



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



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**Walter Reed Army
Institute of Research**

**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

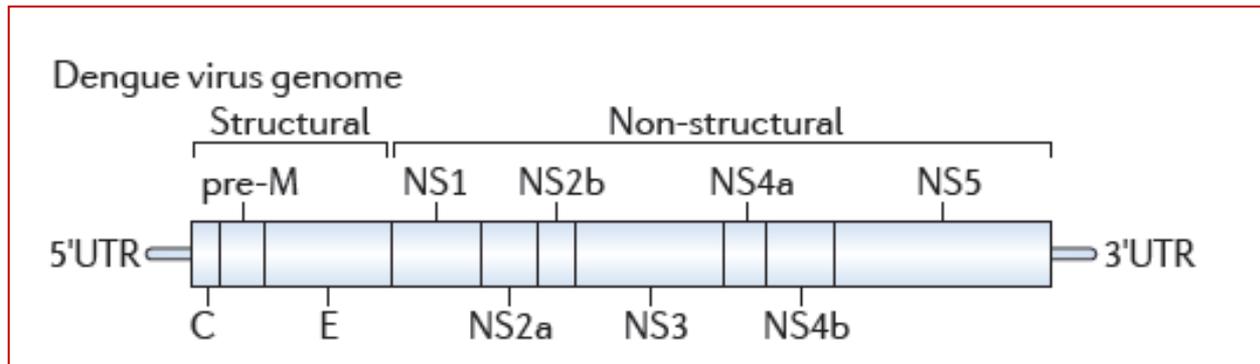
- 18 year old native from Thailand (moved to US at 10 years of age) presents in August with 2 days of illness including fever, headache, bone pain, and nausea 3 days after returning from a vacation in Puerto Rico. He vacationed in San Juan, stayed in a hotel, ate and drank local foods and beverages from 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



Is dengue a threat to the blood supply?

Transfusion Medicine, 2009, 19, 66–77

D. Teo,*¹ L. C. Ng†¹ & S. Lam* *Blood Services Group, Health Sciences Authority, and †Environmental Health Institute, National Environment Agency, Singapore

• Transmission

- Feeding vector
- Laboratory acquired
- Blood supply?
- Organ donation?

• Vector

- *Aedes aegypti*
- *Aedes albopictus*



Table 3. Dengue and donor deferral

Country	Donor deferral measures for dengue
Singapore*	6 months deferral for history of dengue infection 3 weeks deferral for history of fever No travel-related deferral for dengue
Hong Kong*	6 months deferral for history of dengue infection 2 weeks deferral for history of fever No travel-related deferral for dengue
Sri Lanka*	No specific deferral for history of dengue infection 2 weeks deferral for history of fever No travel-related deferral for dengue
Australia†	4 weeks deferral for history of dengue infection No travel-related deferral for dengue
New Zealand‡	4 weeks deferral for history of dengue infection No travel-related deferral for dengue
UK‡	2 weeks deferral for history of dengue infection No travel-related deferral for dengue
United States‡	4 weeks deferral for history of dengue infection No travel-related deferral for dengue

*Endemic for dengue.

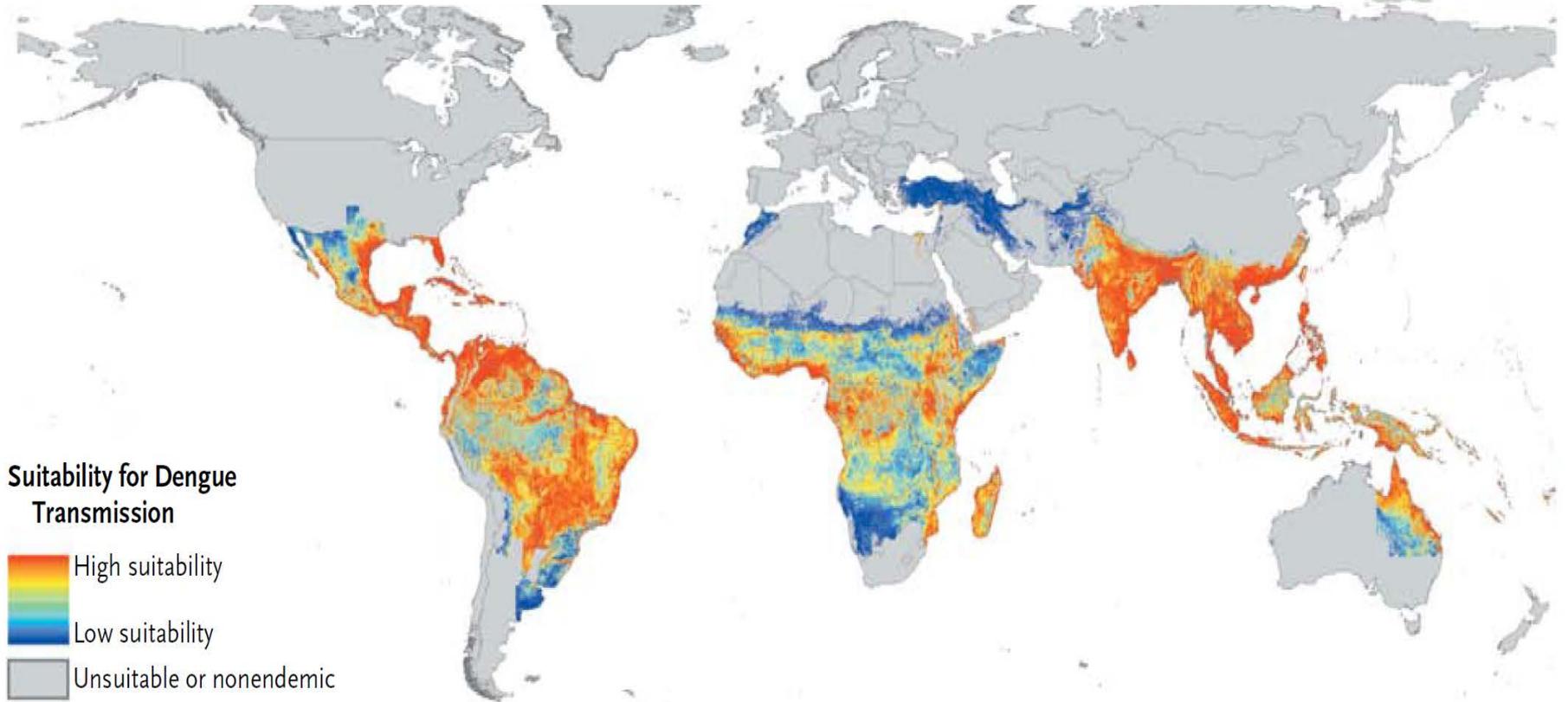
†Non-endemic except parts of Northern Australia.

‡Non-endemic.

Dengue

N Engl J Med 2012;366:1423-32.

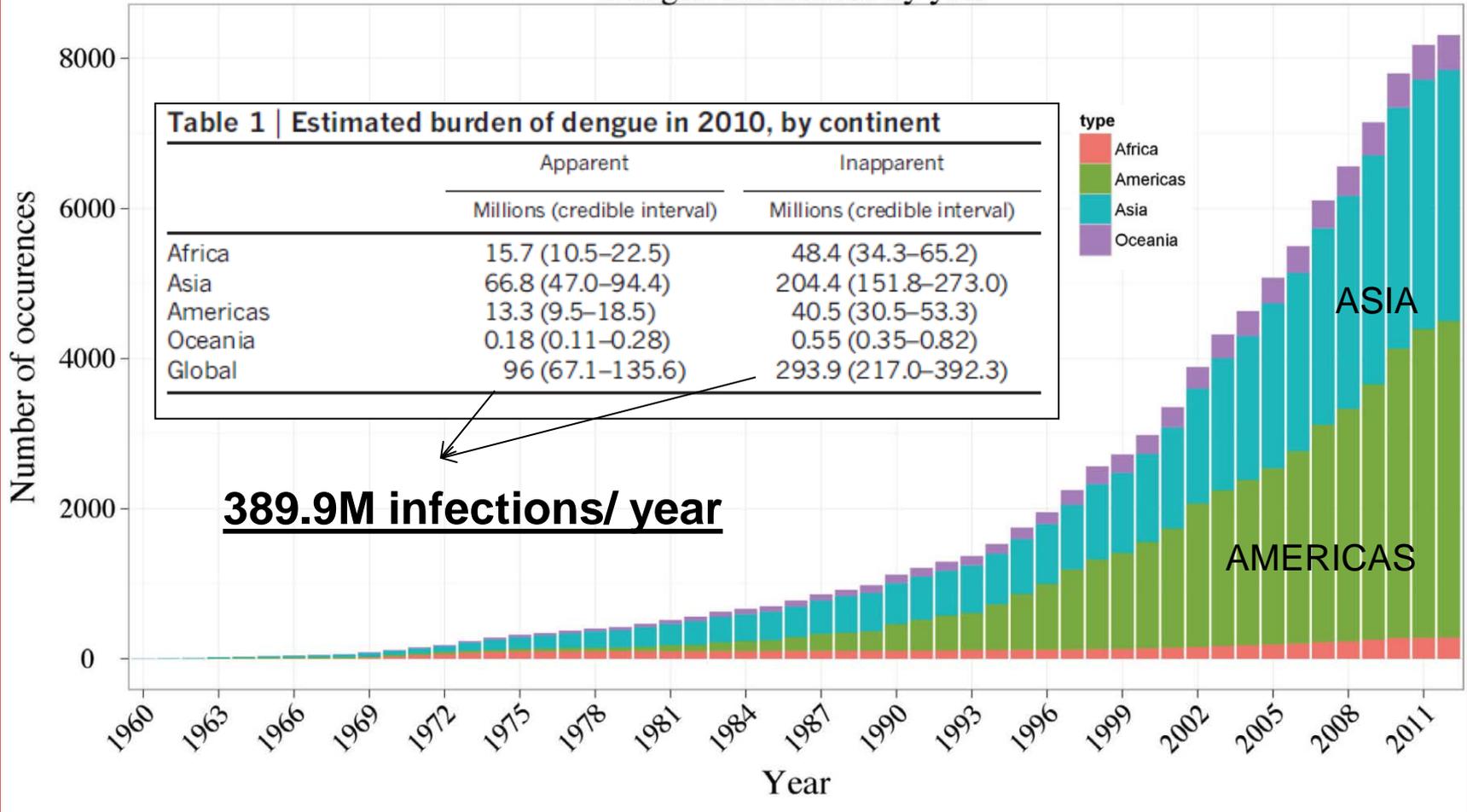
Areas supporting dengue virus transmission.



Dengue Burden

Under-estimated and under-reported

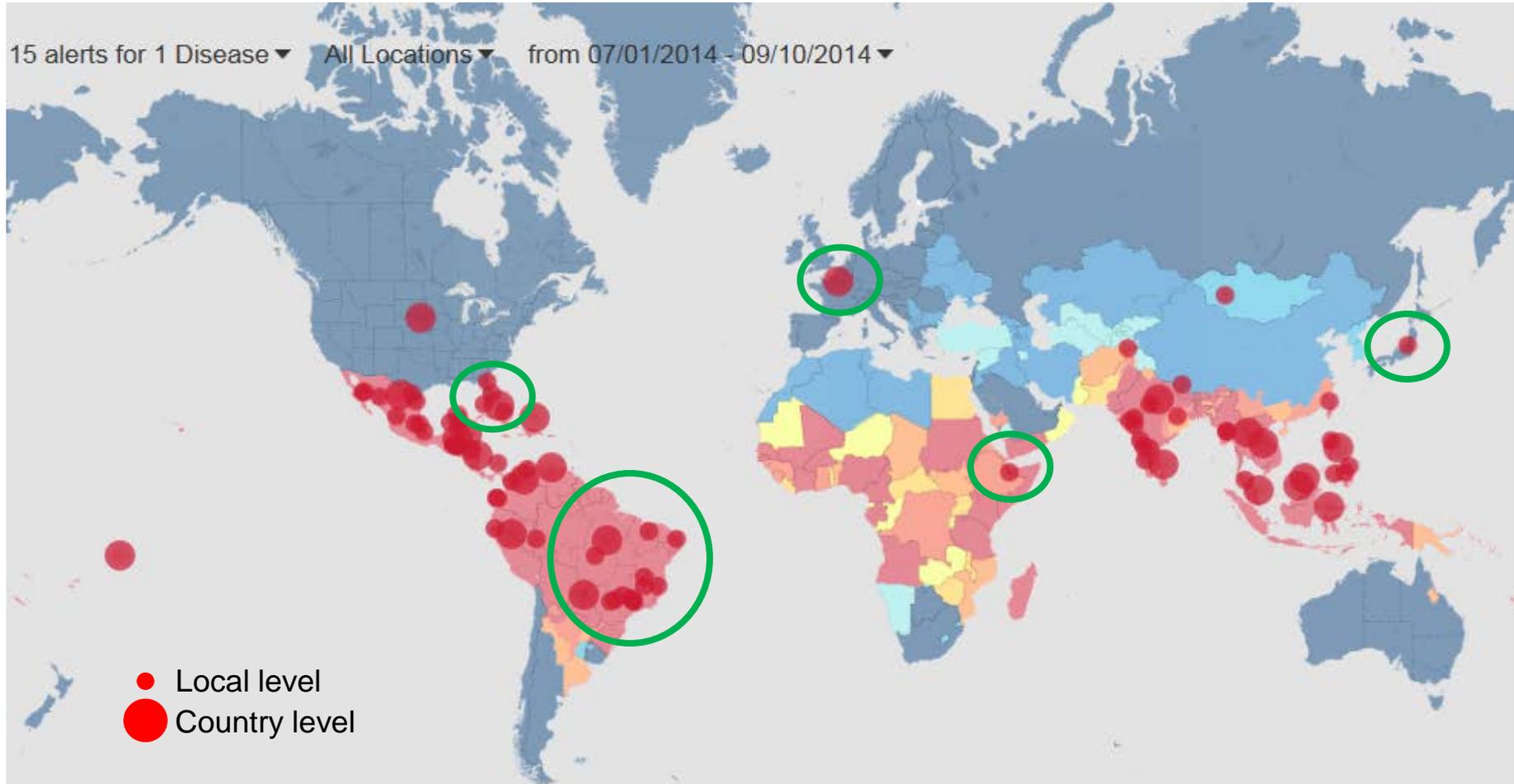
Dengue occurrences by year



Bhatt et al., Nature. 2013 Apr 7.



CDC Dengue Map – 1 JUL – 10 SEP 2014



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

Paul V. Effler,* Lorrin Pang,* Paul Kitsutani,† Vance Vorndam,† Michele Nakata,* Tracy Ayers,*
Joe Elm,* Tammy Tom,* Paul Reiter,† José G. Rigau-Perez,† John M. Hayes,† Kristin Mills,*
Mike Napier,‡ Gary G. Clark,† and Duane J. Gubler*
for the Hawaii Dengue Outbreak Investigation Team¹

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

VECTOR-BORNE AND ZOOONOTIC DISEASES
Volume 13, Number 0, 2013
© Mary Ann Liebert, Inc.
DOI: 10.1089/vbz.2013.1413

Centers for Disease Control and Prevention

MMWR

Morbidity and Mortality Weekly Report

Weekly / Vol. 59 / No. 19

May 21, 2010

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



ONE
SQUARE
METER

in association with



8 dengue cases detected in Florida

By **Joe Sutton** and **Catherine E. Shoichet**, CNN
August 28, 2013 – Updated 1407 GMT (2207 HKT)

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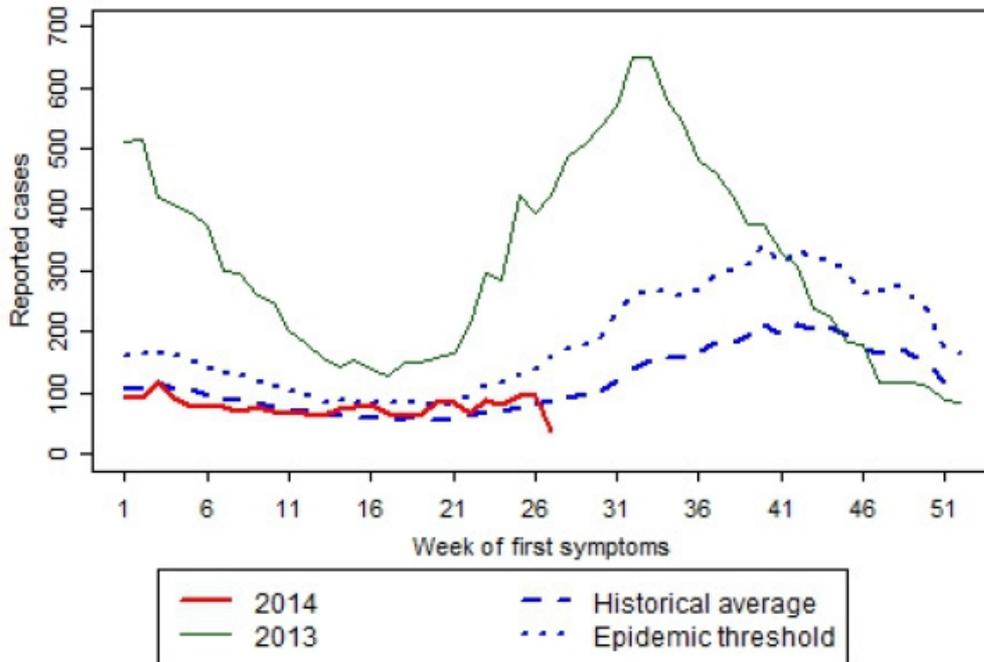
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- Email
- More sharing

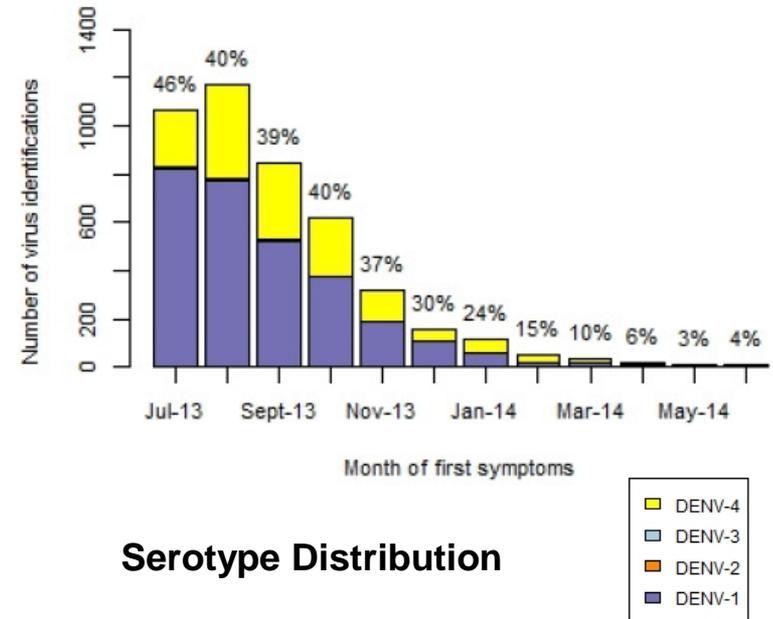


Puerto Rico

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



Spectrum of Disease and Relation to Place of Exposure among Ill Returned Travelers

David O. Freedman, M.D., Leisa H. Weld, Ph.D., Phyllis E. Kozarsky, M.D., Tamara Fisk, M.D.,* Rachel Robins, M.D., Frank von Sonnenburg, M.D., Jay S. Keystone, M.D., Prativa Pandey, M.D., and Martin S. Cetron, M.D., for the GeoSentinel Surveillance Network†

Table 3. Etiologic Diagnoses within Selected Syndrome Groups, According to Travel Region.*

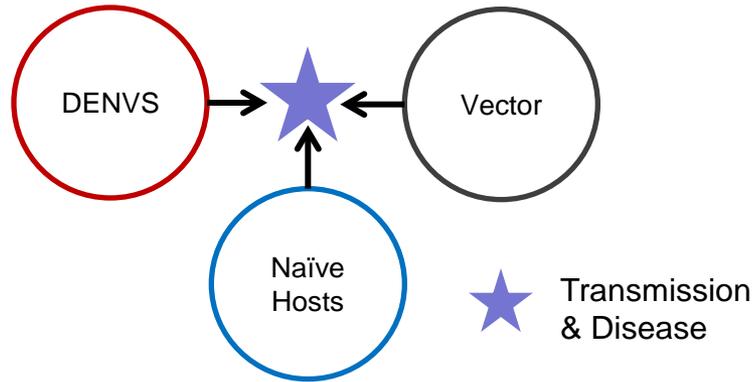
Syndrome and Cause	All Regions	Caribbean	Central America	South America	Sub-Saharan Africa	South Central Asia	Southeast Asia	Other or Multiple Regions†
			number of cases per 1000 patients with syndrome					
Systemic febrile illness (n= 3907)								
Specific pathogen or cause reported‡	594	459	527	446	718	522	547	454
Malaria‡	352	65	133	133	622	139	130	234
Dengue‡	104	238	123	138	7	142	315	35
Mononucleosis (due to Epstein-Barr virus or cytomegalovirus)‡	32	70	69	79	10	17	32	63
Rickettsial infection‡	31	0	0	0	56	10	16	24
Salmonella typhi or S. paratyphi infection‡	29	22	25	17	7	141	26	24
No specific cause reported‡	406	541	473	554	282	478	453	546

‡ P<0.01 for the comparison among regions.

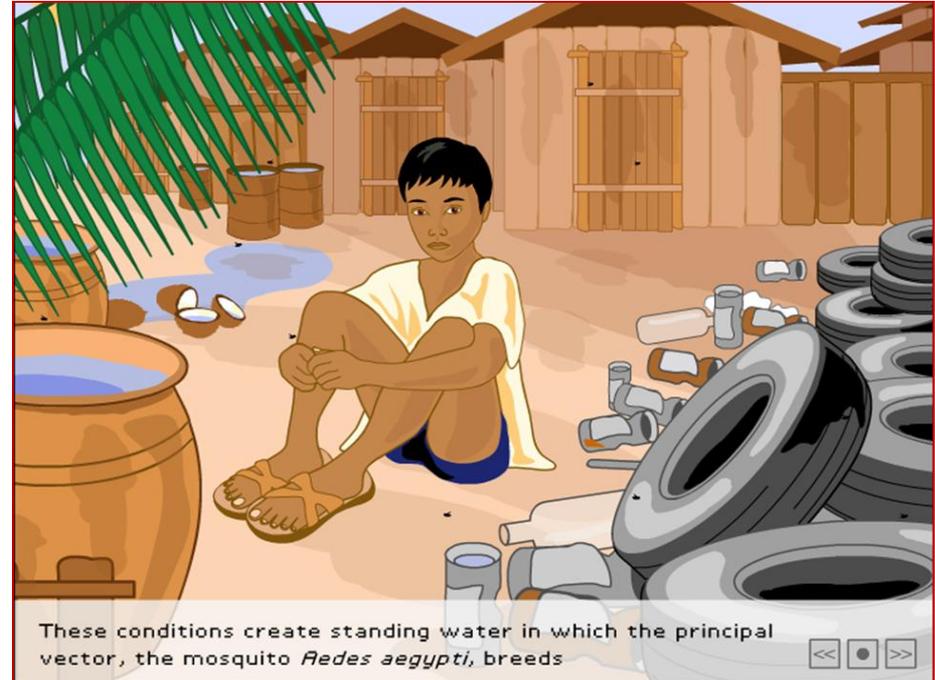
*“With respect to specific diagnoses, malaria was one of the three most frequent causes of systemic febrile illness among travelers from every region, **although travelers from every region except sub-Saharan Africa and Central America had confirmed or probable dengue more frequently than malaria.**”*



Factors Driving Transmission



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

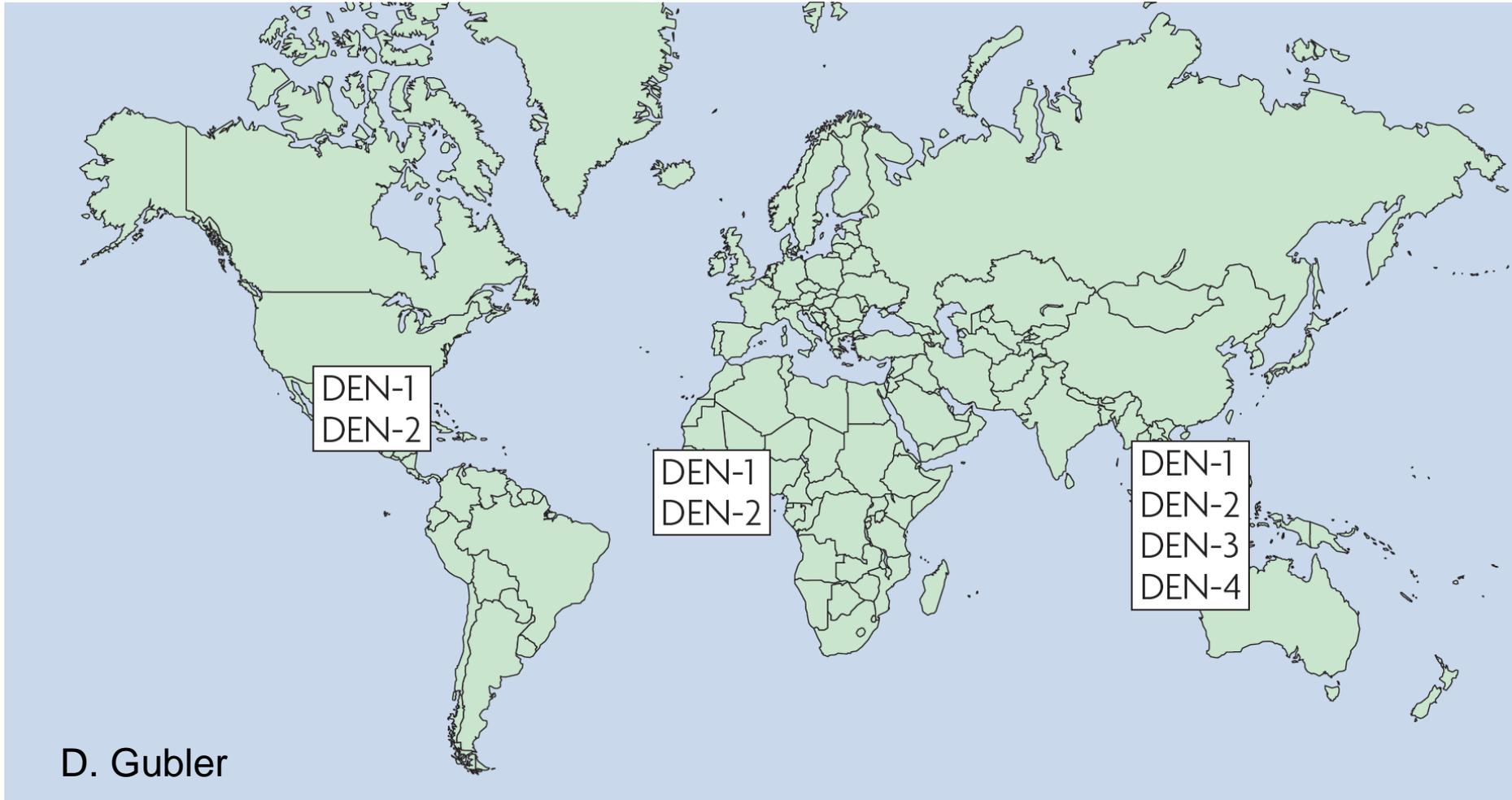


Factors Driving Disease

- 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



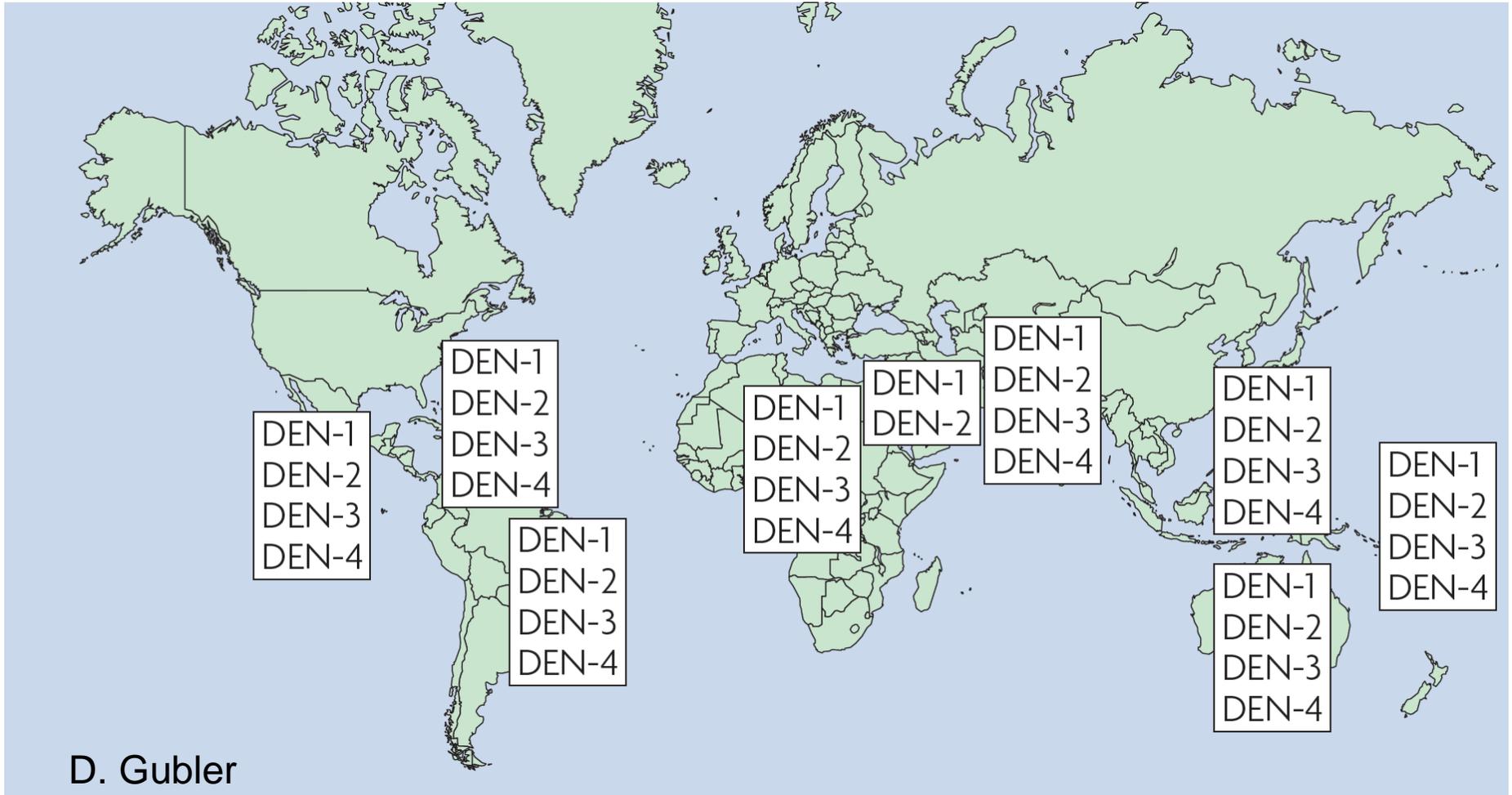
Global Air Travel Flight Patterns



<http://upload.wikimedia.org/wikipedia/commons/a/ac/World-airline-routemap-2009.png>



DENV Type Distribution - 2004



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 (102.5F) 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

GUIDELINES FOR DIAGNOSIS,
TREATMENT, PREVENTION AND CONTROL



New edition
2009

TDR

For research on
diseases of poverty
UNICEF • UNDP • World Bank • WHO

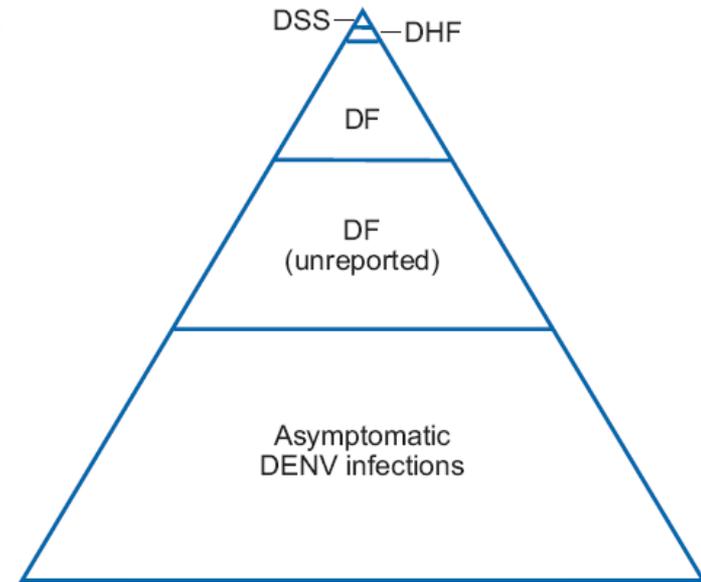


World Health
Organization

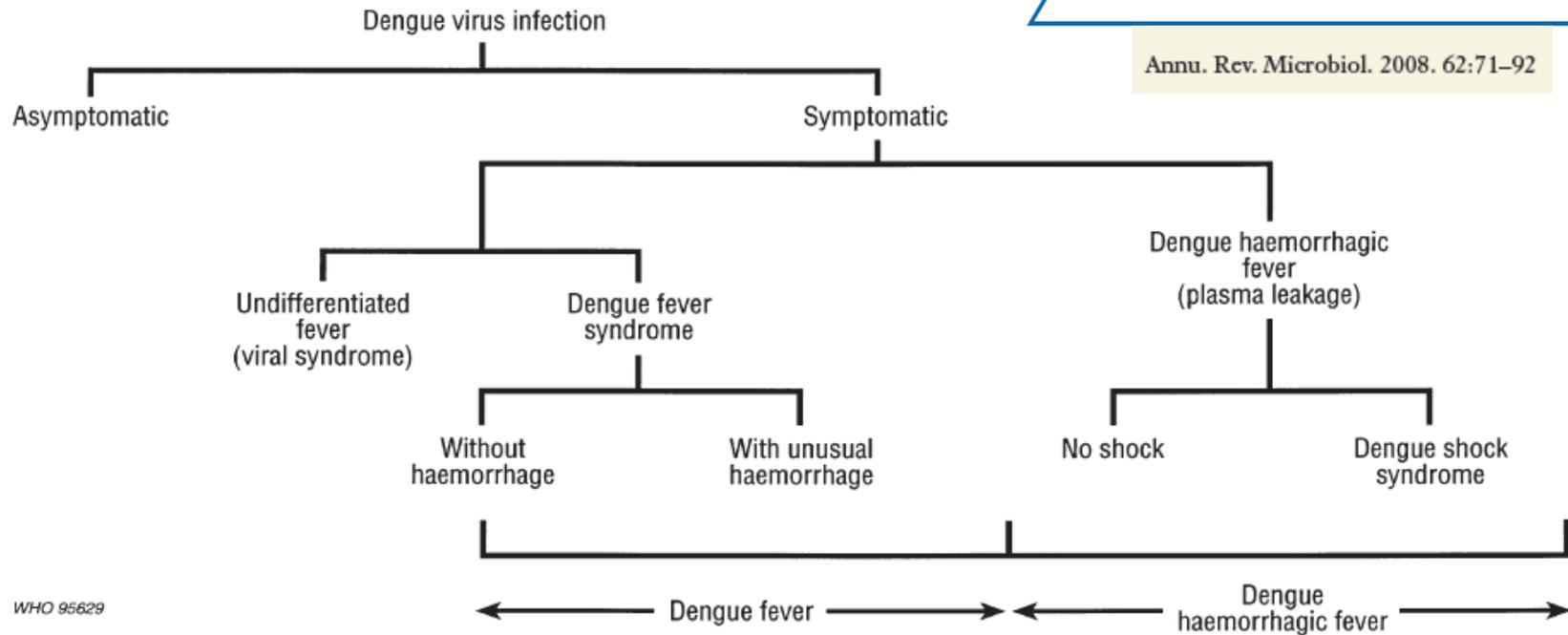
http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf



Dengue Infection Clinical Phenotypes



Annu. Rev. Microbiol. 2008. 62:71-92



WHO 95629



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 1

1997 World Health Organization (WHO) case definition for dengue hemorrhagic fever and dengue shock syndrome*

DHF, the following must all be present:

Fever, or history of acute fever, lasting 2–7 days, occasionally biphasic

Hemorrhagic tendencies, evidenced by at least one of the following:

A positive tourniquet test

Petechiae, ecchymoses, or purpura

Bleeding from the mucosa, gastrointestinal tract, injection sites, or other locations

Hematemesis or melaena

Thrombocytopenia (100,000 cells/mm³ or less)

Evidence of plasma leakage caused by increased vascular permeability, manifested by at least one of the following:

A rise in the hematocrit equal to or > 20% above average for age, sex, and population

A drop in the hematocrit following volume replacement treatment equal to or > 20% of baseline

Signs of plasma leakage such as pleural effusion, ascites, and hypoproteinemia

Case definition for dengue shock syndrome:

All of the above four criteria for DHF must be present, plus evidence of circulatory failure manifested by:

Rapid and weak pulse, and

Narrow pulse pressure (< 20 mm Hg)

or manifested by:

Hypotension for age, and

Cold, clammy skin and restlessness.

*HF = dengue hemorrhagic fever.



2009 WHO dengue case definitions

TABLE 2

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

Probable dengue

Live in or travel to dengue endemic area, fever and two of the following:
 Nausea, vomiting
 Rash
 Aches and pains
 Tourniquet test positive
 Leucopenia
 Any "Warning Sign"
 Dengue with 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

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 $\geq 1,000$
 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.

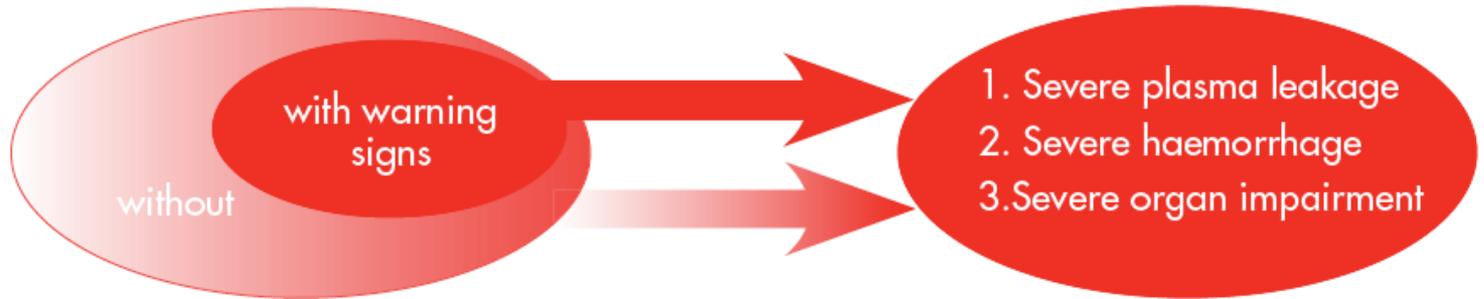
*HCT = hematocrit; DSS = dengue shock syndrome; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CNS = central nervous system.



Figure 1.4 Suggested dengue case classification and levels of severity

DENGUE ± WARNING SIGNS

SEVERE DENGUE



CRITERIA FOR DENGUE ± WARNING SIGNS

CRITERIA FOR SEVERE DENGUE

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)

Severe plasma leakage
 leading to:

- Shock (DSS)
- Fluid accumulation with respiratory distress

Severe bleeding
 as evaluated by clinician

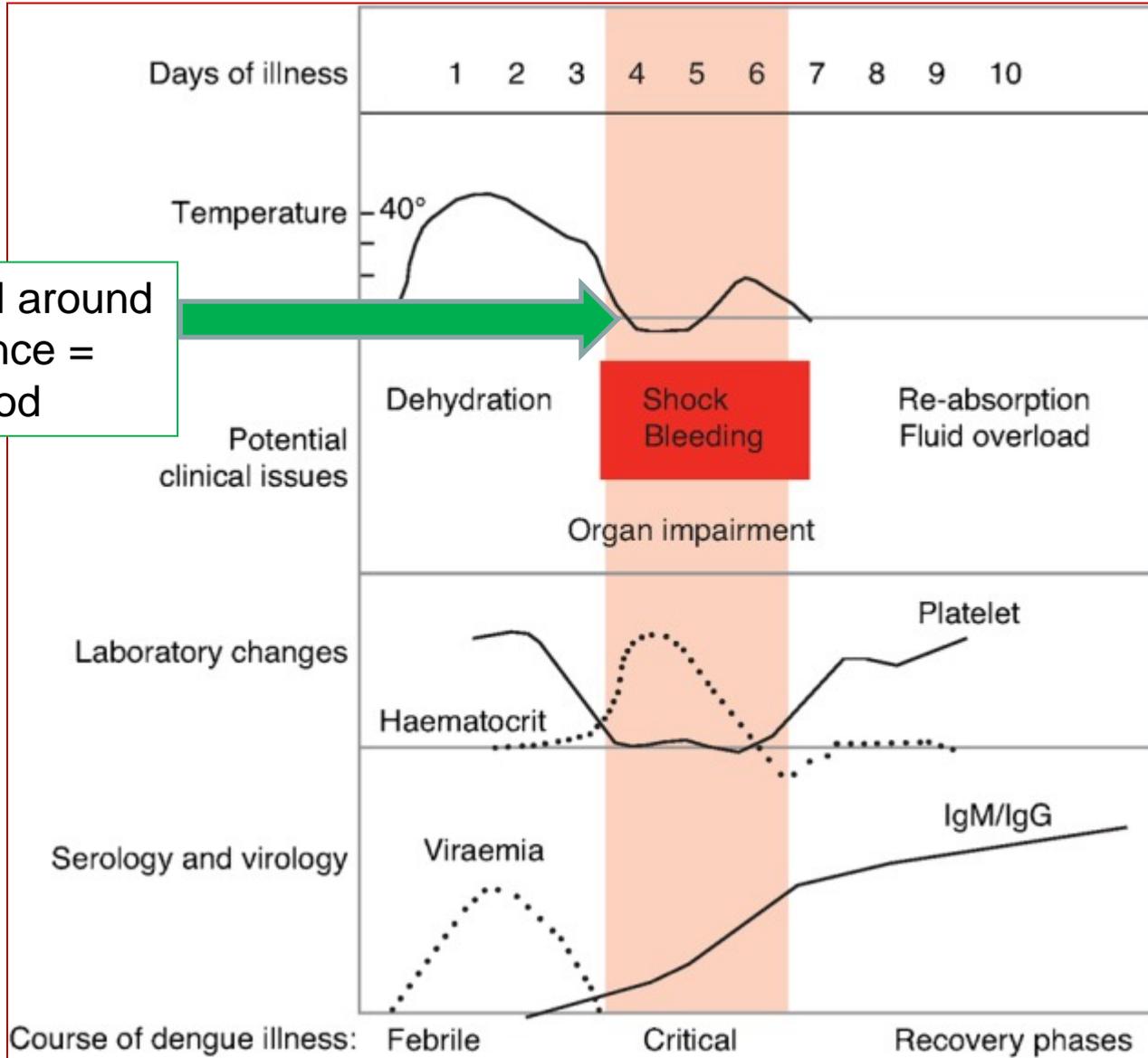
Severe organ involvement

- Liver: AST or ALT \geq 1000
- CNS: Impaired consciousness
- Heart and other organs



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. Cardoso, 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, Nidia Rizzo, Susana Vázquez and Sutee Yoksan

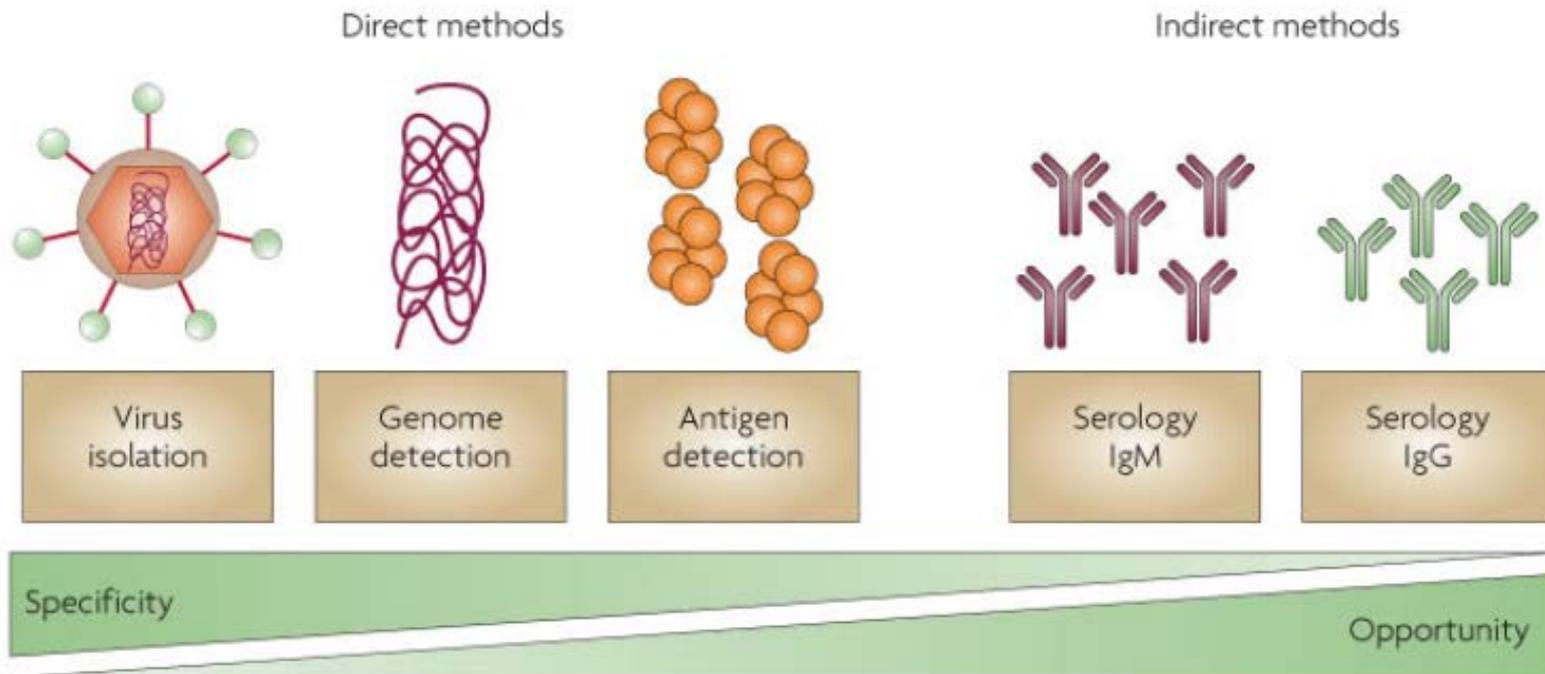


Figure 1 | **Comparative merits of direct and indirect laboratory methods for the diagnosis of dengue infections.** Opportunity refers to the fact that antibody testing is usually the most practical diagnostic option available.

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 cm^2 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).



For research on
diseases of poverty
UNICEF - UNDP - World Bank - WHO





DENGUE WITH WARNING SIGNS

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.



Figure 2.2 Algorithm for fluid management in compensated shock

- 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

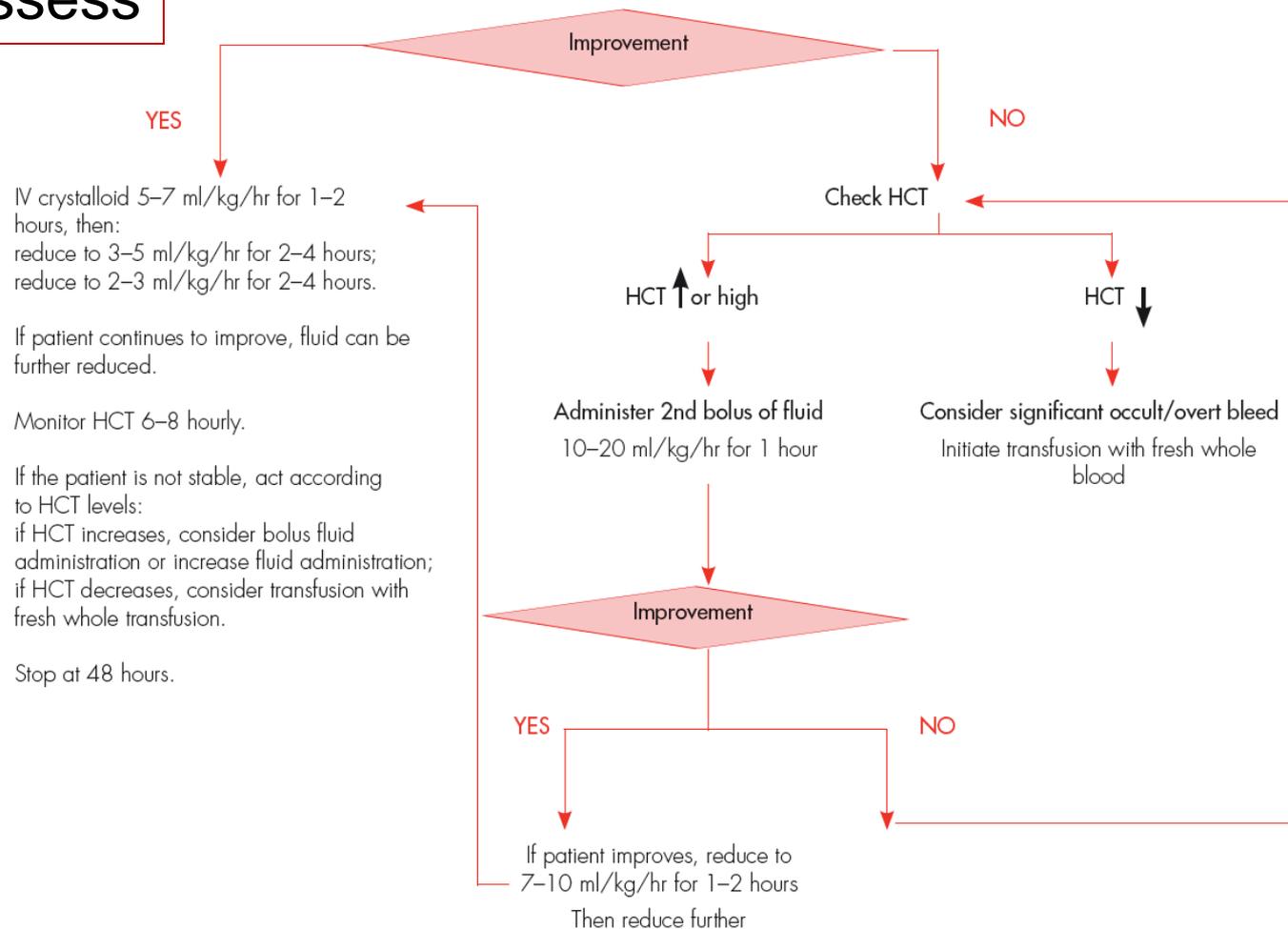
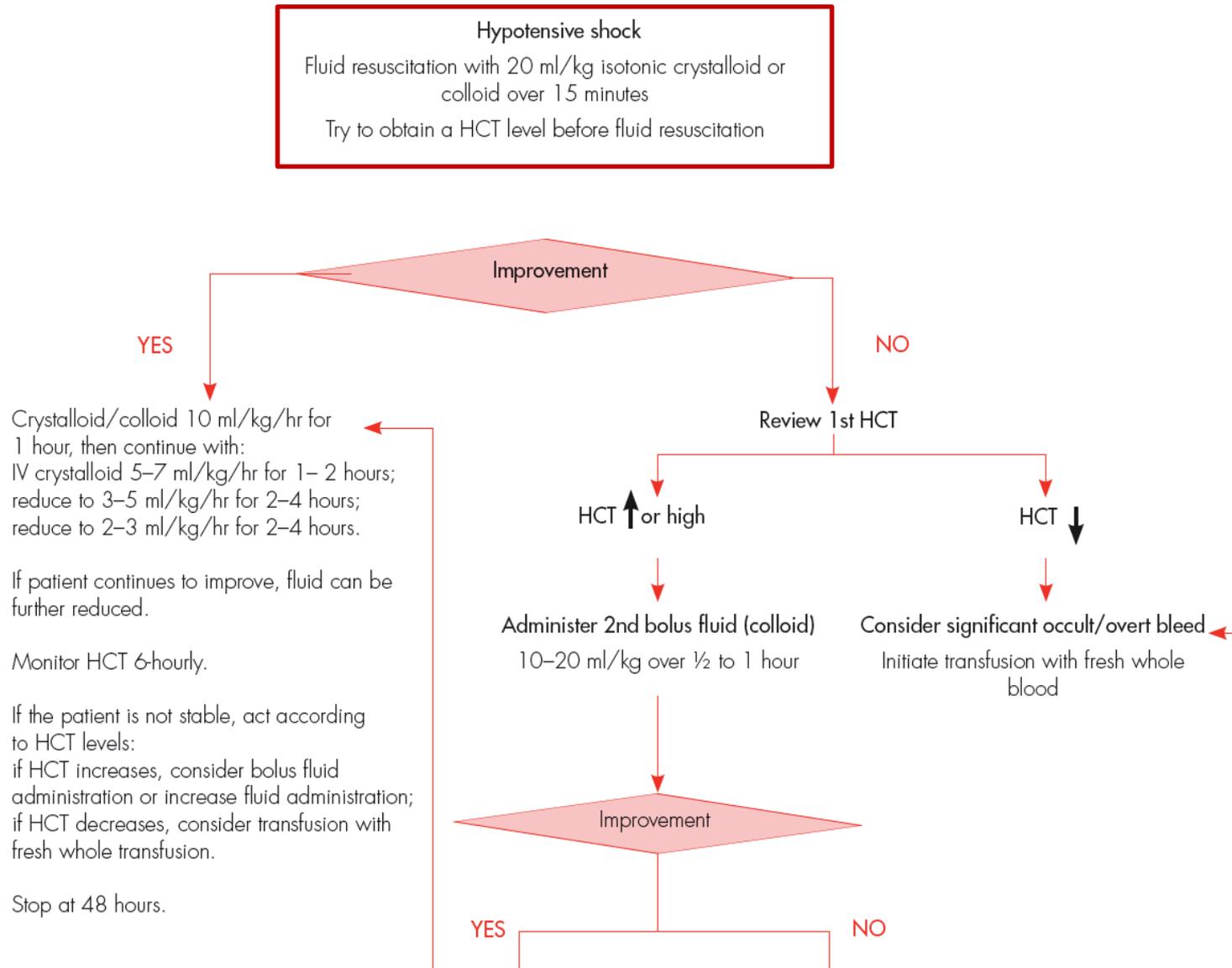
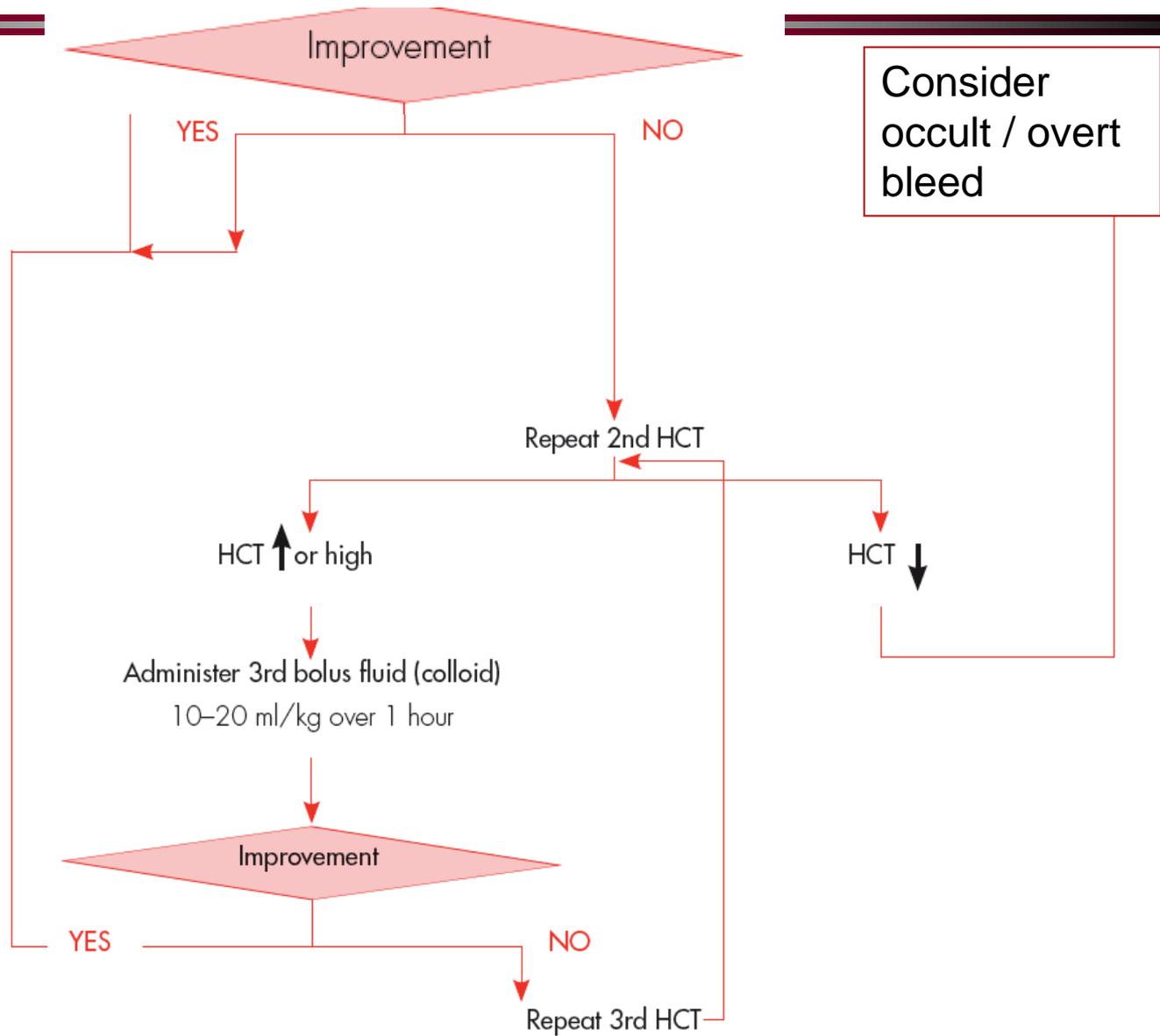


Figure 2.3 Algorithm for fluid management in hypotensive shock



- Assess
- Intervene
- Re-assess



Textbox A. Good clinical practice and bad clinical practice

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



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

Case Report: Richard W. Douce *Am. J. Trop. Med. Hyg.*, 82(4), 2010, pp. 740–742



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

Bali Pulendran

NATURE REVIEWS | IMMUNOLOGY

VOLUME 9 | OCTOBER 2009 | 741

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.

1648 1762 1793 1802 1878 1881 1898 1899–1901 1927 1937 1945 1951 2006–2009

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 substrains 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



Havana in 1900



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

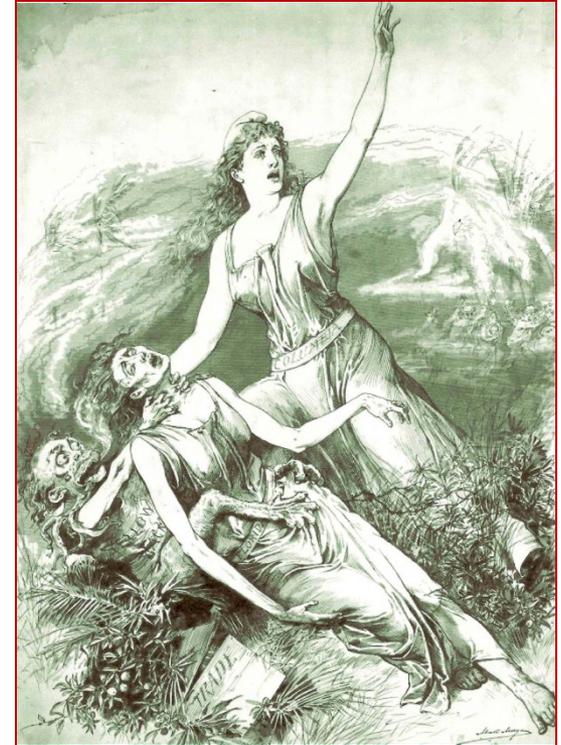
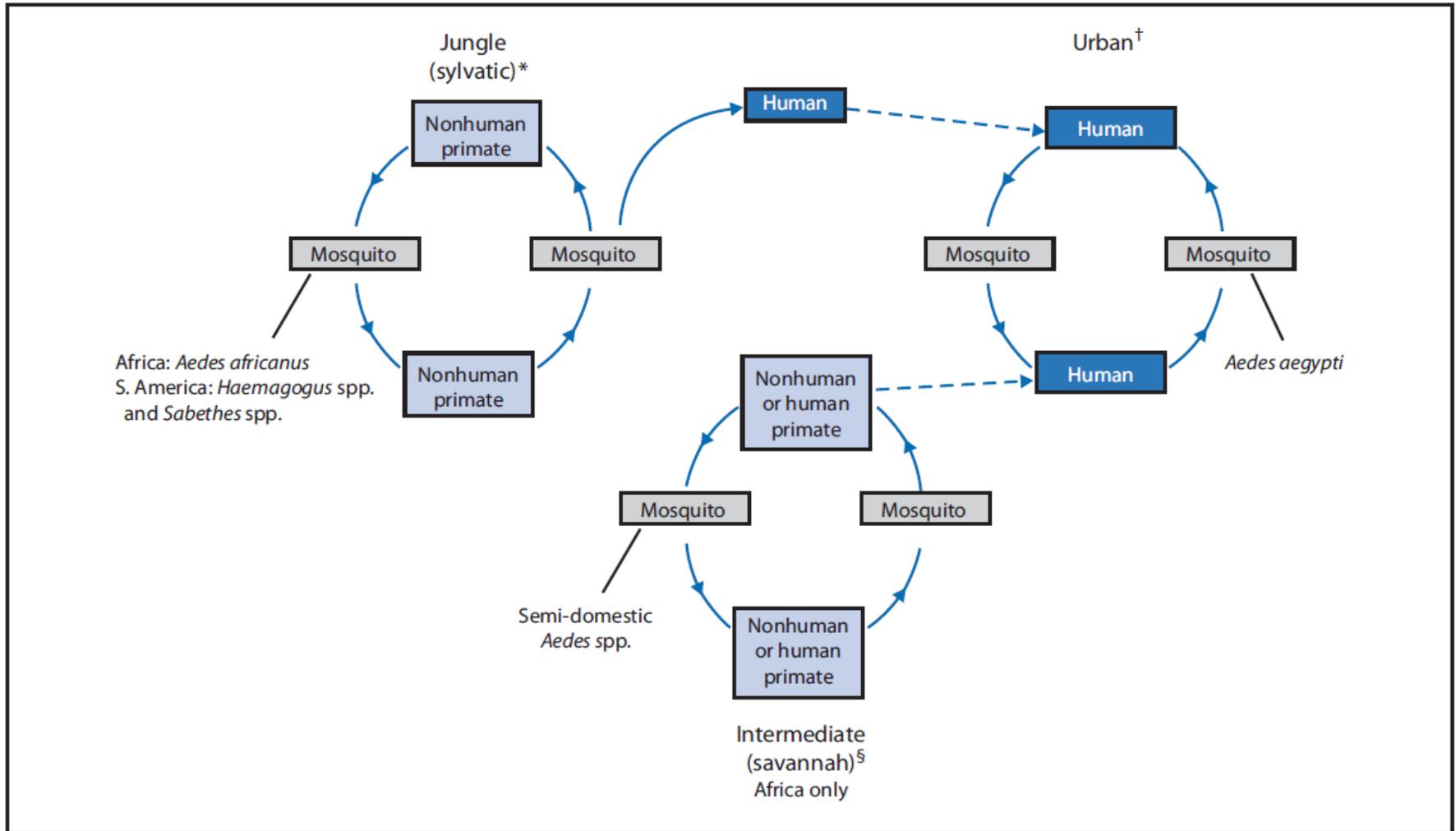


FIGURE 1. Transmission cycles for yellow fever virus



* 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



Figure 3: Areas with risk of yellow fever virus transmission in South America, 2010

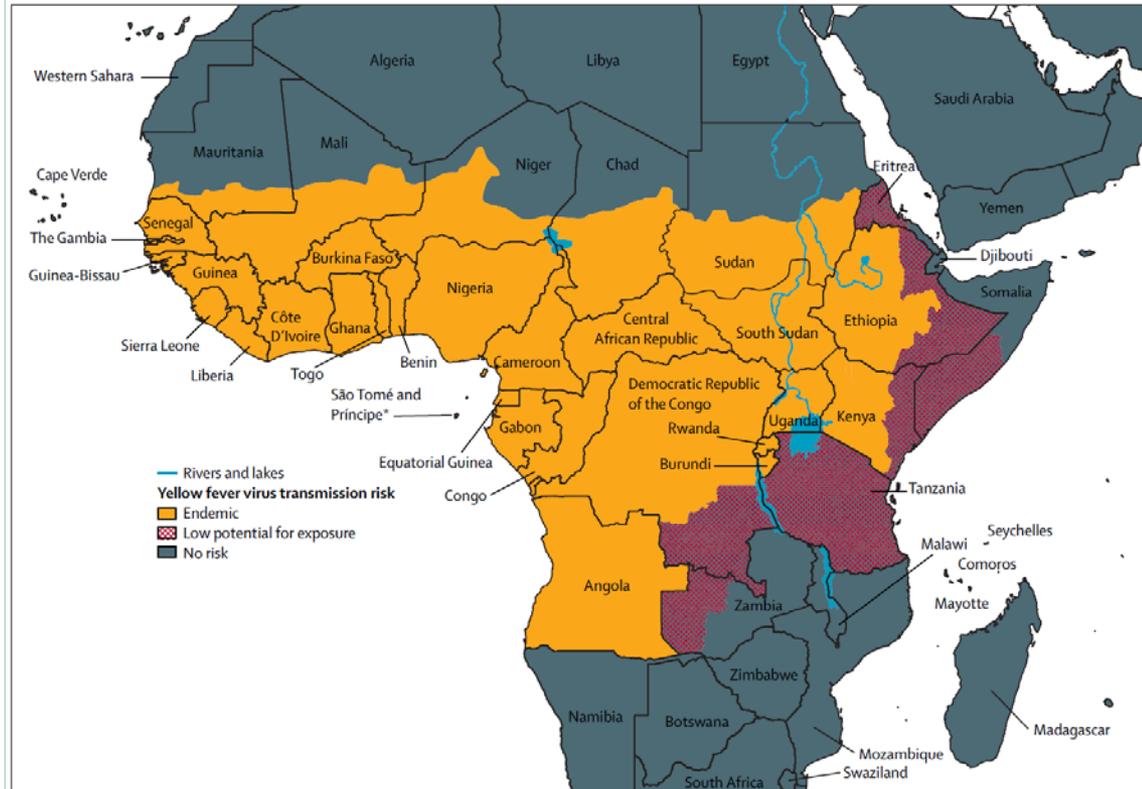


Figure 4: Areas with risk of yellow fever virus transmission in Africa, 2010
*São Tomé and Príncipe was classified as low potential for exposure.

Map is from the following publication: Jentes ES, Pomeroy G, Gershman MD, et al. The revised global yellow fever risk map and recommendations for vaccination, 2010: consensus of the Informal WHO Working Group on Geographic Risk for Yellow Fever. *Lancet Infect Dis.* 2011;11:622-32.



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.

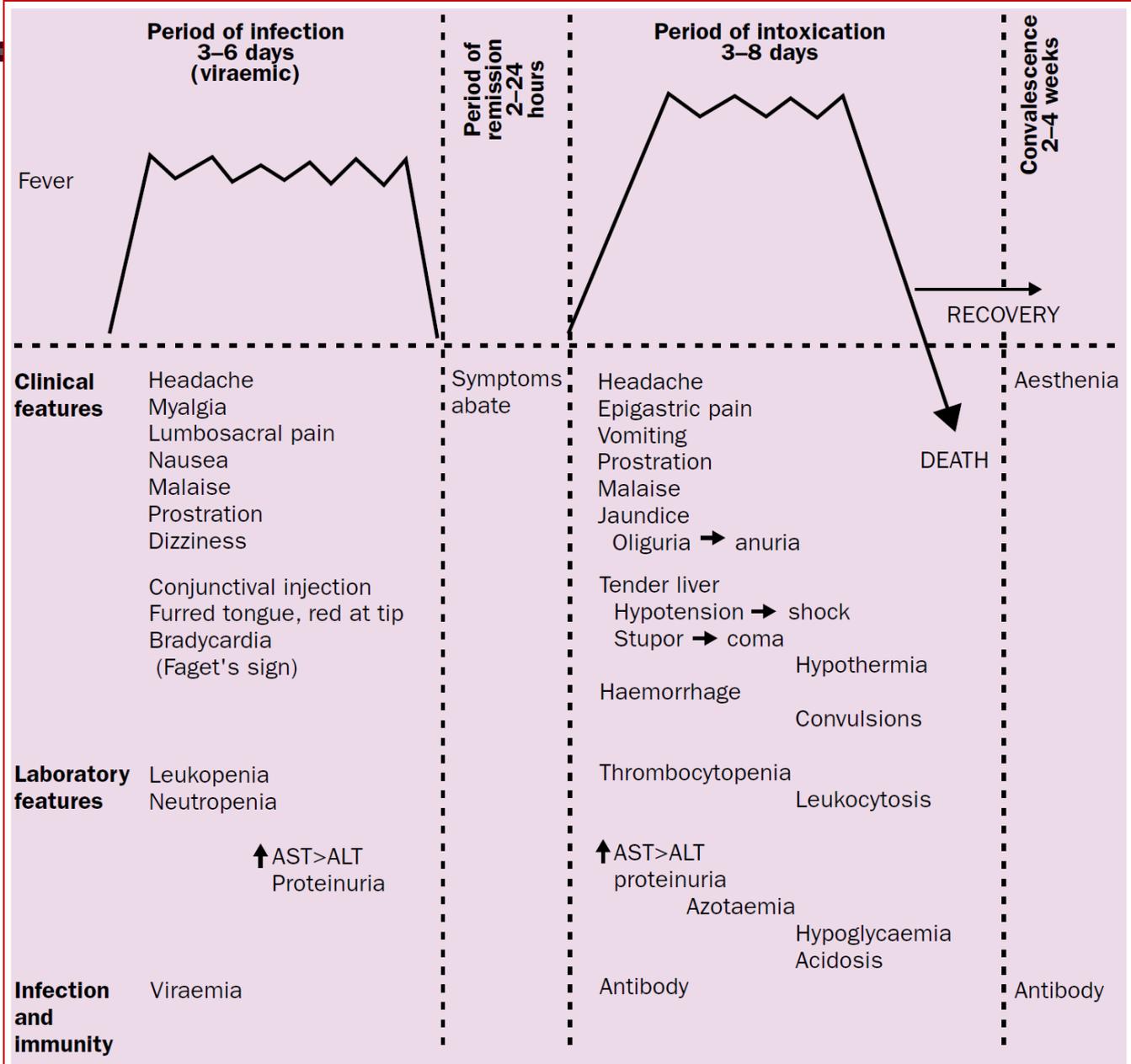


Figure 4. Stages of yellow fever infection, showing the major clinical and laboratory features of the disease.



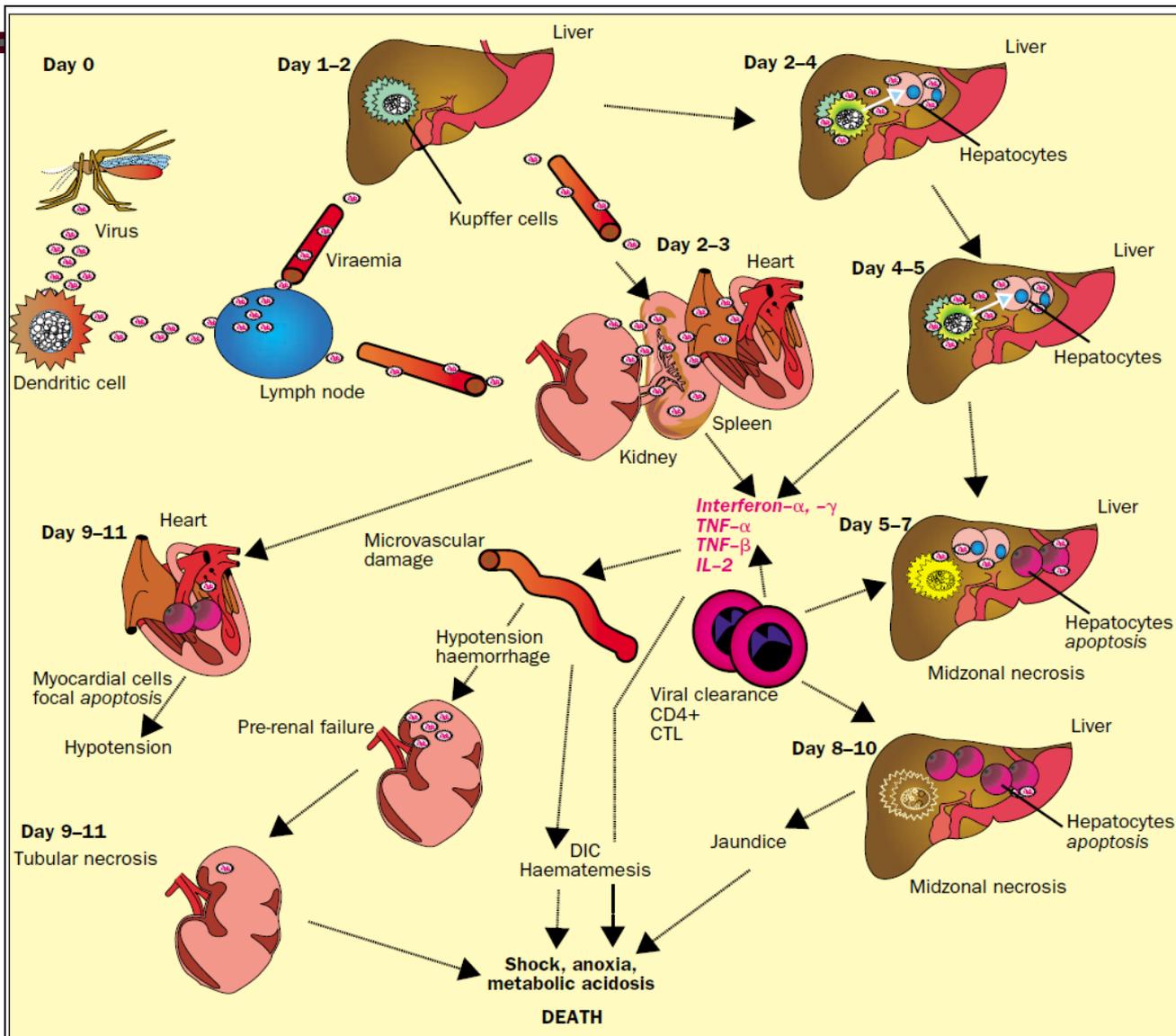


Figure 6. Pathogenesis of yellow fever based on studies in experimentally infected monkeys and human case reports (*bold*). Speculative mechanisms shown in *italics* are drawn from *in-vitro* data or reports on other flavivirus infections. CTL=cytotoxic T lymphocyte, DIC=disseminated intravascular coagulation, IL=interleukin.



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

Case Report: Richard W. Douce *Am. J. Trop. Med. Hyg.*, 82(4), 2010, pp. 740–742



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.
2. Poland JD, Calisher CH, Monath TP. Persistence of neutralizing antibody 30-35 years after immunization with 17D yellow fever vaccine. Bull World Health Organ 1981;59:895-900.



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 ($>101.5^{\circ}\text{F}$ $> 24\text{h}$) and headache ($>24\text{h}$ duration)
- focal neurological dysfunction (aphasia, paresis, etc)
- mental status change
- new-onset seizure or recurrence
- CSF pleocytosis ($> 5 \text{ WBC}/\text{mm}^3$) 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, \geq 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. Polyarthrititis
 - 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

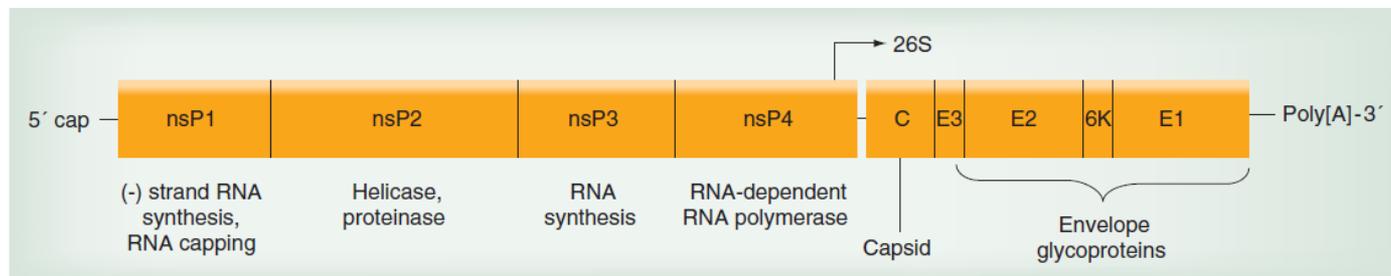


Figure 1. Organization of the chikungunya virus genome, including the nonstructural and structural polyprotein open reading frames, and the 26S or subgenomic promoter.

Expert Rev. Vaccines 11(9), (2012)



Historic Movement of Chikungunya

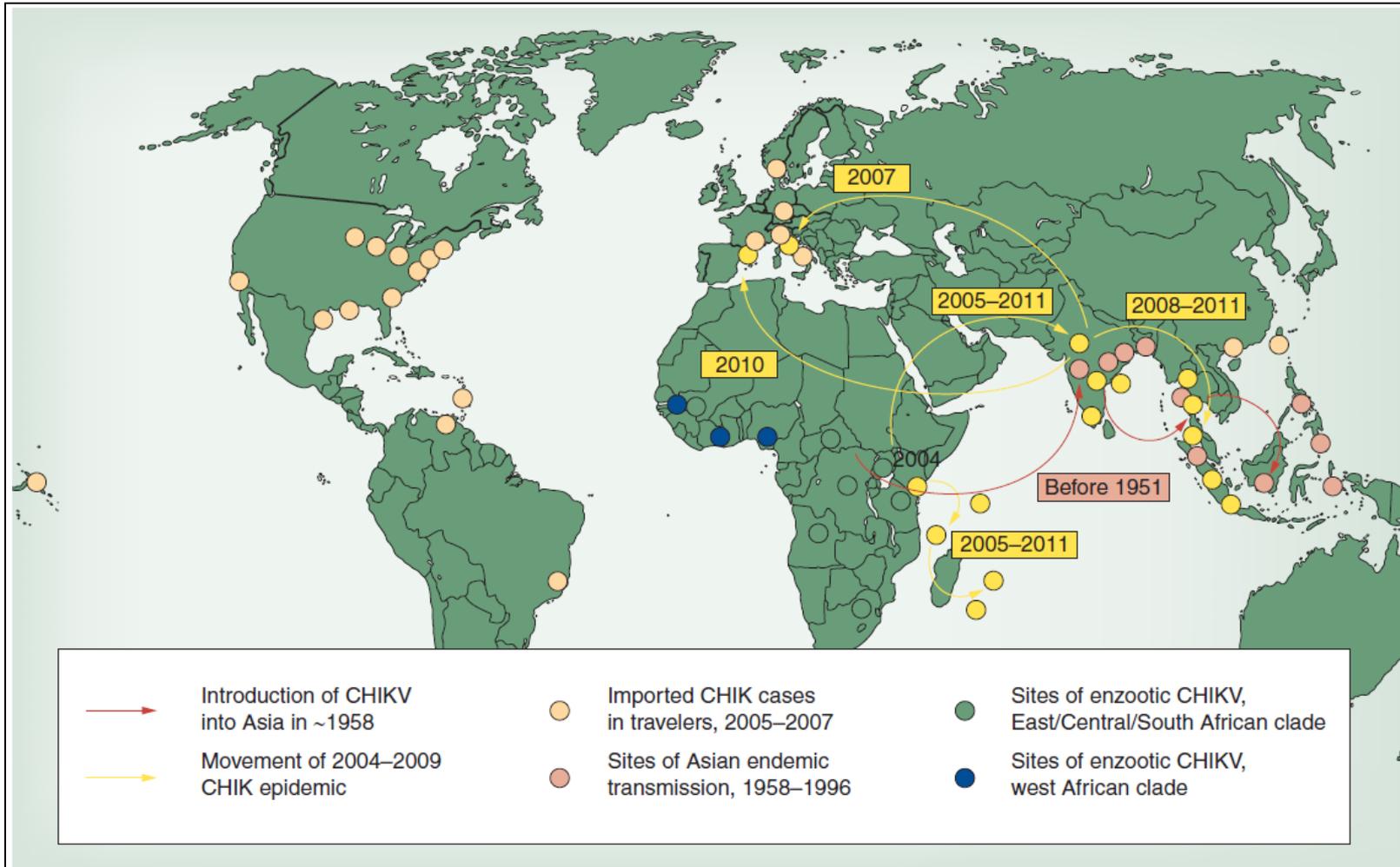


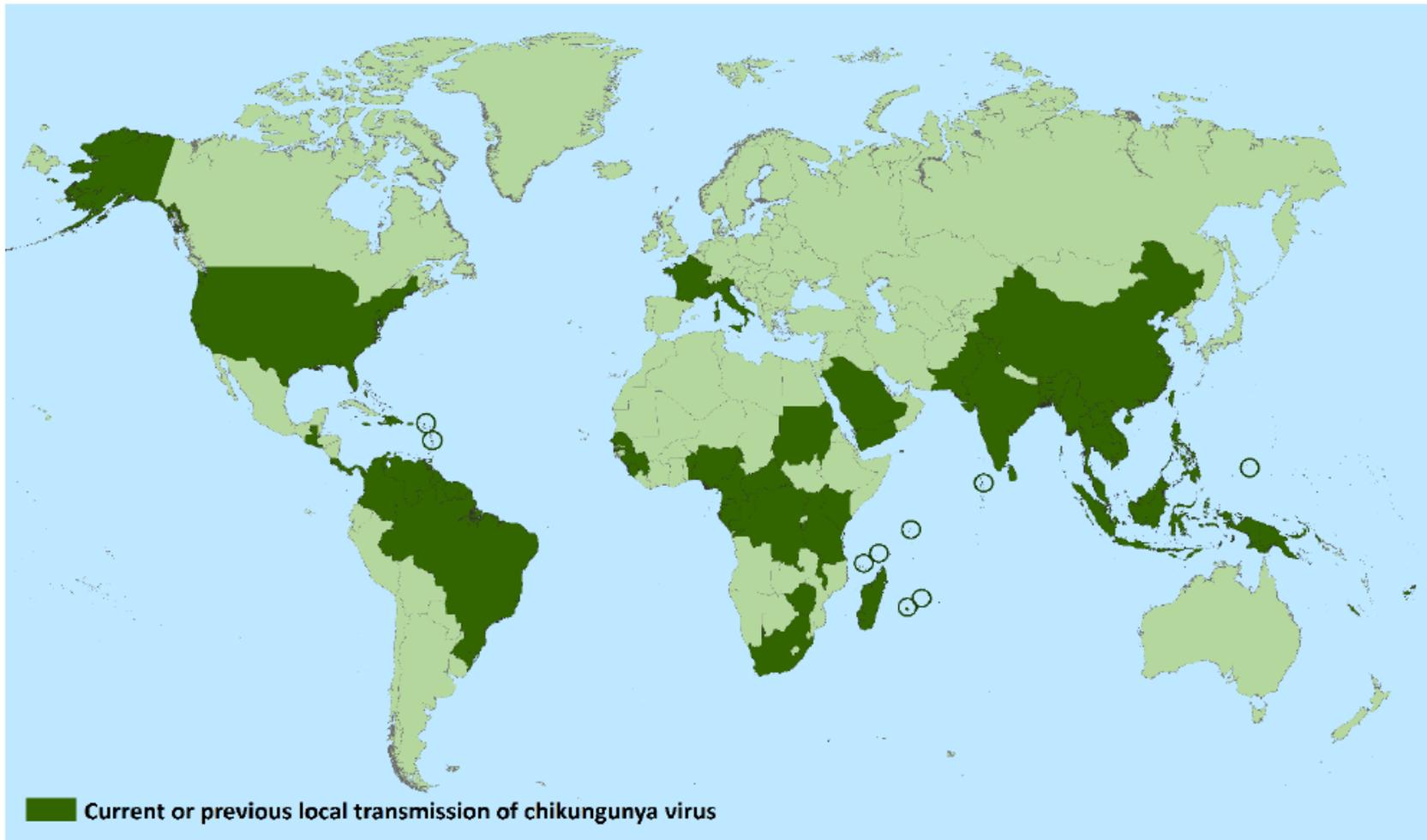
Figure 5. Distribution of chikungunya virus strains and movement of outbreaks inferred from the phylogenetic analysis depicted in FIGURE 4.

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

Symptom or sign	Frequency range (% of symptomatic patients)
Fever	76–100
Polyarthralgias	71–100
Headache	17–74
Myalgias	46–72
Back pain	34–50
Nausea	50–69
Vomiting	4–59
Rash	28–77
Polyarthrititis	12–32
Conjunctivitis	3–56

^aTable 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



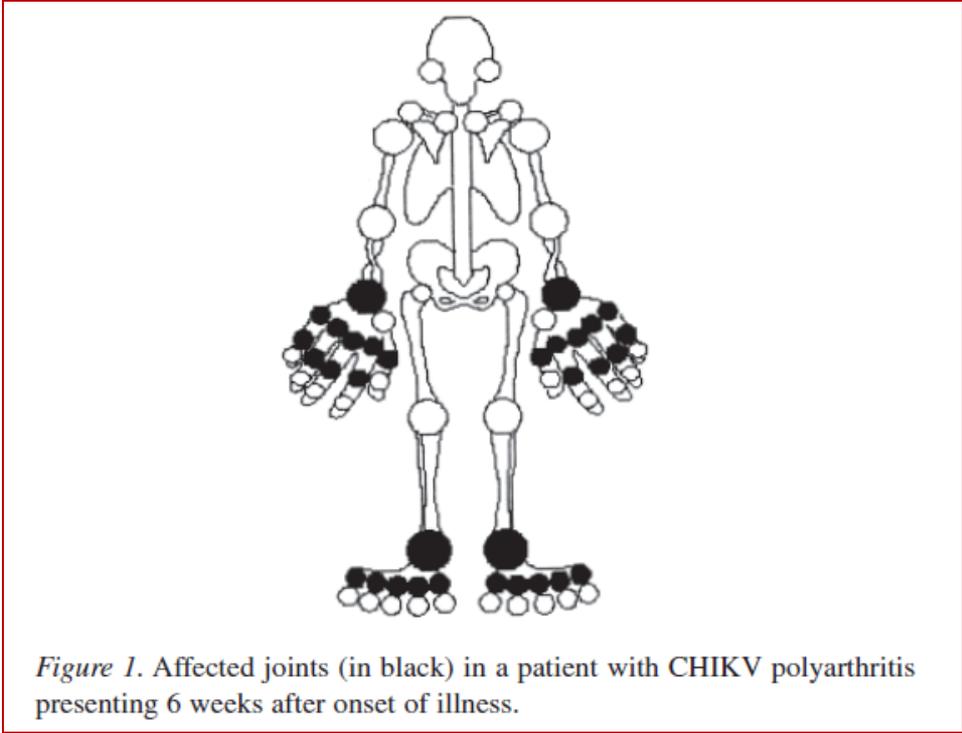
Chikungunya vs. Dengue

Clinical and laboratory features	Chikungunya virus infection	Dengue virus infection
Fever (>102°F or 39°C)	+++	++
Myalgias	+	++
Arthralgias	+++	+/-
Headache	++	++ ^b
Rash	++	+
Bleeding dyscrasias	+/-	++
Shock	-	+
Leukopenia	++	+++
Neutropenia	+	+++
Lymphopenia	+++	++
Elevated hematocrit	-	++
Thrombocytopenia	+	+++

^a 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%^{32,33}

^b Often retroorbital

Table modified from Staples et al.³⁴



Chikungunya viral polyarthrititis.

Raj J Carmona, Saeed Shaikh and Nader A Khalidi

J Rheumatol 2008;35:935-936



Chikungunya: A Potentially Emerging Epidemic?

Michelle M. Thiboutot^{1,2}, Senthil Kannan², Omkar U. Kawalekar², Devon J. Shedlock², Amir S. Khan³, Gopalsamy Sarangan⁴, Padma Srikanth⁴, David B. Weiner², Karupppiah Muthumani^{2*}

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

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

doi:10.1371/journal.pntd.0000623.t001



Diagnosis

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

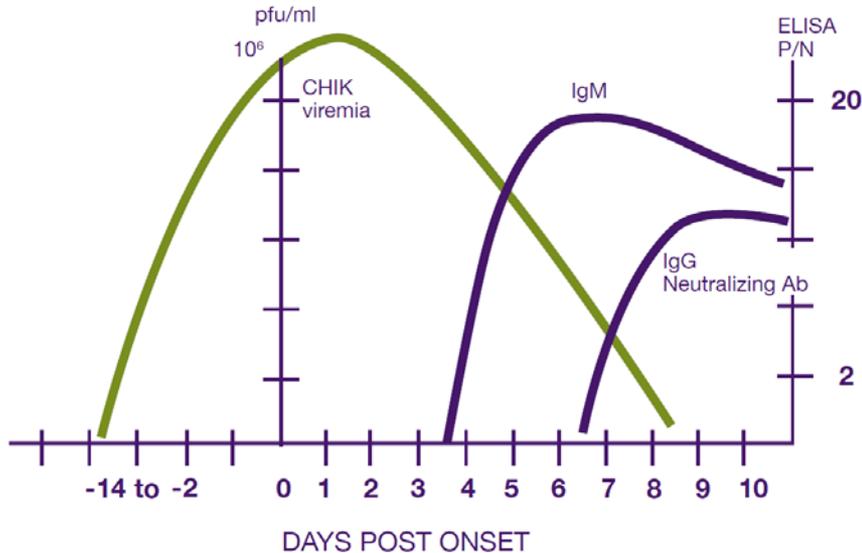


Table 6. Typical results of samples tested at various time points post-infection.

Days post illness onset	Virus testing	Antibody testing
Day 1-3	RT-PCR = Positive Isolation = Positive	IgM = Negative PRNT = Negative
Day 4-8	RT-PCR = Positive Isolation = Negative	IgM = Positive PRNT = Negative
>Day 8	RT-PCR = Negative Isolation = Negative	IgM = Positive PRNT = Positive

- 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



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. 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

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

Adapted from Rajapakse et al. ²⁰



Persistent Chikungunya

Three clinical components, singly / in combination:

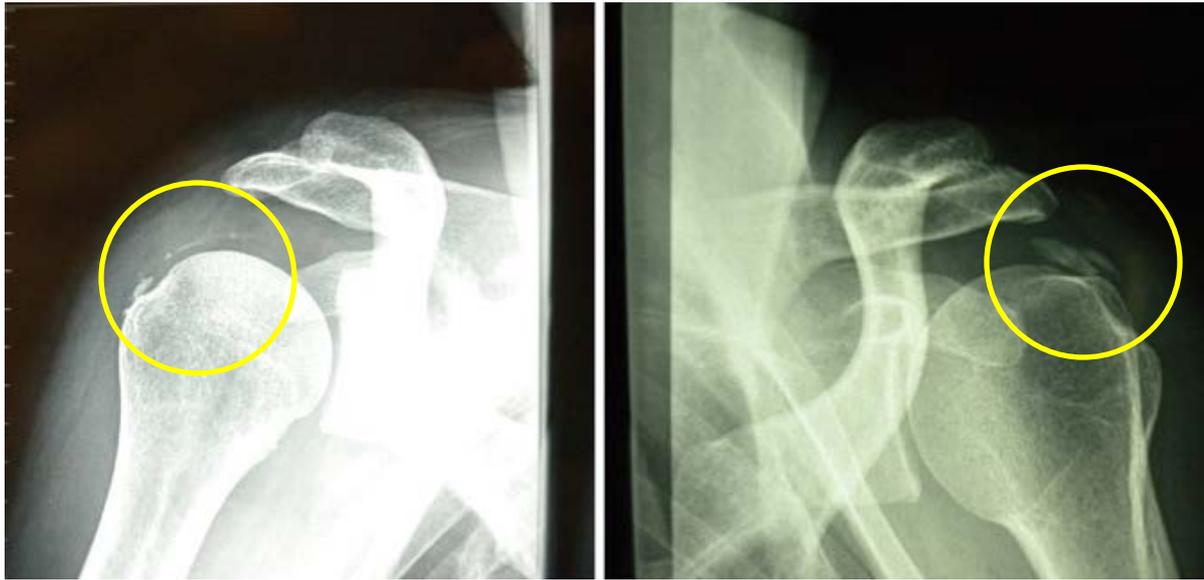
1. Distal polyarthritis / monoarthritis improved with NSAIDs;
2. Frequent tenosynovitides in the hands, wrists, or ankles, sometimes responsible for carpal or tarsal tunnel syndromes, highly sensitive to short-term systemic corticotherapy; and
3. Exacerbation of pain in previously injured joints and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



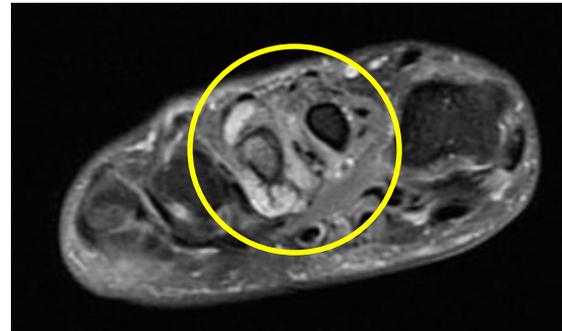
Persistent Chikungunya



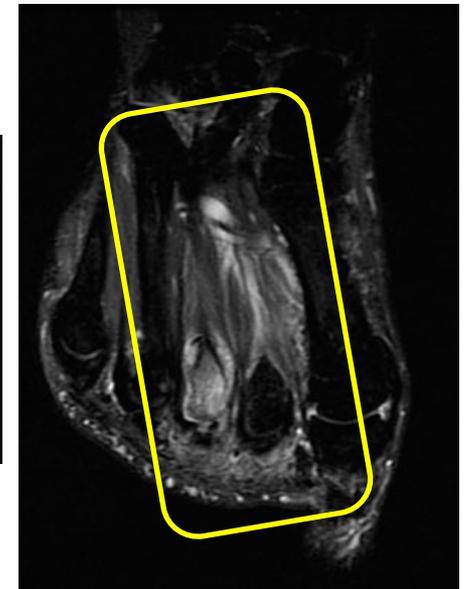
Persistent Chikungunya



Calcifications in shoulder tendon 18 months after infection



Inflammatory osteoarthritis, foot, 5 years after infection



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. 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 \geq 50K cases (2.5 cases/10,000 pop.)
- Risk of JE following infection
 - \sim 1/200 in indigenous pop.; \sim 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 \sim 25 -30%
 - Long-term neuropsychiatric disability 45 - 50%

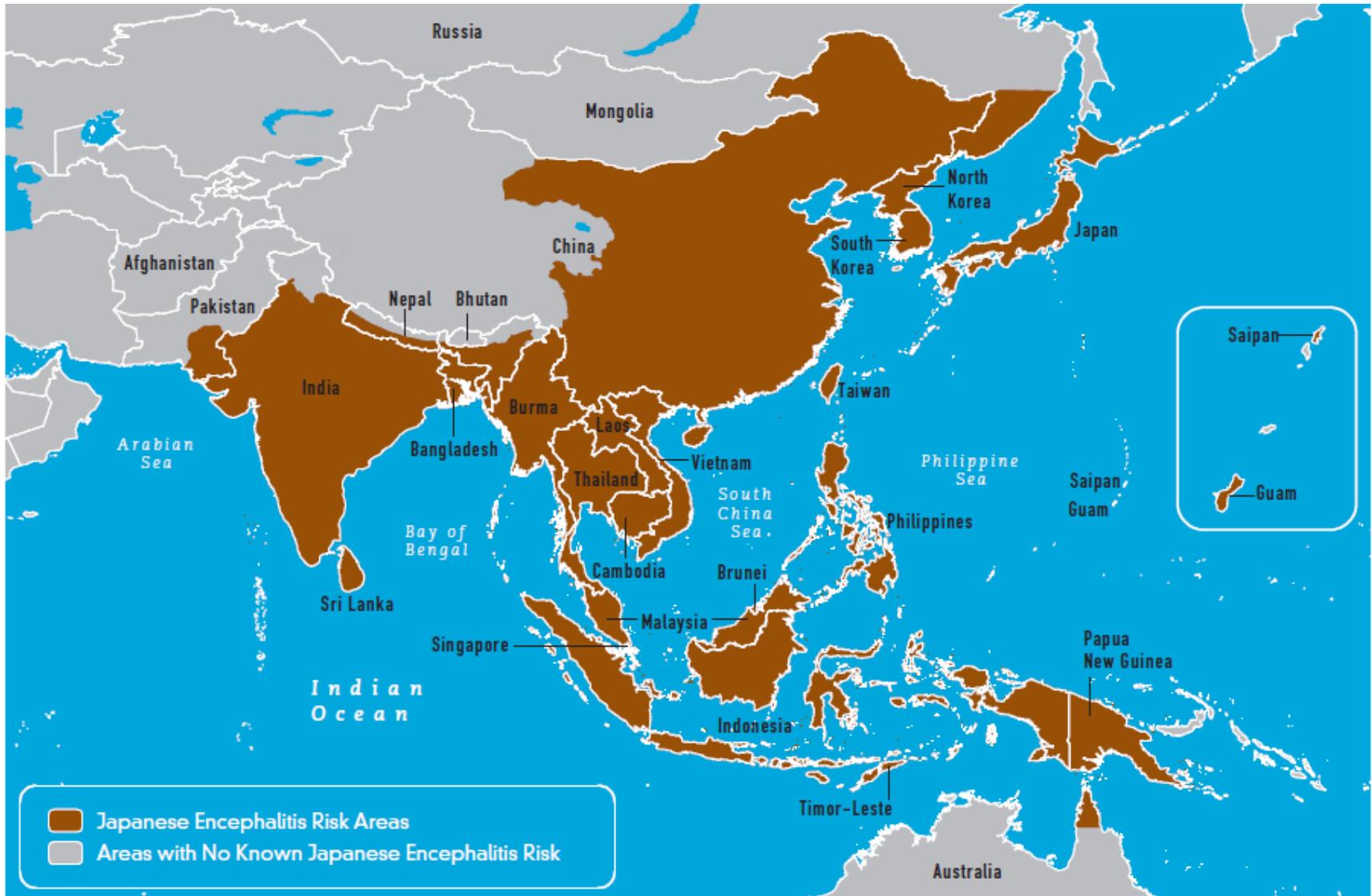


Transmission

- Vector: *Culex* mosquitoes
 - *Culex tritaeniorhynchus*
 - 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

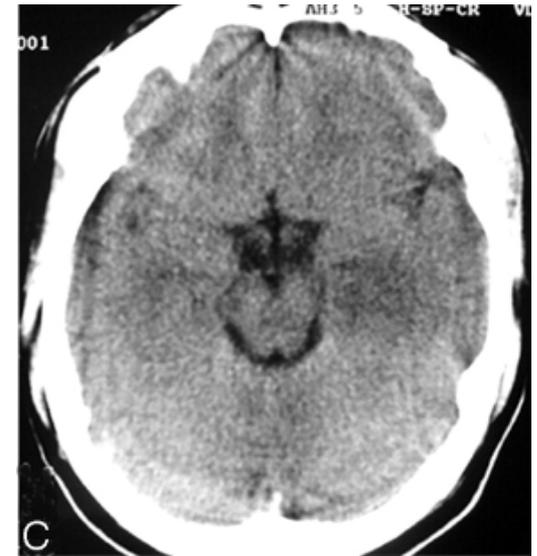
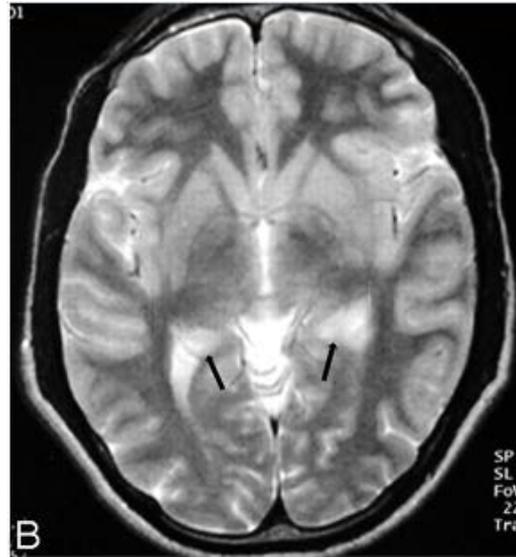
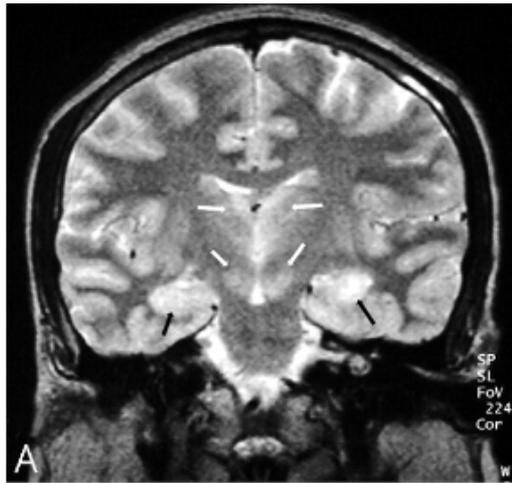
- Peripheral leukocytosis, up to $30\text{k}/\text{mm}^3$ with a left shift
- Hyponatremia
- CSF
 - Opening Pressure $>$ in $\sim 50\%$
 - Pleocytosis <10 to several thousand cells (lymphocytic)
 - Protein may be normal or elevated up to 100 mg/dL
- EEG
 - Diffuse slow waves (theta or delta), seizure 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.

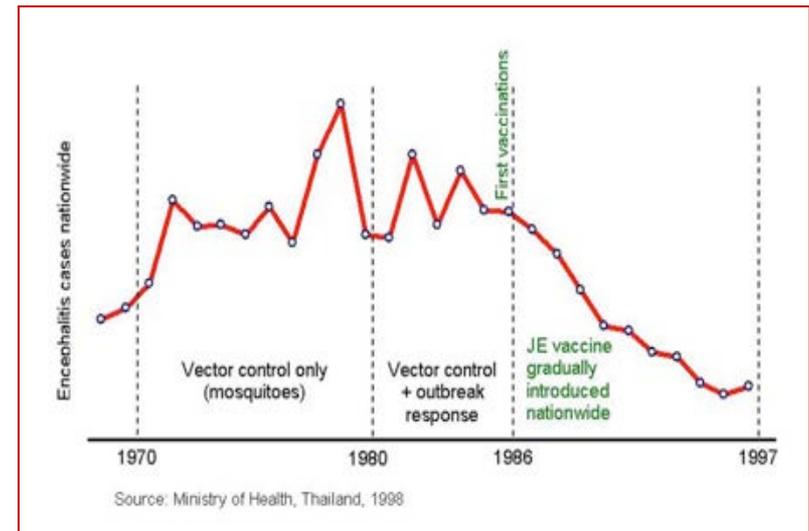
Question

- 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.

BOOSTER DOSE

2 MONTHS TO <3 YEARS	2 MONTHS TO <17 YEARS	2 TO 11 MONTHS
Primary immunization with IXIARO consists of two (2) 0.25 mL doses, administered 28 days apart.	The safety and immunogenicity of a booster dose has not been evaluated.	The anterolateral aspect of the thigh
≥3 YEARS	≥17 YEARS	1 TO <3 YEARS
Primary immunization with IXIARO consists of two (2) 0.5 mL doses, administered 28 days apart.	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.	The anterolateral aspect of the thigh (or the deltoid muscle if muscle mass is adequate)
		≥3 YEARS
		The deltoid muscle

ADMINISTRATION

IXIARO is administered intramuscularly. Do not administer intravenously, intradermally, or subcutaneously. Preferred site for intramuscular injection is as follows:

<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[§]

	Post Dose 1 (N=55‡) % of subjects	Post Dose 2 (N=49‡) % of subjects
Injection Site Reactions		
Pain	18.2	16.3
Itching	3.6	2.0
Tenderness	30.9	24.5
Hardening	0.0	2.0
Swelling	0.0	0.0
Redness	5.5	0.0
Solicited Systemic Reaction		
Irritability	0.0	6.1
Nausea	1.8	2.0
Vomiting	0.0	2.0
Diarrhea	1.8	0.0
Flu-like symptoms	0.0	0.0
Excessive fatigue	12.7	0.0
Muscle pain	27.3	2.0
Rash	1.8	2.0
Headache	1.8	4.1
Loss of appetite	1.8	0.0
Fever $\geq 37.7^{\circ}\text{C}$ ($\geq 99.9^{\circ}\text{F}$)	5.5	2.0
37.7-38.6 $^{\circ}\text{C}$ (99.9-101.5 $^{\circ}\text{F}$)	3.6	2.0
38.7-39.3 $^{\circ}\text{C}$ (101.6-102.7 $^{\circ}\text{F}$)	1.8	0.0
39.4-40.5 $^{\circ}\text{C}$ (102.8-104.9 $^{\circ}\text{F}$)	0.0	0.0
$>40.5^{\circ}\text{C}$ ($>104.9^{\circ}\text{F}$)	0.0	0.0

[§]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[§]

Adverse Event	Post Dose 1 (Day 0 to Day 28) % of subjects		Post Dose 2 (Day 28 to Day 56) % of subjects		Post Dose 1 or Dose 2 (Day 0 to Day 56) % of subjects	
	IXIARO N‡=1993	PBS + Al(OH) ₃ N‡=657	IXIARO N‡=1968	PBS + Al(OH) ₃ N‡=645	IXIARO N‡=1993	PBS + Al(OH) ₃ N‡=657
Headache†	21.6	20.2	13.4	13.0	27.9	26.2
Myalgia†	13.3	12.9	5.6	5.3	15.6	15.5
Fatigue†	8.6	8.7	5.2	5.9	11.3	11.7
Influenza-like illness†	8.2	8.5	5.8	4.3	12.3	11.7
Nausea†	4.7	5.3	2.6	3.7	6.6	7.5
Nasopharyngitis	2.3	1.8	2.6	2.3	4.7	4.0
Fever†	1.9	2.1	1.5	1.7	3.2	3.0
Rhinitis	1.0	0.8	0.5	0.6	1.4	1.4
Upper Respiratory Tract Infection	0.9	0.9	0.8	0.9	1.7	2.0
Back Pain	0.8	0.9	0.6	0.2	1.3	1.1
Pharyngolaryngeal Pain	0.8	0.9	1.0	0.5	1.6	1.4
Rash†	0.8	0.9	0.7	0.8	1.3	1.5
Diarrhea	0.8	0.8	0.7	0.3	1.5	1.1
Cough	0.8	0.8	0.6	0.6	1.2	1.2
Vomiting†	0.6	0.8	0.8	0.9	1.4	1.7

[§]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[§]**

Age Group	2 months – <6 months	6 months – <12 months	1 year – <3 years	3 years – <12 years	12 years – <18 years
Time Point	Proportion of Subjects with PRNT₅₀ Titer ≥1:10 (n/N) [95% CI]				
Pre-Vaccination Screen	30% (3/10) [10.8, 60.3]	0% (0/20) [0.0, 16.1]	3.2% (4/125) [1.3, 7.9]	16.8% (17/101) [10.8, 25.3]	45.7% (64/140) [37.7, 54.0]
Day 56 (28 days after vaccine dose 2)	100% (9/9) [70.1, 100.0]	100% (19/19) [83.2, 100.0]	99.2% (119/120) [95.4, 99.9]	100% (100/100) [96.3, 100.0]	100% (137/137) [97.3, 100.0]
Month 7 (6 months after vaccine dose 2)	100% (10/10) [72.2, 100.0]	100% (18/18) [82.4, 100.0]	85.5% (106/124) [78.2, 90.6]	91.0% (91/100) [83.8, 95.2]	97.1% (133/137) [92.7, 98.9]
Time Point	Geometric Mean Titers[†] (N) [95% CI]				
Pre-Vaccination Screen	8.4 (10) [4.3, 16.7]	5.0 (20) [5.0, 5.0]	5.5 (124) [5.0, 6.1]	6.5 (101) [5.8, 7.4]	13.1 (140) [10.7, 16.1]
Day 56 (28 days after vaccine dose 2)	687.4 (9) [263.2, 1795.1]	377.8 (19) [210.3, 678.8]	258.9 (121) [214.4, 312.6]	213.7 (100) [175.6, 260.0]	175.6 (137) [147.8, 208.7]
Month 7 (6 months after vaccine dose 2)	159.3 (10) [110.0, 230.7]	64.0 (18) [39.4, 104.1]	38.9 (125) [31.8, 47.7]	43.6 (100) [35.6, 53.4]	86.6 (137) [70.7, 106.0]

[§]NCT01041573

*Infants and children ≥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 PRNT₅₀ titer ≥1:10

[†]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[§]

Proportion of Subjects with PRNT₅₀ Titer ≥1:10			
Time Point	IXIARO (n/N) [95% CI]	JE-VAX (n/N) [95% CI]	Rate difference [95% CI]
Day 56 (28 days after vaccine dose 2)	96.4% (352/365) [94.0, 97.9]	93.8% (347/370) [90.9, 95.8]	2.6% [-0.5, 6.0]†
Geometric Mean Titers‡			
Time Point	IXIARO (N**=361) [95% CI]	JE-VAX (N**=364) [95% CI]	GMT ratio [95% CI]
Day 56 (28 days after vaccine dose 2)	243.6 [216.4, 274.1]	102.0 [90.3, 115.2]	2.33 [1.97, 2.75]‡

[§]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 ≥1:10 (IXIARO minus JE-VAX) was >-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 ≥1:10

N=number of subjects in the Per Protocol Population

**N=Number of subjects with immunogenicity data

‡ 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[§]

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

[§]NCT00596271

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

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 IXIARO Administered 14 Months After Completion of the Primary Series Among Adults Residing in Non-Endemic Areas, Intent to Treat Population*, Study 5^s

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

^sNCT00595309

*The Intent to Treat population consisted of all subjects who received the booster vaccination

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

N=number of subjects with immunogenicity data

A Single Dose of Vero Cell–Derived Japanese Encephalitis (JE) Vaccine (Ixiaro) Effectively Boosts Immunity in Travelers Primed With Mouse Brain–Derived JE Vaccines
 Erra, et al. Clin Infect Dis. 2012 September 15; 55(6): 825–834.

	Response Rate After 1 Dose of JE-VC ^a		Protection Rate After 1 Dose of JE-VC ^b		Geometric Mean Titers After 1 Dose of JE-VC ^b	
	Nonprimed	Primed	Nonprimed	Primed	Nonprimed	Primed
PRNT Nakayama	39% (10/26)	98% (41/42)	40% (10/25)	100% (17/17)	<10	236
PRNT SA14-14-2	42% (11/26)	95% (40/42)	40% (10/25)	100% (17/17)	12	236



Questions?

