 2 3 4

Months

Urine

Semen

NA (PRNT)

IgM

Viremia

Symptoms

? >62 d
Zika Diagnosis

- Reverse transcriptase PCR of plasma (urine, saliva, CSF) in first 7 d post-symptom onset
  - Blood PCR may remain positive longer than urine or plasma

- IgM serology cross reacts with dengue—need negative dengue or 4X higher Zika titer plus ideally PRNT confirmation

- 4 fold rise titer by PRNT acute vs. convalescent sera

- Direct viral detection in amniotic fluid or tissue
Screening of Pregnant Women with Zika Exposure

Pregnant woman with history of travel to an area with Zika virus transmission

Pregnant woman reports clinical illness consistent with Zika virus disease during or within 2 weeks of travel

Test for Zika virus infection

Positive or inconclusive test for Zika virus infection
Fetal ultrasound to detect microcephaly or intracranial calcifications
Offer amniocentesis for Zika virus testing

Negative test(s) for Zika virus infection
Fetal ultrasound to detect microcephaly or intracranial calcifications

Either finding present
Consider amniocentesis for Zika virus testing

No findings present
Test pregnant woman for Zika virus infection
Consider amniocentesis for Zika virus testing

Fetal ultrasound to detect microcephaly or intracranial calcifications

Either finding present
No findings present

Consider serial ultrasounds to detect development of microcephaly or intracranial calcifications

Either finding develops

Pregnant woman does NOT report clinical illness consistent with Zika virus disease during or within 2 weeks of travel

Either finding present

No findings present

Consider serial ultrasounds to detect development of microcephaly or intracranial calcifications

Either finding develops
Treatment of Zika Virus Disease

- Supportive treatment with acetaminophen, hydration, and rest
- Avoid aspirin and NSAIDs until dengue has been ruled out
- Urgent medical care needed if symptoms of GBS develop
- No specific antiviral therapy available
  - In vitro data suggest sofosbuvir, chloroquine, and azithromycin active against Zika
  - Hamer DH et al. Curr Infect Dis Rep 2017
Recent Emerging Infectious Diseases

Paules CI et al. Ann Int Med 2017
Estimated 75% of EIDs are zoonoses
Cutler SJ et al. EID 2010
Factors Influencing Infectious Disease Emergence and Spread

- Demographic
- Social and behavioral
- Technology and industry
- Environment and land use changes
- International travel and commerce
- Microbial adaptation
- Breakdown in public health measures
Global Aviation Network
(civil traffic, 500 largest airports, 100 countries)
Global Travel and World Population

From: Murphy and Nathanson Sems. Virol. 5, 87, 1994
Emergence and Spread of Chikungunya Asian and Indian Ocean Lineages from Africa

http://www.plosntd.org/article/info:doi/10.1371/journal.pntd.0002921
Chikungunya in the Americas

More than 1.7 million estimated cases reported to PAHO
Factors Responsible for Chikungunya Emergence

**Biologic and genetic:**
- Virus adaptation: mutation at residue 226 of membrane fusion glycoprotein E1
  - May have resulted in improved virus adaptation to *A. albopictus*

**Ecological:**
- East Africa drought increased standing water and provided ideal conditions for vector breeding
- Warm European summer with high vector abundance
Factors Responsible for Chikungunya Re-emergence (2)

- Physical environment:
  - Artificial vector breeding sites (household water stores, manholes, used tires)
  - Previous introduction of *A. albopictus* to Indian Ocean islands and Italy

- Social, political, and economic:
  - International and domestic travel
  - Human population migrations in the Indian Ocean region
  - Delayed identification and control of initial outbreaks
Three Theories on How Zika Was Introduced into Brazil

- 2014 World Cup in Brazil
- Va’a canoe event in Rio de Janeiro in August 2014 included participants from French Polynesia
- Confederation Cups soccer tournament in June 2013
  - Phylogenetic analyses suggest single introduction of Zika virus May-Dec 2013
    - Faria NR et al Science 2016
Why has Zika emerged now?

- Naïve populations in South Pacific amplified virus and facilitated spread via global mobility
- Abundance of competent vectors in the Americas
- Antibody-dependent enhancement in a heavily dengue-exposed population
- Mutational change (‘Asia strain’) - enhanced viral infectivity of Aedes vectors
- Mutational change
  - Enhanced human viremia and improved transmission efficiency
  - Increased infectivity to neural progenitor cells
Previously established DENV infected cohort in NE Thailand

- Panel monoclonal antibodies generated from DENV-infected subjects
- Blood sample collection done during acute illness, convalescent phase (6m after hospital discharge) 2002-2004
DENV plasma potently induces ADE 1-2 weeks after recovery
Mutation of the prM Protein of Zika Virus

- S139N mutant virus (Asian lineage) emerged in May 2013 shortly before the French Polynesia outbreak
  - Based on Bayesian phylogenetic tree estimates
- S139N mutant causes more robust infection of neuroprogenital cells of embryonic mouse brains
- Mutation associated with severe fetal microcephaly

Yuan L et al. Science 2017
S139N mutant virus enhanced virulence in neonatal mouse model

Graph showing survival and virus titer of different mutants post infection.
Pregnant?

Warning: Zika might be linked to birth defects
There is no vaccine to prevent Zika virus infection

Protect yourself from mosquito bites

Daytime is most dangerous
Mosquitoes that spread chikungunya, dengue, and Zika are aggressive daytime biters. They can also bite at night.

Use insect repellent
It works!
Look for the following active ingredients:
- DEET
- PICARIDIN
- IR3535

Wear protective clothes
Wear long-sleeved shirts and long pants and use insect repellent. For extra protection, treat clothing with permethrin.

Mosquito-proof your home
Use screens on windows and doors. Use air conditioning when available. Keep mosquitoes from laying eggs in and near standing water.

For more information:
Mosquito Vectors – Most Common

Aedes aegypti

Aedes albopictus
Options for Prevention

- As weather permits, wearing long-sleeved shirts and long pants
- Insect repellents
- Air conditioning or window/door screens to keep mosquitoes outside
  - If no screens, sleep under a mosquito bed net or in a tent (can treat with permethrin)
- Reduce number of mosquitoes outside by emptying standing water from containers (community mobilization)
Antivector Measures

- **Insect repellents**
  - Repellents containing DEET (30%), picaridin, IR3535, and oil of lemon eucalyptus and para-menthane-diol products provide long lasting protection.
  - If you use both sunscreen and insect repellent, apply sunscreen first and then repellent.
  - Treat clothing with permethrin.

- **Dark colors, strong smells, CO, PW attract**

- **Protected sex post-travel!**

- **Screening of blood products**
Genetically modified mosquitoes?
Travel Precautions

- Women who are pregnant should avoid travel to areas with ongoing Zika transmission
  - Modified (based on epidemiological data on *Ae. aegypti* distribution and dengue risk) to avoid travel to elevations <2000 m

- Pregnant women who have traveled to Zika-infected areas should be screened for infection upon return

- Male partners of women who are pregnant should use barrier methods after travel to countries with Zika for 6 months
Dengue Vaccine (Dengvaxia)

**Table 2. Vaccine Efficacy against Any Serotype of Dengue.**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Vaccine Group</th>
<th>Control Group</th>
<th>Vaccine Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases/Events*</td>
<td>Person-Yr at Risk†</td>
<td>Incidence Density (95% CI)‡</td>
</tr>
<tr>
<td></td>
<td>no.</td>
<td>no./100 person-yr</td>
<td></td>
</tr>
<tr>
<td>Per-protocol analysis</td>
<td>176/176</td>
<td>11,793</td>
<td>1.5 (1.3–1.7)</td>
</tr>
<tr>
<td>Intention-to-treat analysis</td>
<td>277/280§</td>
<td>26,883</td>
<td>1.0 (0.9–1.2)</td>
</tr>
</tbody>
</table>

- Recombinant live attenuated tetravalent vaccine
- Protective efficacy varied from 42% for serotype 2 to 78% serotype (higher protection severe dengue – 95%)
  - Villar L et al NEJM 2014
- Much less effective in Thailand-only 30% (9% and 100% for serotypes 2 and 4)
  - Sabchareon A et al Lancet 2012
Dengvaxia - Challenges

- Pooled vaccine efficacy across both trials 59.2% in year following primary series (PP analysis)
  - 79% vs. severe dengue

- Efficacy varied by age at vaccination and serostatus at baseline (i.e., previous exposure to dengue prior to vaccination)

- Today Sanofi Pasteur announced risk of severe dengue in those seronegative at baseline
  - Excess of hospitalization and severe dengue in year 3 post vaccination
  - Excess mainly observed in those vaccinated aged 2-5 years in CYD14 in Asia, (RR hospitalized dengue in vaccinees 7.45 (95% CI 1.15, 313.80) in year 3)
Chikungunya Vaccines

- CHIKV elicits long-lasting protective immunity
- Animal models show some cross-protection between CHIKV and other alphaviruses
- Candidate vaccines have been tested in humans
  - MV-CHIKV (measles vaccine modified to express CHIKV proteins; Themis Bioscience) beginning phase 1/2 trial
    - 2 dose vaccine; testing different intervals
  - Virus-like particle vaccine in phase 2
Zika Vaccine Strategies

- Live attenuated vaccine
  - Phase 1 soon in Baltimore and Burlington
- Purified inactivated virus
  - ZPIV (Walter Reed) in phase 1 trials
- DNA
  - DNA virus completed phase 1 trials 2016 (NIAID)
  - Phase 2 trial (VRC 705) ongoing – safety and immunogenicity
- mRNA
- Virus like particles
- Vesicular stomatitis virus
- Recombinant subunit
Zika Vaccines – Design Issues

Where?
→ Multiple sites

- Epidemics may wane & recur
- Flavivirus-naïve & non-naïve populations

Who is at priority?

- Women of childbearing age with no previous ZIKV infection & not pregnant
- Risk for fetus during incident pregnancies
- sexual partners

Placebo group?

- Controversial during Ebola vaccine trials
- Probably acceptable for Zika in healthy adults (usually non-severe)

Lipsitch & Cowling Science 2016
Marston et al. NEJM 2016
Any Questions?

Futuras Madres