Mosquito-Borne Viral Diseases with a Focus on Dengue and Yellow Fever
Disclaimer

The views expressed during this presentation are the personal views of the presenter and do not necessarily represent the views of the U.S. Army or the U.S. Department of Defense.

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Silver Spring, Maryland
Objectives

• Increase knowledge of:
  
  – Arbovirology
  
  – Epidemiology of tropical viral disease threats
  
  – Arboviral clinical syndromes
  
  – Key points related to prevention and treatment
Case Presentation

• 18 year old native from Thailand (moved to US at 10 years of age) presents in August with 2 days of illness including fever, headache, bone pain, and nausea 3 days after returning from a vacation in Puerto Rico. He vacationed in San Juan, stayed in a hotel, ate and drank local foods and beverages from the hotel and restaurants, and swam in the hotel pool and ocean. He does not recall mosquito exposure and did not reliably use DEET. He had no animal exposures. He has no past medical history except for a single STI. He takes no medications to include those acquired OTC. He does not abuse ETOH nor use illicit drugs.

• The LEAST likely diagnosis would be:
  - 1. chikungunya
  - 2. dengue
  - 3. leptospirosis
  - 4. acute HIV
Dengue

- **Basics**
  - Family Flaviviridae, Genus Flavivirus, Species Dengue
    - Same family as WNV, YF, JE, Zika
  - RNA virus, 3 structural and 7 non-structural genes
    - Different functions during infection process
    - Different targets for drugs/vaccines
  - 4 dengue virus types: DENV-1-4
    - Multiple genotypes within each dengue virus type
• Transmission
  – Feeding vector
  – Laboratory acquired
  – Blood supply?
  – Organ donation?

• Vector
  – *Aedes aegypti*
  – *Aedes albopictus*

<table>
<thead>
<tr>
<th>Country</th>
<th>Donor deferral measures for dengue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singapore*</td>
<td>6 months deferral for history of dengue infection</td>
</tr>
<tr>
<td></td>
<td>3 weeks deferral for history of fever</td>
</tr>
<tr>
<td></td>
<td>No travel-related deferral for dengue</td>
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<tr>
<td>Hong Kong*</td>
<td>6 months deferral for history of dengue infection</td>
</tr>
<tr>
<td></td>
<td>2 weeks deferral for history of fever</td>
</tr>
<tr>
<td></td>
<td>No travel-related deferral for dengue</td>
</tr>
<tr>
<td>Sri Lanka*</td>
<td>No specific deferral for history of dengue infection</td>
</tr>
<tr>
<td></td>
<td>2 weeks deferral for history of fever</td>
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<tr>
<td></td>
<td>No travel-related deferral for dengue</td>
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<tr>
<td>Australia†</td>
<td>4 weeks deferral for history of dengue infection</td>
</tr>
<tr>
<td></td>
<td>No travel-related deferral for dengue</td>
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<tr>
<td>New Zealand‡</td>
<td>4 weeks deferral for history of dengue infection</td>
</tr>
<tr>
<td></td>
<td>No travel-related deferral for dengue</td>
</tr>
<tr>
<td>UK‡</td>
<td>2 weeks deferral for history of dengue infection</td>
</tr>
<tr>
<td></td>
<td>No travel-related deferral for dengue</td>
</tr>
<tr>
<td>United States‡</td>
<td>4 weeks deferral for history of dengue infection</td>
</tr>
<tr>
<td></td>
<td>No travel-related deferral for dengue</td>
</tr>
</tbody>
</table>

*Endemic for dengue.
†Non-endemic except parts of Northern Australia.
‡Non-endemic.
Areas supporting dengue virus transmission.
Dengue Burden

Under-estimated and under-reported

389.9M infections/ year

Reporting sources – WHO, MOHs, ProMed, GeoSentinel, EuroSurveillance, World Org
Dengue Fever, Hawaii, 2001–2002
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 11, No. 5, May 2005

Identification of Dengue Fever Cases in Houston, Texas, with Evidence of Autochthonous Transmission Between 2003 and 2005

Locally Acquired Dengue — Key West, Florida, 2009–2010

8 dengue cases detected in Florida
Puerto Rico

Suspected cases reported compared to the historical average

Total viral identifications in the last 12 months

Serotype Distribution

Totals through 29 JUL 2014: 2,468 suspected, 381 confirmed, 2 severe (DHF), 0 deaths
Totals through 31 DEC 2013: 18,164 suspected, 9,032 confirmed, 50 severe (DHF), 12 deaths

References: 1) CDC Website  4) Dengue Surveillance Weekly Report, CDC, December 2013
With respect to specific diagnoses, malaria was one of the three most frequent causes of systemic febrile illness among travelers from every region, although travelers from every region except sub-Saharan Africa and Central America had confirmed or probable dengue more frequently than malaria.
Factors Driving Transmission

- **DENVs**
  - Travel in hosts
  - Viral evolution
- **Naïve hosts**
  - Population growth
  - Increased urbanization
- **Vector**
  - Ecologic changes
  - Evolution

These conditions create standing water in which the principal vector, the mosquito *Aedes aegypti*, breeds.
Factors Driving Disease

• There is a significantly increased risk of severe dengue disease (dengue hemorrhagic fever) when infected a second time with a different DENV type than what you were infected with during your first infection (i.e. DENV-4 during first infection, DENV-2 during second).

• Co-circulation of numerous DENV types in similar time and space increases risk of experiencing multiple infections with different DENV types.
DENV Type Distribution - 1970

D. Gubler
Global Air Travel Flight Patterns

http://upload.wikimedia.org/wikipedia/commons/a/ac/World-airline-routemap-2009.png
DENV Type Distribution - 2004

D. Gubler
Case Presentation

• 18 year old native from Thailand (moved to US at 10 years of age) presents in August with 2 days of illness including fever, headache, bone pain, and nausea 3 days after returning from a vacation in Puerto Rico. You suspect he has a dengue infection. He is tolerating PO intake without vomiting and is urinating. Vital signs except for temperature (102.5°F) are in the range of normal. Mucous membranes are moist, skin turgor is normal, abdominal exam is normal, and lungs are clear. A CBC reveals a low WBC (3.5k) but otherwise is within normal limits. Electrolytes are normal.

• What is the most reasonable initial management strategy?

  – 1. treat as outpt, provide NSAIDS, encourage PO fluids
  – 2. treat as inpt, provide 1L NS bolus, monitor in ICU setting
  – 3. treat as outpt, provide acetaminophen, encourage po fluids, F/U
  – 4. treat as inpt, encourage PO fluids, perform q6 hr HCT evaluations
Dengue haemorrhagic fever
Diagnosis, treatment, prevention and control

SECOND EDITION

World Health Organization
Geneva
1997

Dengue Infection
Clinical Phenotypes

Dengue virus infection

Asymptomatic

Symptomatic

Undifferentiated fever (viral syndrome)

Dengue fever syndrome

Dengue haemorrhagic fever (plasma leakage)

Without haemorrhage

With unusual haemorrhage

No shock

Dengue shock syndrome

Dengue fever

Dengue haemorrhagic fever

1997 WHO dengue fever case definition

• Probable dengue infection
  – Acute febrile illness and at least 2 of the following:
    • Headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, and leukopenia
• Confirmed dengue infection
  – Above + lab confirmation (at least one method below)
    • DENV isolation (blood, autopsy samples)
    • 4 fold rise in IgM or IgG to any of the four DENV antigens in paired blood samples
    • Demonstration of DENV antigen (tissue, CSF, serum) by ELISA, Immunohistochemistry, immunofluorescence
• PCR +
1997 WHO case definition for DHF/DSS

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td>1997 World Health Organization (WHO) case definition for dengue hemorrhagic fever and dengue shock syndrome*</td>
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<tr>
<td>---------</td>
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<tr>
<td><strong>DHF, the following must all be present:</strong></td>
</tr>
<tr>
<td>Fever, or history of acute fever, lasting 2–7 days, occasionally biphasic</td>
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<tr>
<td>Hemorrhagic tendencies, evidenced by at least one of the following:</td>
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<tr>
<td>A positive tourniquet test</td>
</tr>
<tr>
<td>Petechiae, ecchymoses, or purpura</td>
</tr>
<tr>
<td>Bleeding from the mucosa, gastrointestinal tract, injection sites, or other locations</td>
</tr>
<tr>
<td>Hematemesis or melena</td>
</tr>
<tr>
<td>Thrombocytopenia (100,000 cells/mm³ or less)</td>
</tr>
<tr>
<td>Evidence of plasma leakage caused by increased vascular permeability, manifested by at least one of the following:</td>
</tr>
<tr>
<td>A rise in the hematocrit equal to or &gt; 20% above average for age, sex, and population</td>
</tr>
<tr>
<td>A drop in the hematocrit following volume replacement treatment equal to or &gt; 20% of baseline</td>
</tr>
<tr>
<td>Signs of plasma leakage such as pleural effusion, ascites, and hypoproteinemia</td>
</tr>
<tr>
<td><strong>Case definition for dengue shock syndrome:</strong></td>
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<tr>
<td>All of the above four criteria for DHF must be present, plus evidence of circulatory failure manifested by:</td>
</tr>
<tr>
<td>Rapid and weak pulse, and</td>
</tr>
<tr>
<td>Narrow pulse pressure (&lt; 20 mm Hg)</td>
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<tr>
<td>or manifested by:</td>
</tr>
<tr>
<td>Hypotension for age, and</td>
</tr>
<tr>
<td>Cold, clammy skin and restlessness.</td>
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*HF = dengue hemorrhagic fever.
**2009 WHO dengue case definitions**

**Table 2**

2009 World Health Organization (WHO) dengue case definitions*14

<table>
<thead>
<tr>
<th>Probable dengue</th>
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<tbody>
<tr>
<td>Live in or travel to dengue endemic area, fever and two of</td>
</tr>
<tr>
<td>the following:</td>
</tr>
<tr>
<td>Nausea, vomiting</td>
</tr>
<tr>
<td>Rash</td>
</tr>
<tr>
<td>Aches and pains</td>
</tr>
<tr>
<td>Tourniquet test positive</td>
</tr>
<tr>
<td>Leucopenia</td>
</tr>
<tr>
<td>Any “Warning Sign”</td>
</tr>
<tr>
<td>Dengue with Warning Signs</td>
</tr>
<tr>
<td>Abdominal pain or tenderness</td>
</tr>
<tr>
<td>Persistent vomiting</td>
</tr>
<tr>
<td>Clinical fluid accumulation</td>
</tr>
<tr>
<td>Mucosal bleed</td>
</tr>
<tr>
<td>Lethargy, restlessness</td>
</tr>
<tr>
<td>Liver enlargement &gt; 2 cm</td>
</tr>
<tr>
<td>Laboratory increase in HCT concurrent with rapid decrease in platelet count</td>
</tr>
</tbody>
</table>

| Severe dengue (short form)                                                      |
| Severe plasma leakage                                                           |
| Shock (DSS)                                                                      |
| Fluid accumulation with respiratory distress                                    |
| Severe bleeding (as evaluated by clinician)                                     |
| Severe organ involvement                                                        |
| Liver AST or ALT >= 1,000                                                        |
| CNS impaired consciousness                                                      |
| Heart and other organs                                                          |

Severe dengue (long form)

There is evidence of plasma leakage, such as:
- High or progressively rising hematocrit;
- Pleural effusions or ascites;
- Circulatory compromise or shock (tachycardia, cold and clammy extremities, capillary refill time greater than 3 seconds, weak or undetectable pulse, narrow pulse pressure or, in late shock, unrecordable blood pressure).

There is significant bleeding
- There is an altered level of consciousness (lethargy or restlessness, coma, convulsions).
- There is severe gastrointestinal involvement (persistent vomiting, increasing or intense abdominal pain, jaundice).
- There is severe organ impairment (acute liver failure, acute renal failure, encephalopathy or encephalitis, or other unusual manifestations, cardiomyopathy) or other unusual manifestations.

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*HCT = hematocrit; DSS = dengue shock syndrome; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CNS = central nervous system.
Figure 1.4 Suggested dengue case classification and levels of severity

**DENGUE ± WARNING SIGNS**

- with warning signs
- without

**SEVERE DENGUE**

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

**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)*

**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 >= 1000
- CNS: Impaired consciousness
- Heart and other organs
Dengue Clinical and Lab Parameters

24 hr period around defervescence = danger period
Diagnosing Dengue

• Maintain high degree of suspicion
  – Geographic location
  – Clustering of cases
• History and physical
  – Clinical presentation
  – Vital signs (HR, BP, Temp)
  – Dengue tourniquet test (TT)
• Clinical lab assessment
  – CBC (WBC, HCT, PLT), AST/ALT
• Dengue tests in US
  – IgM capture ELISA
  – CDC RT-PCR
• Dengue area, +Clinical, +TT, WBC<5k = High PPV (~70%)
Evaluation of diagnostic tests: dengue

Rosanna W. Peeling, Harvey Artsob, Jose Luis Pelegrino, Philippe Buchy, Mary J. Cardosa, Shamala Devi, Delia A. Enria, Jeremy Farrar, Duane J. Gubler, Maria G. Guzman, Scott B. Halstead, Elizabeth Hunsperger, Susie Kliks, Harold S. Margolis, Carl M. Nathanson, Vinh Chau Nguyen, Nicia Rizzo, Susana Vázquez and Suthee Yoksan

Figure 1 | Comparative merits of direct and indirect laboratory methods for the diagnosis of dengue infections. Opportunity refers to the fact that antibody testing is usually the most practical diagnostic option available.
Dengue Tourniquet Test

- Measure BP
- SBP + DBP / 2 = target insufflation pressure for test
- Inspect area near antecubital fossa
  - You will assess delta before / after
- Inflate to target pressure
- Hold for 5 minutes
- Remove cuff
- Reassess antecubital fossa
- Count # of petechiae in 2.5 cm² area
- ≥10 new petechiae is positive

• TT measures capillary fragility, severe disease predictor?
Case Presentation

• 18 year old native from Thailand (moved to US at 10 years of age) presents in August with 2 days of illness including fever, headache, bone pain, and nausea 3 days after returning from a vacation in Puerto Rico. You decide to manage him as an outpatient. He fails to follow up as requested but does return day 6 of his illness afebrile with resolution of some symptoms but he now has abdominal pain, slight shortness of breath, and lethargy.

• What is the most reasonable management strategy at this point?
  – 1. Continue close follow up as outpatient, encourage PO fluid intake, this is the natural history of a resolving dengue infection
  – 2. Admit to the hospital, approach as a critically ill patient, this is a severe dengue virus infection
  – 3. Admit to the hospital, evaluate for another infectious disease process, these new symptoms represent a new medical problem
  – 4. Prescribe doxycycline, he probably has leptospirosis
Dengue Treatment

**Dengue Without Warning Signs**

**Group A**
(May be sent home)

- **Group criteria**
  - Patients who do not have warning signs AND who are able:
  - to tolerate adequate volumes of oral fluids
  - to pass urine at least once every 6 hours

- **Laboratory tests**
  - full blood count (FBC)
  - haematocrit (HCT)

**Treatment**
Advice for:
- adequate bed rest
- adequate fluid intake
- Paracetamol, 4 gram maximum per day in adults and accordingly in children.

Patients with stable HCT can be sent home.

**Monitoring**
Daily review for disease progression:
- decreasing white blood cell count
- defervescence
- warning signs (until out of critical period).

Advice for immediate return to hospital if development of any warning signs, and
- written advice for management (e.g. home care card for dengue).
Group B
(Referred for in-hospital care)

Group criteria
Patients with any of the following features:
• co-existing conditions such as pregnancy, infancy, old age, diabetes mellitus, renal failure
• social circumstances such as living alone, living far from hospital

Laboratory tests
• full blood count (FBC)
• haematocrit (HCT)

Treatment
• Encouragement for oral fluids. If not tolerated, start intravenous fluid therapy 0.9% saline or Ringer’s Lactate at maintenance rate.

Monitoring
Monitor:
• temperature pattern
• volume of fluid intake and losses
• urine output (volume and frequency)
• warning signs
• HCT, white blood cell and platelet counts.
• Assess
• Intervene
• Re-assess

Compensated shock (systolic pressure maintained but has signs of reduced perfusion)
Fluid resuscitation with isotonic crystalloid
5–10 ml/kg/hr over 1 hour

Yes

Improvement

IV crystalloid 5–7 ml/kg/hr for 1–2 hours, then:
reduce to 3–5 ml/kg/hr for 2–4 hours;
reduce to 2–3 ml/kg/hr for 2–4 hours.

If patient continues to improve, fluid can be further reduced.

Monitor HCT 6–8 hourly.

If the patient is not stable, act according to HCT levels:
if HCT increases, consider bolus fluid administration or increase fluid administration;
if HCT decreases, consider transfusion with fresh whole blood.

Stop at 48 hours.

No

Check HCT

HCT ↓↓

Consider significant occult/overt bleed
Initiate transfusion with fresh whole blood

HCT ↑↑ or high

Administer 2nd bolus of fluid
10–20 ml/kg/hr for 1 hour

Improvement

YES

If patient improves, reduce to 7–10 ml/kg/hr for 1–2 hours
Then reduce further

NO
Figure 2.3 Algorithm for fluid management in hypotensive shock

**Hypotensive shock**
Fluid resuscitation with 20 ml/kg isotonic crystalloid or colloid over 15 minutes
Try to obtain a HCT level before fluid resuscitation

**Improvement**

**YES**
Crystalloid/colloid 10 ml/kg/hr for 1 hour, then continue with:
IV crystalloid 5-7 ml/kg/hr for 1-2 hours; reduce to 3-5 ml/kg/hr for 2-4 hours; reduce to 2-3 ml/kg/hr for 2-4 hours.

If patient continues to improve, fluid can be further reduced.
Monitor HCT 6-hourly.
If the patient is not stable, act according to HCT levels:
if HCT increases, consider bolus fluid administration or increase fluid administration;
if HCT decreases, consider transfusion with fresh whole transfusion.
Stop at 48 hours.

**NO**
Review 1st HCT

**HCT↑ or high**
Administer 2nd bolus fluid (colloid)
10-20 ml/kg over ½ to 1 hour

**Improvement**

**YES**

**NO**
Consider significant occult/overt bleed
Initiate transfusion with fresh whole blood

**HCT↓**
Consider occult / overt bleed

- Assess
- Intervene
- Re-assess

[Diagram]

Improvement

YES

Repeat 2nd HCT

HCT↑ or high

Administer 3rd bolus fluid (colloid)
10-20 ml/kg over 1 hour

Improvement

YES

NO

Repeat 3rd HCT

HCT↓
## Textbox A. Good clinical practice and bad clinical practice

<table>
<thead>
<tr>
<th></th>
<th>Good practice</th>
<th>Bad practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Assessment and follow-up of patients with non-severe dengue and careful instruction of warning signs to watch out for</td>
<td>Sending patients with non-severe dengue home with no follow-up and inadequate instructions</td>
</tr>
<tr>
<td>2</td>
<td>Administration of paracetamol for high fever if the patient is uncomfortable</td>
<td>Administration of acetylsalicylic acid (aspirin) or ibuprofen</td>
</tr>
<tr>
<td>3</td>
<td>Obtaining a haematocrit level before and after fluid boluses</td>
<td>Not knowing when haematocrit levels are taken with respect to fluid therapy</td>
</tr>
<tr>
<td>4</td>
<td>Clinical assessment of the haemodynamic status before and after each fluid bolus</td>
<td>No clinical assessment of patient with respect to fluid therapy</td>
</tr>
<tr>
<td>5</td>
<td>Interpretation of haematocrit levels in the context of fluid administered and haemodynamic assessment</td>
<td>Interpretation of haematocrit levels independent of clinical status</td>
</tr>
<tr>
<td>6</td>
<td>Administration of intravenous fluids for repeated vomiting or a high or rapidly rising haematocrit</td>
<td>Administration of intravenous fluids to any patient with non-severe dengue</td>
</tr>
<tr>
<td>7</td>
<td>Use of isotonic intravenous fluids for severe dengue</td>
<td>Use of hypotonic intravenous fluids for severe dengue</td>
</tr>
<tr>
<td>8</td>
<td>Giving intravenous fluid volume just sufficient to maintain effective circulation during the period of plasma leakage for severe dengue</td>
<td>Excessive or prolonged intravenous fluid administration for severe dengue</td>
</tr>
<tr>
<td>9</td>
<td>Avoiding intramuscular injections in dengue patients</td>
<td>Giving intramuscular injections to dengue patients</td>
</tr>
<tr>
<td>10</td>
<td>Intravenous fluid rate and frequency of monitoring and haematocrit measurement adjusted according to the patient’s condition</td>
<td>Fixed intravenous fluid rate and unchanged frequency of monitoring and haematocrit measurement during entire hospitalization for severe dengue</td>
</tr>
<tr>
<td>11</td>
<td>Close monitoring of blood glucose, i.e. tight glycaemic control</td>
<td>Not monitoring blood glucose, unaware of the hyperglycaemic effect on osmotic diuresis and confounding hypovolaemia</td>
</tr>
<tr>
<td>12</td>
<td>Discontinuation or reducing fluid therapy once haemodynamic status stabilizes</td>
<td>Continuation and no review of intravenous fluid therapy once haemodynamic status stabilizes</td>
</tr>
</tbody>
</table>
Question

• A 67 yo male presents with a five-day history of a febrile illness, headache, severe abdominal pain, nausea and vomiting, jaundice, leukopenia, and thrombocytopenia. He recently immigrated from the U.S. to Peru near the Amazon basin. He does not abuse ETOH or use tobacco. He is up to date on all immunizations including yellow fever vaccine received 4 days prior to onset of symptoms. On admission, his BP was 110/70, HR of 72, a RR of 20, a temp. of 36°C, and O2 saturation rate of 74% on room air.

• What additional information would most inform your differential diagnosis?
  – 1. History of past dengue virus infection
  – 2. History of past yellow fever vaccinations
  – 3. Use of personal protective measures to prevent arthropod exposure
  – 4. Use of malaria prophylaxis

Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology

Bali Pulendran

Timeline | Events in the development and understanding of the YF-17D vaccine

- The first recorded description of an epidemic thought to be yellow fever is made by Mayans in Yucatan.
- A yellow fever epidemic kills ~10% of the population of Philadelphia, USA.
- 20,000 inhabitants are killed in the Mississippi river valley, USA.
- Spanish-American War: 968 American soldiers are killed in combat but over 5,000 die of yellow fever.
- Stokes and colleagues isolate a strain of yellow fever virus from an infected individual named Asibi, in Ghana. French researchers in Dakar, Senegal, isolate the yellow fever virus from an infected Syrian.
- The Nobel Prize in Medicine and Physiology is awarded to Max Theiler.


- Thousands of British and American troops die in the British expedition to Cuba. Epidemics in coastal and island communities kill ~10% of the population.
- 40,000 French soldiers are killed by yellow fever in Haiti.
- Carlos Finlay, a Cuban physician, proposes that yellow fever is carried by the mosquito.
- Walter Reed shows that yellow fever is spread by mosquitoes.
- Max Theiler develops the yellow fever vaccine YF-17D.
- The WHO grants the use of two substraits of the YF-17D vaccine: 17DD for use in South America and 17D-204 for use in the rest of the world.
- The T cell immunogenicity of YF-17D is shown to depend on signalling through multiple TLRs, and systems biology approaches reveal the complexity of the innate immune responses to YF-17D and can predict the immunogenicity of YF-17D. In addition, there are insights into the dynamics of CD8+ T cell response.

TLR, Toll-like receptor; WHO, World Health Organization.
Walter Reed Yellow Fever Commission

- Experiment summary
  - 14 non-fatal human YF challenge experiments
  - Transmission cycle revealed
  - Reed et al. publish results in JAMA, 1901
  - Army orders Gorgas to complete source reduction
  - In 90 days Havana is free of YF
Yellow Fever Virus

- Virus
  - Flavivirus (YF, JE, WNV, DENV)
  - 1 serotype
    - 5 genotypes within serotype

- Vector
  - Mosquito (*Aedes* spp.)

- Phylogenetic analyses
  - Evolved over 3000 yrs
  - YF virus originated in Africa
  - Divided into West and East African lineages
  - W. African lineage
    - Imported into S. America and New World
FIGURE 1. Transmission cycles for yellow fever virus

* The jungle (sylvatic) transmission cycle involves transmission of the virus between nonhuman primates and mosquito species found in the forest canopy. The virus is transmitted via mosquitoes from nonhuman primates to human when the humans encroach into the jungle during occupational or recreational activities.

† The urban transmission cycle involves transmission of the virus between human and urban mosquitoes, primarily *Ae. aegypti*. Viremic humans traveling from one region to another can feed into and serve as a source of infection for mosquitoes in other transmission cycles (dotted line).

§ In Africa, an intermediate (savannah) cycle involves transmission of YFV from tree hole-breeding *Aedes* spp. to humans living or working in jungle border areas. In this cycle, the virus can be transmitted from nonhuman primate to humans or from human to human via these mosquitoes.
Yellow Fever Risk Map

Disease Time-course

- Incubation period: 3-6 days
- Symptoms for ~ 3 days (viremia)
- Defervescence and short term improvement (remission)
- Fever and symptoms return (intoxication)
- Improvement (convalescence)

*Figure 5. Yellow fever patient during the period of infection. The patient is febrile and acutely ill, with prominent conjunctival congestion. During this pre-icteric phase, the illness is difficult to differentiate from many other infectious diseases. Virus is present in the blood and the patient is infectious for blood-feeding mosquitoes.*
Figure 4. Stages of yellow fever infection, showing the major clinical and laboratory features of the disease.
Figure 6. Pathogenesis of yellow fever based on studies in experimentally infected monkeys and human case reports (bold). Speculative mechanisms shown in italics are drawn from in-vitro data or reports on other flavivirus infections. CTL = cytotoxic T lymphocyte; DIC = disseminated intravascular coagulation; IL = interferon.
Diagnosis

• Clinical Diagnosis
  – h/o travel to endemic area within the incubation period
  – Consistent clinical picture

• Advanced Diagnostics
  – Virus Isolation (culture)
  – Rapid Diagnostics
    • PCR
    • Antibody or Antigen detection (ELISA)
      – IgM for acute phase, coupled with convalescent antibodies (IgM/IgG)
    • Neutralization Ab are more specific for YF
Treatment Overview

• Supportive Care -- no specific therapy
  – Maintain nutrition and prevent hypoglycemia
  – NG tube to prevent gastric distention
  – Treatment of hypotension (IVF, pressors)
  – Supplemental oxygen
  – Correction of bleeding abnormalities
  – Dialysis
  – Treatment of secondary infections
  – Treatment of DIC

– PROTECT FROM FURTHER MOSQUITO EXPOSURE

Certain medications should be avoided, such as aspirin or other non-steroidal anti-inflammatory drugs (such as ibuprofen and naproxen), because these may increase the risk for bleeding.
Question

• A 67 yo male presents with a five-day history of a febrile illness, headache, severe abdominal pain, nausea and vomiting, jaundice, leukopenia, and thrombocytopenia. On admission, his BP was 110/70, HR of 72, a RR of 20, a temp. of 36°C, and O2 saturation rate of 74% on room air. The pt went on to develop a YF like illness and died. The final diagnosis is YF vaccine associated viscerotropic disease (YF-AVD).

• What represents a know risk factor for development of YF-AVD?

  – 1. First time YF vaccine recipient
  – 2. Age greater than 50 years
  – 3. Potential of previous non-YF flavivirus infections (dengue, West nile)
  – 4. Concomitant NSAID use

Yellow Fever Vaccine 17D

• Has remained in continuous use since 1936
  – Over 500 million doses given
  – Protects 90%/10 days, 99%/30 days

• Long-lasting immunity
  – Countries may require boosting every 10 years
  – Studies have shown neutralizing Ab decades after dose
    • 81% of US WWII veterans with Ab after > 30yrs

1. WHO. The Immunological Basis for Immunization Series. Module 8: Yellow Fever.
Yellow Fever Vaccine Reactions

• Common
  – Fever, Headache, body aches 5-10 days
  – Injection site inflammation 1-5 days

• Severe
  – Hypersensitivity reactions (including anaphylaxis)
  – YF vaccine-associated neurologic disease (YEL-AND)
  – YF vaccine-associated viscerotropic disease (YEL-AVD)
Yellow Fever Vaccine Reactions

Viscerotropic (hepatotropic) infection:
- transient viremia
- damage to liver, spleen, kidneys and heart
- hemorrhage
- in nature, occurs only in humans and non-human primates
- molecular mechanisms of infection type are poorly understood

Neurotropic infection:
- infects brain parenchyma and causes encephalitis
- in nature, occurs in susceptible rodents
- in “nature” wild-type viruses do not result in neurotropic disease
- can occur in primates when vaccine strain “reverts” to virulent phenotype → Vaccine Associated Neurotropic Disease

Current Opinion in Immunology
Yellow Fever Vaccine Reactions

**YEL-AND**
- primary vaccinees
- 2 to 30 days post-vaccination
- fever (>101.5 °F > 24h) and headache (>24h duration)
- focal neurological dysfunction (aphasia, paresis, etc)
- mental status change
- new-onset seizure or recurrence
- CSF pleocytosis (> 5 WBC/mm³) or elevated protein (>1.5 times normal)
- three distinct clinical entities
  - neurotropic disease
  - auto-immune CNS disease
  - auto-immune PNS disease
- recovery in 95% (CFR <5%)

**YEL-AVD**
- primary vaccinees
- 2 to 5 days post yellow fever vaccination
- fever, myalgia and arthralgia
- elevated liver enzymes and bilirubin, sometimes progressing to liver failure
- thrombocytopenia, lymphocytopenia
- rhabdomyolysis
- hypotension, requiring vasopressors
- renal failure, requiring dialysis
- respiratory failure, requiring intubation
- recovery in 40% (CFR > 60%), with higher CRF in women

Current Opinion in Immunology
Table 1. Yellow fever vaccine contraindications and precautions.

Contraindications
- Age, <6 months
- Thymus disease or history of thymus disease
- Immunosuppression

Precautions
- Age, 6–12 months
- Age, ≥60 years for first-time vaccinees
- Pregnancy
- Lactation
- Asymptomatic HIV infection with laboratory verification of adequate immune system function
- Hypersensitivity to eggs
- Hypersensitivity to gelatin
- Family history of adverse events associated with yellow fever vaccine
Question

• 50 yo Indian male presents with complaints of 3 days of fever, headache, fatigue, rash, and severe joint pains which started 2 days after returning from a Caribbean cruise. Vital signs are normal except for fever (>102.5F). Exam is normal except for a light erythematous rash of the face and trunk which is difficult to appreciate and painful ROM of his hands and feet. His right ankle is appears swollen. CBC is normal except for lymphopenia. His ESR is in the upper range of normal.

• What is a distinguishing clinical feature of this man’s diagnosis?
  – 1. Rash
  – 2. Fatigue
  – 3. Polyarthritis
  – 4. Headache
Chikungunya

- **Virus**
  - Family Togaviridae, Genus Alphavirus
- **Mosquito-transmitted**
  - *Aedes aegypti, Aedes albopictus*
- **Emerging and Re-emerging disease**
  - Cyclic epidemics, 7-20 yrs between outbreaks
  - Enzootic sylvatic cycle / endemic-epidemic urban cycle
- **Phylogenetic groups**
  - West African, Asian, Eastern/Central Africa

---

*Figure 1. Organization of the chikungunya virus genome, including the nonstructural and structural polyprotein open reading frames, and the 26S or subgenomic promoter.*

*Expert Rev. Vaccines 11(9), (2012)*
Historic Movement of Chikungunya

**Figure 5.** Distribution of chikungunya virus strains and movement of outbreaks inferred from the phylogenetic analysis depicted in Figure 4.

CHIK: Chikungunya fever; CHIKV: Chikungunya virus.
Reproduced with permission from [24].

*Expert Rev. Vaccines 11(9), (2012)*
Countries and territories where chikungunya cases have been reported*  
(as of September 30, 2014)

*Does not include countries or territories where only imported cases have been documented. This map is updated weekly if there are new countries or territories that report local chikungunya virus transmission.
Clinical Manifestations

• Majority of infected people become symptomatic
• Incubation period usually 3–7 days (range 1–12 days)
• Acute onset of fever and polyarthralgia
• Joint symptoms usually symmetric
  – Hands and feet
  – Can be severe and debilitating
• Headache, myalgia, arthritis, conjunctivitis, nausea/vomiting, maculopapular rash
• Lymphopenia, thrombocytopenia, elevated creatinine, and elevated hepatic transaminases
## Clinical Manifestations

<table>
<thead>
<tr>
<th>Symptom or sign</th>
<th>Frequency range (% of symptomatic patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>76–100</td>
</tr>
<tr>
<td>Polyarthralgias</td>
<td>71–100</td>
</tr>
<tr>
<td>Headache</td>
<td>17–74</td>
</tr>
<tr>
<td>Myalgias</td>
<td>46–72</td>
</tr>
<tr>
<td>Back pain</td>
<td>34–50</td>
</tr>
<tr>
<td>Nausea</td>
<td>50–69</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4–59</td>
</tr>
<tr>
<td>Rash</td>
<td>28–77</td>
</tr>
<tr>
<td>Polyarthritis</td>
<td>12–32</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>3–56</td>
</tr>
</tbody>
</table>

*Table compiled from a number of different studies.8, 9, 12–17

Pan American Health Organization
Preparedness and Response for Chikungunya Virus: Introduction in the Americas
Washington, D.C.: PAHO; © 2011
Clinical Manifestations - Rash
# Chikungunya vs. Dengue

<table>
<thead>
<tr>
<th>Clinical and laboratory features</th>
<th>Chikungunya virus infection</th>
<th>Dengue virus infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (≥102°F or 39°C)</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Myalgias</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arthralgias</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>Headache</td>
<td>++</td>
<td>++&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rash</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Bleeding dyscrasias</td>
<td>+/-</td>
<td>++</td>
</tr>
<tr>
<td>Shock</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Elevated hematocrit</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean frequency of symptoms from studies where the two diseases were directly compared among patient seeking care; +++ = 70-100% of patients; ++ = 40-69%; + = 10-39%; +/- = <10%; - = 0%<sup>32,33</sup>

<sup>b</sup> Often retroorbital

Table modified from Staples et al.<sup>34</sup>  

*Figure 1. Affected joints (in black) in a patient with CHIKV polyarthritis presenting 6 weeks after onset of illness.*

Chikungunya viral polyarthritis.  
Raj J Carmona, Saeed Shaikh and Nader A Khalidi  
J Rheumatol 2008;35:935-936
Chikungunya: A Potentially Emerging Epidemic?

Michelle M. Thiboutot\textsuperscript{1,2}, Senthil Kannan\textsuperscript{2}, Omkar U. Kawalekar\textsuperscript{2}, Devon J. Shedlock\textsuperscript{2}, Amir S. Khan\textsuperscript{3}, Gopalsamy Sarangan\textsuperscript{4}, Padma Srikanth\textsuperscript{4}, David B. Weiner\textsuperscript{2}, Karuppijah Muthumani\textsuperscript{2*}

Table 1. Comparison of clinical features of Chikungunya and Dengue virus.

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Chikungunya Virus (CHIKV)</th>
<th>Dengue Virus (DENV)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Fever, asthenia</td>
<td>Common</td>
<td>Common</td>
<td>[6,8]</td>
</tr>
<tr>
<td>2) Myalgia</td>
<td>Possible</td>
<td>Very common</td>
<td>[6]</td>
</tr>
<tr>
<td>3) Polyarthritis</td>
<td>Very Common, edematous</td>
<td>None</td>
<td>[56]</td>
</tr>
<tr>
<td>4) Tenosynovitis</td>
<td>Yes</td>
<td>None</td>
<td>[57]</td>
</tr>
<tr>
<td>5) Leukopenia</td>
<td>None</td>
<td>Yes</td>
<td>[58]</td>
</tr>
<tr>
<td>6) Thrombocytopenia</td>
<td>None</td>
<td>Yes</td>
<td>[59]</td>
</tr>
<tr>
<td>7) Rash</td>
<td>Days 1–4, important skin edema</td>
<td>Days 3–7</td>
<td>[6,35,58]</td>
</tr>
<tr>
<td>8) Retro-orbital pain</td>
<td>Rare</td>
<td>Common</td>
<td>[60]</td>
</tr>
<tr>
<td>9) Hypotension</td>
<td>Possible</td>
<td>Common, Days 5–7</td>
<td>[60,61]</td>
</tr>
<tr>
<td>10) Minor bleeding</td>
<td>Chronic polyarthritis up to 1 year</td>
<td>Common</td>
<td>[17,56]</td>
</tr>
<tr>
<td>11) Second stage</td>
<td>Possible; Tenosynovitis at M2–M3 Raynaud’s syndrome at M2–M3</td>
<td>Fatigue up to 3 mo</td>
<td>[6,56,57,58,62,63]</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pntd.0000623.t001
**Diagnosis**

### Figure 2. Viremia and immune response following Chikungunya virus infection.

#### Table 6. Typical results of samples tested at various time points post-infection.

<table>
<thead>
<tr>
<th>Days post illness onset</th>
<th>Virus testing</th>
<th>Antibody testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1-3</td>
<td>RT-PCR = Positive</td>
<td>IgM = Negative</td>
</tr>
<tr>
<td></td>
<td>Isolation = Positive</td>
<td>PRNT = Negative</td>
</tr>
<tr>
<td>Day 4-8</td>
<td>RT-PCR = Positive</td>
<td>IgM = Positive</td>
</tr>
<tr>
<td></td>
<td>Isolation = Negative</td>
<td>PRNT = Negative</td>
</tr>
<tr>
<td>&gt;Day 8</td>
<td>RT-PCR = Negative</td>
<td>IgM = Positive</td>
</tr>
<tr>
<td></td>
<td>Isolation = Negative</td>
<td>PRNT = Positive</td>
</tr>
</tbody>
</table>

- Confirming a recent CHIKV infection:
  - Isolation of CHIKV, confirmatory identification (IFA, RTPCR, sequencing).
  - Detection of CHIKV RNA by real time RT-PCR.
  - Identification of a positive IgM result + acute symptoms, followed by the demonstration of CHIKV-specific antibody determined by PRNT with viruses in the SFV serogroup.
  - Seroconversion or a four-fold rise in PRNT, HI, or ELISA titers
Treatment

• No specific antiviral therapy; treatment is symptomatic
• Assess hemodynamic status and provide supportive care
• Evaluate for other serious conditions and treat appropriately
• Collect specimens for diagnostic testing
• Acetaminophen or paracetamol for initial fever and pain
• Consider using narcotics or NSAIDs
• If the patient may have dengue, do not use aspirin or other NSAIDs (e.g., ibuprofen, naproxen, toradol) until they have been afebrile ≥48 hours and have no warning signs for severe dengue
• 50 yo Indian male presents with complaints of 3 days of fever, headache, fatigue, rash, and severe joint pains which started 2 days after returning from a Caribbean cruise. You diagnose an acute chikungunya infection and treat with reassurance, rest, and NSAIDS. You see him again in 2 days and he is no longer febrile and feeling overall improved. You recommend continued rest and gradual return to normal ADLs. In 6 months the patient calls you complaining of persistent hand pain and limited ROM. He states his fingers feel swollen. The condition is impacting his quality of life and he requests a prescription for narcotics. He asks if “chik” can be chronic.

• What is the most accurate response?
  – 1. No, it is an acute infection like dengue, this is a new process
  – 2. Yes, but symptoms usually resolve in <3 months
  – 3. Yes, but not manifesting with joint problems
  – 4. Yes, long term (years) joint and tendon issues have been reported
## Atypical Clinical Manifestations

<table>
<thead>
<tr>
<th>System</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological</td>
<td>Meningoencephalitis, encephalopathy, seizures, Guillain-Barré syndrome, cerebellar syndrome, paresis, palsies, neuropathy</td>
</tr>
<tr>
<td>Ocular</td>
<td>Optic neuritis, iridocyclitis, episcleritis, retinitis, uveitis</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Myocarditis, pericarditis, heart failure, arrhythmias, hemodynamic instability</td>
</tr>
<tr>
<td>Dermatological</td>
<td>Photosensitive hyperpigmentation, intertriginous aphthous-like ulcers, vesiculobullous dermatosis</td>
</tr>
<tr>
<td>Renal</td>
<td>Nephritis, acute renal failure</td>
</tr>
<tr>
<td>Other</td>
<td>Bleeding dyscrasias, pneumonia, respiratory failure, hepatitis, pancreatitis, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), hypoadrenalism</td>
</tr>
</tbody>
</table>

Adapted from Rajapakse et al. \(^{20}\)
Persistent Chikungunya

Three clinical components, singly / in combination:

1. Distal polyarthritis / monoarthritis improved with NSAIDs;
2. Frequent tenosynovitides in the hands, wrists, or ankles, sometimes responsible for carpal or tarsal tunnel syndromes, highly sensitive to short-term systemic corticotherapy; and
3. Exacerbation of pain in previously injured joints and bones requiring painkillers.
Persistent Chikungunya
Persistent Chikungunya

Calcifications in shoulder tendon 18 months after infection

Inflammatory osteoarthritis, foot, 5 years after infection
A 43 yo male is traveling on business throughout Southeast Asia. He will be traveling for 3 weeks (August) staying in hotels located in major urban centers. Tourist activities are planned in rural areas in Northern Thailand, Viet Nam and Cambodia. Activities will include elephant treks, river rafting, and camping. He inquires about a vaccine to prevent a “brain infection” you get from mosquitoes.

What is your guidance regarding Japanese encephalitis risk?
- 1. Moderate risk, especially in rural areas with mosquitoes.
- 2. Low risk, he is traveling during the low season for JE.
- 3. Moderate risk, especially in urban areas with mosquitoes.
- 4. Low risk, there is no JE in the areas he is traveling.
Japanese Encephalitis

• Virus
  – Family Flaviviridae, Genus Flavivirus
• Most common viral encephalitis etiology worldwide
  – 160,000 cases 1966, 16,000 in 1996 (vaccination)
  – Annual estimate is ≥ 50K cases (2.5 cases/10,000 pop.)
• Risk of JE following infection
  – ~1/200 in indigenous pop.; ~1/25-50 in foreign born
• Primarily a disease of children
  – Naïve adults at similar risk (i.e., travelers, military)
• Disease has high mortality, morbidity
  – CFR ~25 -30%
  – Long-term neuropsychiatric disability 45 - 50%
Transmission

• Vector: *Culex* mosquitoes
  – *Culex tritaeniorhynchos*
  • Breeds in marshes, rice paddies
  • Night-biting

• Zoonotic amplification
  – Domestic pigs
  – Migratory waterfowl

• Seasonal/climate factors
  – Summertime / post-rainy season
  • Increased vector number
  • Increased feeding behaviors
  • Increased viral replication
Japanese Encephalitis
Clinical Findings

• Incubation period 4 – 14 days
• Earliest symptoms
  – Lethargy, fever, headache, abdominal pain, N & V
• Over several days
  – Lethargy >, agitated delirium, unsteadiness, and abnormal motor movements → somnolence / coma
• Some have sudden convulsion after a brief febrile illness
Clinical Findings

• High fever and altered consciousness
  – Mild disorientation, subtle personality change, severe state of confusion, delirium, and coma
• Nuchal rigidity is a variable finding (1/3 – 2/3 of cases)
• CN palsies, facial paralysis, disconjugate gaze (1/3 of cases)
• Muscular weakness
  – Distributions of flaccid / spastic paralysis
  – Hemiparesis
  – Increased or decreased tone
  – Generalized or asymmetrical
Clinical Findings

• Hyperreflexia, ankle clonus, and other abnormal reflexes

• Disordered movements
  – Flailing, ataxia, tremor, choreoathetosis, rigidity, masked facies, and other extrapyramidal signs

• Seizures
  – Focal or generalized
  – 85% peds
  – 10% adults
Lab and Imaging

• Peripheral leukocytosis, up to 30k/mm³ with a left shift
• Hyponatremia
• CSF
  – Opening Pressure > in ~ 50%
  – Pleocytosis <10 to several thousand cells (lymphocytic)
  – Protein may be normal or elevated up to 100 mg/dL
• EEG
  – Diffuse slow waves (theta or delta), seizure activity, periodic lateralized epileptiform discharges (PLEDS)
• EMG
  – Chronic partial denervation, anterior horn cell destruction
Lab and Imaging

- Imaging
  - Diffuse white matter edema and abnormal signals
  - Thalamus, basal ganglia, cerebellum, midbrain, pons, cord

A, Coronal T2-weighted image, bilateral hippocampal body involvement (black arrows). Note bilateral thalamic and substantia nigra involvement (white arrows).

B, Axial T2-weighted image shows bilateral hippocampal tail involvement (arrows).

C, Axial CT scan done at the same time as A and B shows hypoattenuated left mesial temporal lobe lesion. Note resemblance to Herpes simplex virus encephalitis.

Question

• A 43 yo male is traveling on business throughout Southeast Asia. He will be traveling for 3 weeks (August) staying in hotels located in major urban centers. Tourist activities are planned in rural areas in Northern Thailand, Viet Nam and Cambodia. Activities will include elephant treks, river rafting, and camping. You explain the risk of JE is moderate and believe he should consider vaccination. He produces his medical record indicating he received 2 doses of a JE vaccine 5 years ago. He asks if he needs to repeat the entire vaccination series?

• What is your guidance?
  - 1. Two doses is sufficient, he does not need to be re-vaccinated
  - 2. Two doses was not sufficient, he needs to start the series again, three doses over a 28 day period and use PPMs in country
  - 3. Two doses was not sufficient, he needs to start a series again with two doses over a 28 day period and use PPMs in country
  - 4. There is no US FDA approved JE vaccine, he is limited to PPM
Prevention

• Vector control – difficult in endemic regions
  – Twilight-biting, marsh-breeding mosquitoes
  – Impractical, historically ineffective

• Reservoir control – difficult in endemic regions
  – Swine
    • Segregation impractical
    • Vaccination expensive

• Vaccination
  – Mass pediatric vaccinations
JE Vaccines

- Live, attenuated SA 14-14-2 vaccine
  - Produced and used successfully in China for > 20 years

- Inactivated vero-cell derived JEV (Biken, Kaketsuken)
  - Inactivated Beijing JEV grown on Vero cells

- ChimeriVax-JE vaccine (Acambis/sanofi pasteur)
  - Live, recombinant vaccine (based on Yellow Fever 17D)

- Ixiaro (Intercell/Novartis/Biological E)
  - Inactivated vaccine
  - Derived from SA-14-14-2 JEV cultured in Vero cells
  - 2 doses at day 0 and day 28
  - Licensed in US for adult use 2009
  - US licensure for pediatric use, May 2013
IXIARO®
Manufacturer’s FDA-approved labeling

• Indication: 2 months of age and older
• Dosing
  – 0.5 mL single dose syringes
  – 2 months to <3 years of age, a single dose is 0.25 mL
  – 3 years of age and older, a single dose is 0.5 mL
• Contraindications
  – Severe allergic reaction (e.g, anaphylaxis) after a previous dose of IXIARO, any other JE vaccine, or any component of IXIARO, including protamine sulfate
• Adverse Events
  – Injection-site pain (15%), redness (15%), fever (>10-20%), irritability (>15%), diarrhea (>10%), headache (>20%) and myalgia (>10%)
### IMMUNIZATION SERIES
Complete the primary immunization series at least 1 week prior to potential exposure to JEV.

<table>
<thead>
<tr>
<th>2 MONTHS TO &lt;3 YEARS</th>
<th>2 MONTHS TO &lt;17 YEARS</th>
<th>2 TO 11 MONTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary immunization with IXIARO consists of two (2) 0.25 mL doses, administered 28 days apart.</td>
<td>The safety and immunogenicity of a booster dose has not been evaluated.</td>
<td>The anterolateral aspect of the thigh</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>≥3 YEARS</th>
<th>≥17 YEARS</th>
<th>1 TO &lt;3 YEARS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary immunization with IXIARO consists of two (2) 0.5 mL doses, administered 28 days apart.</td>
<td>If the primary series of two doses was completed more than 1 year previously, a booster dose may be given if ongoing exposure or re-exposure to JEV is expected.</td>
<td>The anterolateral aspect of the thigh (or the deltoid muscle if muscle mass is adequate)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>≥3 YEARS</th>
</tr>
</thead>
<tbody>
<tr>
<td>The deltoid muscle</td>
</tr>
</tbody>
</table>

https://www.novartisvaccinesdirect.com/Ixiaro/IxiaroDosingAdministration
Table 6. Rates of Solicited Adverse Reactions on Days 0-7 After Each IXIARO 0.5 mL Vaccination in Children 3 Years to <18 Years of Age Traveling From Western Countries, Study 2

<table>
<thead>
<tr>
<th>Injection Site Reactions</th>
<th>Post Dose 1 (N=55†)</th>
<th>Post Dose 2 (N=49‡)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of subjects</td>
<td>% of subjects</td>
</tr>
<tr>
<td>Pain</td>
<td>18.2</td>
<td>16.3</td>
</tr>
<tr>
<td>Itching</td>
<td>3.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Tenderness</td>
<td>30.9</td>
<td>24.5</td>
</tr>
<tr>
<td>Hardening</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Swelling</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Redness</td>
<td>5.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Solicited Systemic Reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td>0.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Nausea</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Flu-like symptoms</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Excessive fatigue</td>
<td>12.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>27.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Rash</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Headache</td>
<td>1.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Fever ≥37.7°C (≥99.9°F)</td>
<td>5.5</td>
<td>2.0</td>
</tr>
<tr>
<td>37.7-38.6°C (99.9-101.5°F)</td>
<td>3.6</td>
<td>2.0</td>
</tr>
<tr>
<td>38.7-39.3°C (101.6-102.7°F)</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>39.4-40.5°C (102.8-104.9°F)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>&gt;40.5°C (&gt;104.9°F)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

†NCT01047839
‡N=number of subjects with available diary card data after each dose, used as the denominator to calculate percentages.
Table 7. Rates of Common Solicited and Unsolicited Systemic Adverse Events* in Adults Residing in Non-Endemic Areas After IXIARO or Control [PBS + Al(OH)₃], Safety Population, Study 4†

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Post Dose 1 (Day 0 to Day 28)</th>
<th>Post Dose 2 (Day 28 to Day 56)</th>
<th>Post Dose 1 or Dose 2 (Day 0 to Day 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of subjects</td>
<td>% of subjects</td>
<td>% of subjects</td>
</tr>
<tr>
<td>IXIARO N=1993</td>
<td>PBS + Al(OH)₃ N=657</td>
<td>IXIARO N=1968</td>
<td>PBS + Al(OH)₃ N=645</td>
</tr>
<tr>
<td>Headache†</td>
<td>21.6</td>
<td>20.2</td>
<td>13.4</td>
</tr>
<tr>
<td>Myalgia†</td>
<td>13.3</td>
<td>12.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Fatigue†</td>
<td>8.6</td>
<td>8.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Influenza-like Illness†</td>
<td>8.2</td>
<td>8.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Nausea†</td>
<td>4.7</td>
<td>5.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>2.3</td>
<td>1.8</td>
<td>2.6</td>
</tr>
<tr>
<td>Fever†</td>
<td>1.9</td>
<td>2.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>1.0</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Upper Respiratory Tract Infection</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Back Pain</td>
<td>0.8</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Pharyngolaryngeal Pain</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Rash†</td>
<td>0.8</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Cough</td>
<td>0.8</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Vomiting†</td>
<td>0.6</td>
<td>0.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*NCT00605085
The adverse events in this table are those observed at an incidence of ≥1% in the IXIARO or PBS + Al(OH)₃ groups.
† These symptoms were solicited in a subject diary card. Percentages also include unsolicited events that occurred after the 7 day period covered by the diary card.
‡ N=number of subjects in the safety population (subjects treated with at least one dose) who received the respective dose
Table 9. JEV-Neutralizing Antibody Response After IXIARO® Among Children 2 Months to <18 Years of Age Residing in the Philippines, Intent-To-Treat Population**, Study 1$^€$

<table>
<thead>
<tr>
<th>Age Group</th>
<th>2 months – &lt;6 months</th>
<th>6 months – &lt;12 months</th>
<th>1 year – &lt;3 years</th>
<th>3 years – &lt;12 years</th>
<th>12 years – &lt;18 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Point</td>
<td>Proportion of Subjects with PRNT$_{50}$ Titer $\geq$1:10 (n/N) [95% CI]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Vaccination Screen</td>
<td>30% (3/10) [10.8, 60.3]</td>
<td>0% (0/20) [0.0, 16.1]</td>
<td>3.2% (4/125) [1.3, 7.9]</td>
<td>16.8% (17/101) [10.8, 25.3]</td>
<td>45.7% (64/140) [37.7, 54.0]</td>
</tr>
<tr>
<td>Day 56 (28 days after vaccine dose 2)</td>
<td>100% (9/9) [70.1, 100.0]</td>
<td>100% (19/19) [83.2, 100.0]</td>
<td>99.2% (119/120) [95.4, 99.9]</td>
<td>100% (100/100) [96.3, 100.0]</td>
<td>100% (137/137) [97.3, 100.0]</td>
</tr>
<tr>
<td>Month 7 (6 months after vaccine dose 2)</td>
<td>100% (10/10) [72.2, 100.0]</td>
<td>100% (18/18) [82.4, 100.0]</td>
<td>85.5% (106/124) [78.2, 90.6]</td>
<td>91.0% (91/100) [83.8, 95.2]</td>
<td>97.1% (133/137) [92.7, 98.9]</td>
</tr>
<tr>
<td>Time Point</td>
<td>Geometric Mean Titers (N) [95% CI]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Vaccination Screen</td>
<td>8.4 (10) [4.3, 16.7]</td>
<td>5.0 (20) [5.0, 5.0]</td>
<td>5.5 (124) [5.0, 6.1]</td>
<td>6.5 (101) [5.8, 7.4]</td>
<td>13.1 (140) [10.7, 16.1]</td>
</tr>
<tr>
<td>Day 56 (28 days after vaccine dose 2)</td>
<td>687.4 (9) [263.2, 1795.1]</td>
<td>377.8 (19) [210.3, 678.8]</td>
<td>258.9 (121) [214.4, 312.6]</td>
<td>213.7 (100) [175.6, 260.0]</td>
<td>175.6 (137) [147.8, 208.7]</td>
</tr>
<tr>
<td>Month 7 (6 months after vaccine dose 2)</td>
<td>159.3 (10) [110.0, 230.7]</td>
<td>64.0 (18) [39.4, 104.1]</td>
<td>38.9 (125) [31.8, 47.7]</td>
<td>43.6 (100) [35.6, 53.4]</td>
<td>86.6 (137) [70.7, 106.0]</td>
</tr>
</tbody>
</table>

$^€$NCT01041573
*Infants and children $\geq$ 2 months to <3 years of age received two 0.25 mL doses administered on Days 0 and 28. Individuals 3 years of age and older received two 0.5 mL doses administered on Days 0 and 28.
**The Intent to Treat population consisted of all subjects who received at least one dose of IXIARO.
N=number of subjects with data available
n=number of subjects with a PRNT$_{50}$ titer $\geq$1:10
$\circ$Reciprocal titers <10 were imputed to 5.
### Table 10. JEV-Neutralizing Antibody Response After IXIARO or JE-VAX Among Adults Residing in Non-Endemic Areas, Per Protocol Population*, Study 4$^8$

<table>
<thead>
<tr>
<th>Time Point</th>
<th>IXIARO (n/N) [95% CI]</th>
<th>JE-VAX (n/N) [95% CI]</th>
<th>Rate difference [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 56 (28 days after vaccine dose 2)</td>
<td>96.4% (352/365) [94.0, 97.9]</td>
<td>93.8% (347/370) [90.9, 95.8]</td>
<td>2.6% [-0.5, 6.0]$^+$</td>
</tr>
</tbody>
</table>

**Geometric Mean Titers**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>IXIARO (N**=361) [95% CI]</th>
<th>JE-VAX (N**=364) [95% CI]</th>
<th>GMT ratio [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 56 (28 days after vaccine dose 2)</td>
<td>243.6 [216.4, 274.1]</td>
<td>102.0 [90.3, 115.2]</td>
<td>2.33 [1.97, 2.75]$^+$</td>
</tr>
</tbody>
</table>

$^*$NCT00604708

*The Per Protocol population consisted of subjects with no major protocol deviations and a PRNT50 titer <1:10 at baseline

$^+$ Non-Inferiority was demonstrated if the lower bound of the 2-sided 95% confidence interval (CI) for the difference in proportion of subjects with PRNT50 titer $\geq$1:10 (IXIARO minus JE-VAX) was $>\text{-}10$% at Day 56.

$^+$ Non-Inferiority was demonstrated if the lower bound of the 2-sided 95% CI for the GMT ratio (IXIARO /JE-VAX) was $>1/1.5$ at Day 56.

n=number of subjects with a PRNT50 titer $\geq$1:10

N=number of subjects in the Per Protocol Population

**N=Number of subjects with immunogenicity data

$\diamond$ Reciprocal titers $<10$ were imputed to 5.

### Table 11. JEV-Neutralizing Antibody Response During the Vaccination Series (IXIARO on Days 0 and 28) Among Adults Residing in Non-Endemic Areas, Per Protocol Population*, Study 6$^6$

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Proportion of Subjects with PRNT50 Titer $\geq$1:10 (n/N) [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10 (10 days after vaccine dose 1)</td>
<td>21.1% (24/114) [13.6%; 28.5%]</td>
</tr>
<tr>
<td>Day 28 (28 days after vaccine dose 1)</td>
<td>39.8% (45/113) [30.8%; 48.8%]</td>
</tr>
<tr>
<td>Day 35 (7 days after vaccine dose 2)</td>
<td>97.3% (110/113) [94.4%; 100.0%]</td>
</tr>
<tr>
<td>Day 56 (28 days after vaccine dose 2)</td>
<td>97.3% (110/113) [94.4%, 100%]</td>
</tr>
</tbody>
</table>

$^*$NCT00596271

*The Per Protocol population consisted of all subjects with no major protocol deviations

n=number of subjects with a PRNT50 titer $\geq$1:10

N=number of subjects with immunogenicity data

<table>
<thead>
<tr>
<th>Time Point</th>
<th>% PRNT Titer ≥1:10 (n/N) [95% CI]</th>
<th>Geometric Mean Titers (N) [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-booster, Day 0</td>
<td>69.2% (137/198) [62.4%, 75.2%]</td>
<td>22.5 (198) [19.0, 26.7]</td>
</tr>
<tr>
<td>Day 28</td>
<td>100.0% (198/198) [98.1%, 100.0%]</td>
<td>900.1 (198) [742.4, 1091.3]</td>
</tr>
<tr>
<td>Month 6</td>
<td>98.5% (194/197) [95.6%, 99.5%]</td>
<td>487.4 (197) [390.7, 608.1]</td>
</tr>
<tr>
<td>Month 12</td>
<td>98.5% (191/194) [95.6%, 99.5%]</td>
<td>361.4 (194) [294.5, 443.5]</td>
</tr>
</tbody>
</table>

*The Intent to Treat population consisted of all subjects who received the booster vaccination
n=number of subjects with a PRNT50 titer ≥1:10
N=number of subjects with immunogenicity data

---

**Table 12. JEV-Neutralizing Antibody Response Following a Booster Dose of IIXIARO Administered 14 Months After Completion of the Primary Series Among Adults Residing in Non-Endemic Areas, Intent to Treat Population*, Study 5**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Response Rate After 1 Dose of JE-VC(^a)</th>
<th>Protection Rate After 1 Dose of JE-VC(^b)</th>
<th>Geometric Mean Titers After 1 Dose of JE-VC(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonprimed</td>
<td>Primed</td>
<td>Nonprimed</td>
</tr>
<tr>
<td>PRNT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakayama</td>
<td>39% (10/26)</td>
<td>98% (41/42)</td>
<td>40% (10/25)</td>
</tr>
<tr>
<td>SA14-14-2</td>
<td>42% (11/26)</td>
<td>95% (40/42)</td>
<td>40% (10/25)</td>
</tr>
</tbody>
</table>
Questions?