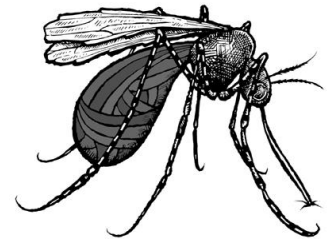
Dengue:
The Breakbone Fever



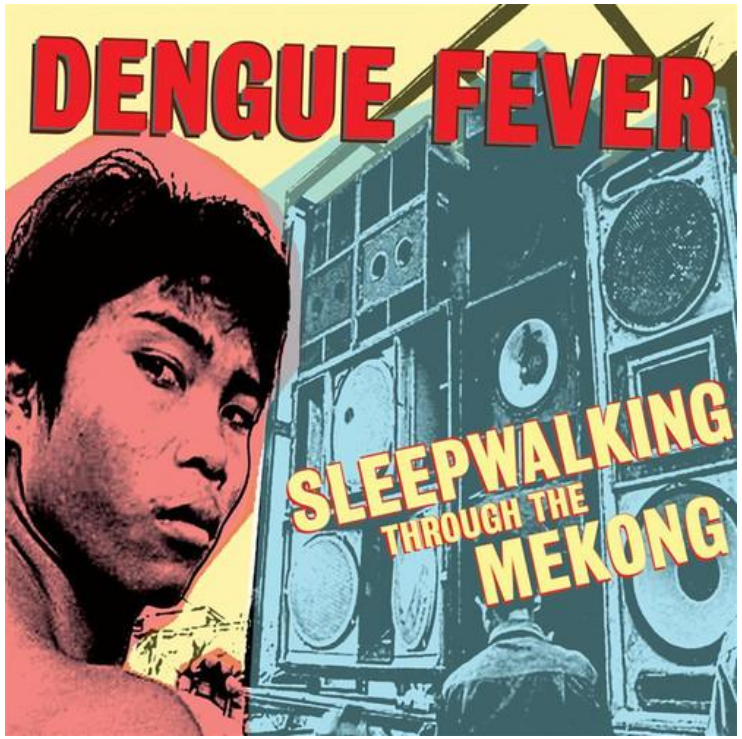
WRAIR Tropical
Medicine Course

Arthur Lyons, COL, MC

Viral Disease Branch

Walter Reed Army Institute of Research

NOT the Band


DENGUE FEVER
SLEEPWALKING
THROUGH THE
MEKONG


DENGUE FEVER
VENUS ON EARTH

Disclaimer

- The points made in this presentation are solely the views/opinions of the author and do not reflect the views/opinions of the US Government, US Department of Defense, US Army or the US Army Medical Research and Materiel Command.

Take Home Points

- Mosquito-borne illness
  - Not spread person-to-person
- First infection can be a bad experience
- Second infection can be deadly
- No antiviral treatment
- No vaccine (yet)

Case (1)

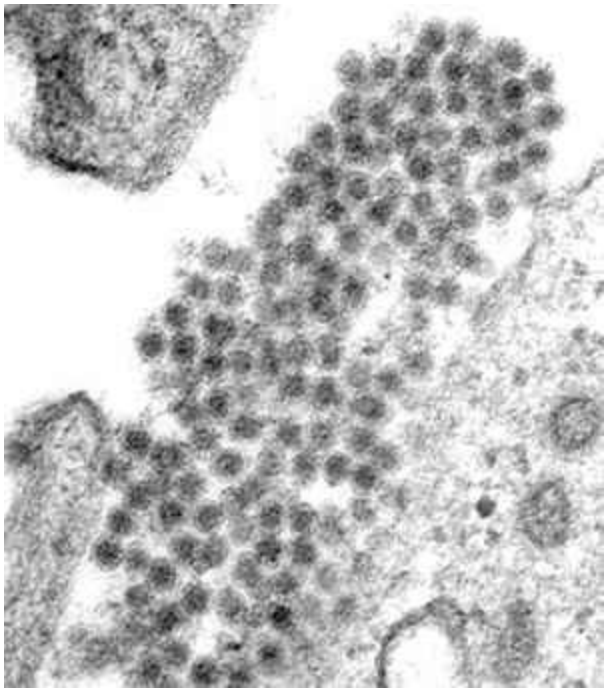
- 25 y/o male Indiana Jones type presents to your clinic in June @ Ft. Bragg, NC with c/o headache, abdominal pain, nausea and vomiting for the past 24 hours. Took pepto bismol and tylenol without relief.
- He returned from leave OCONUS 2 days ago (SubSaharan Africa , Latin America and SE Asia) where he swam in the ocean, ate adventurously, suffered numerous different insect bites, partook of some “horizontal refreshment” with local talent, and volunteered to be a cow herder for 2 weeks in the Pampas. He has 2 cats, a dog, tropical fish and several ferrets as pets at home. 3 weeks ago he cleaned out his aquarium, and stated it was a “bloody chore”. He did not take appropriate prophylaxis prior to/during the trip.
- What do you do?
- A) Bellyache, GOMER, discharge
- B) Give him extra-strength PB and discharge with instruction to f/u with primary HCP next week,
- C) Admit, evaluate for, among other things, malaria, dengue and RMSF
- D) Consult psychiatry

Introduction to Dengue

- Definition: Mosquito-borne flaviviral disease.

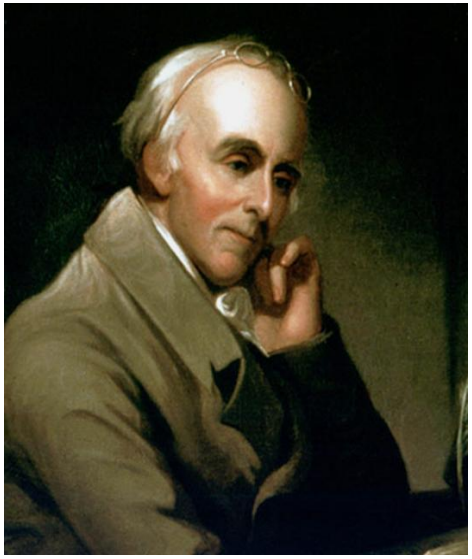
- Etiology:
  Infection with one of four types of dengue virus:
  - DEN-1
  - DEN-2
  - DEN-3
  - DEN-4

- Transmission:
  - Vector: *Aedes* mosquito
    - *Aedes aegypti*
    - *Aedes albopictus*
  - Blood transfusion
  - Organ transplantation
  - No person-to-person transmission documented



History of Dengue

- Clinical descriptions date as far back as 992 AD in China
- David Bylon (Batvia) in 1779
  - “knokkelkoorts” – joint fever
- Benjamin Rush
  - Termed “breakbone fever”
  - Comes from Swahili “ka dinga pepo” meaning a sudden cramp like seizure and plague



Thomas S, Advances in Virus Research 2003.
Kyle J Annu Rev Microbiol 2008. 62:71-92.

http://www91.homepage.villanova.edu/eli.greenbaum/peru.htm

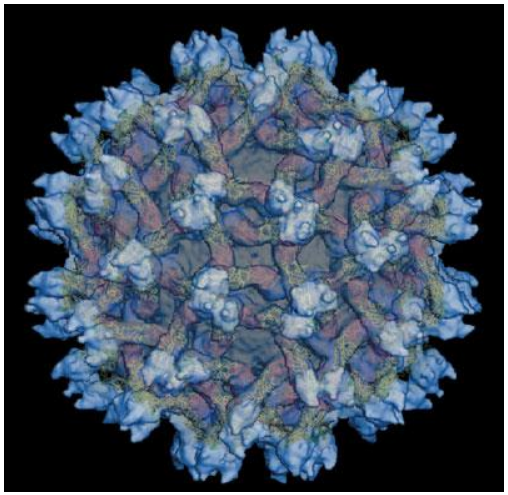
History of Dengue (2)

- Viral etiology suggested in early 1900's by Ashburn and Craig
- Virus types 1 and 2 isolated during World War II
- 1956 outbreak in Manila led to identification of Den-3 and DEN-4
- Dengue hemorrhagic fever recognized since 1950's

Thomas S Advances in Virus Research 2003.
Kyle J Annu Rev Microbiol 2008. 62:71-92.

Dengue Virus

- Flavivirus (single-stranded RNA virus)
- Spherical, 40-50 nm (dia.) viral particle
  - 3 Structural (E, C, M) proteins
  - 7 Nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5)
- 4 serotypes
  - DENV 1 through 4
  - Multiple genotypes per serotype
- Common progenitor 1,000 years ago
- Serotypes have further divergence
  - 62 to 67% homology based on amino acid sequence
- Varying pathogenicity based on serotype



Case 2

- 50 y/o man with multiple mosquito bites after exploring the Amazon during a recent (2 weeks ago) trip. Had been recently web surfing and found out about dengue. He asks you if he should take prophylaxis against dengue. He has been asymptomatic. What do you do?
- A) Admit, put on ribavirin
- B) Reassure

Vector

- *Aedes aegypti* and *Ae. albopticus*
  - Highly susceptible to dengue
    - Efficient vectors
  - Prefers human blood
  - Daytime feeder: interrupted, between laying
    - 0800-1300; 1500-1700
  - Bite goes unnoticed
  - Multiple bites per blood meal; one mosquito can infect several persons
  - Adapted to urban life; breeds in containers
- **RAPID TRANSMISSION, EXPLOSIVE EPIDEMICS**

Larvae

A water sample is teeming with mosquito larvae after it was collected from a fountain outside a vacant house July 15 in Miami Beach, Fla. Miami-Dade County health officials are reporting the first suspected local case of dengue fever, a potentially serious mosquito-borne illness that had once disappeared from the United States.

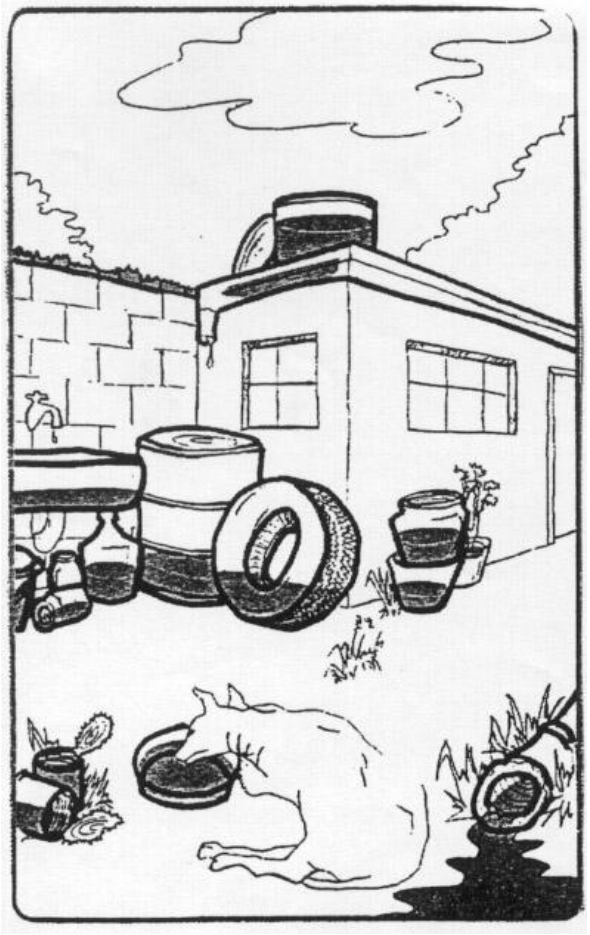
http://www.usatoday.com/news/health/2010-08-03-dengue-fever_N.htm

Life Cycle

http://www.centromedico.com.uy/Novedades/dengue.htm

Habitat

- Breeds in clean, still, stagnant water
  - Discarded tires
  - Water tanks
  - Storage appliances

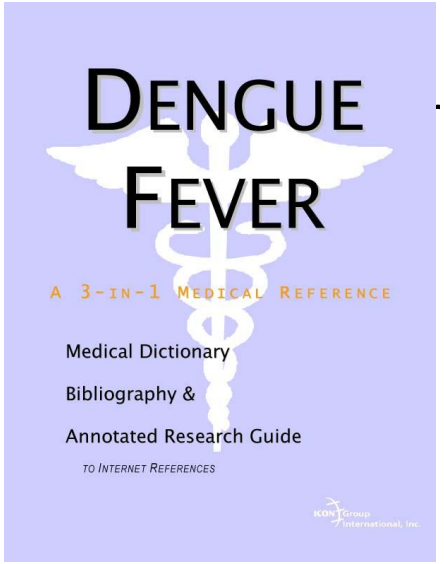


http://www.fcen.uba.ar/habitat/dengue.htm

Breeding sites

Dengue: Epidemiology Assessment

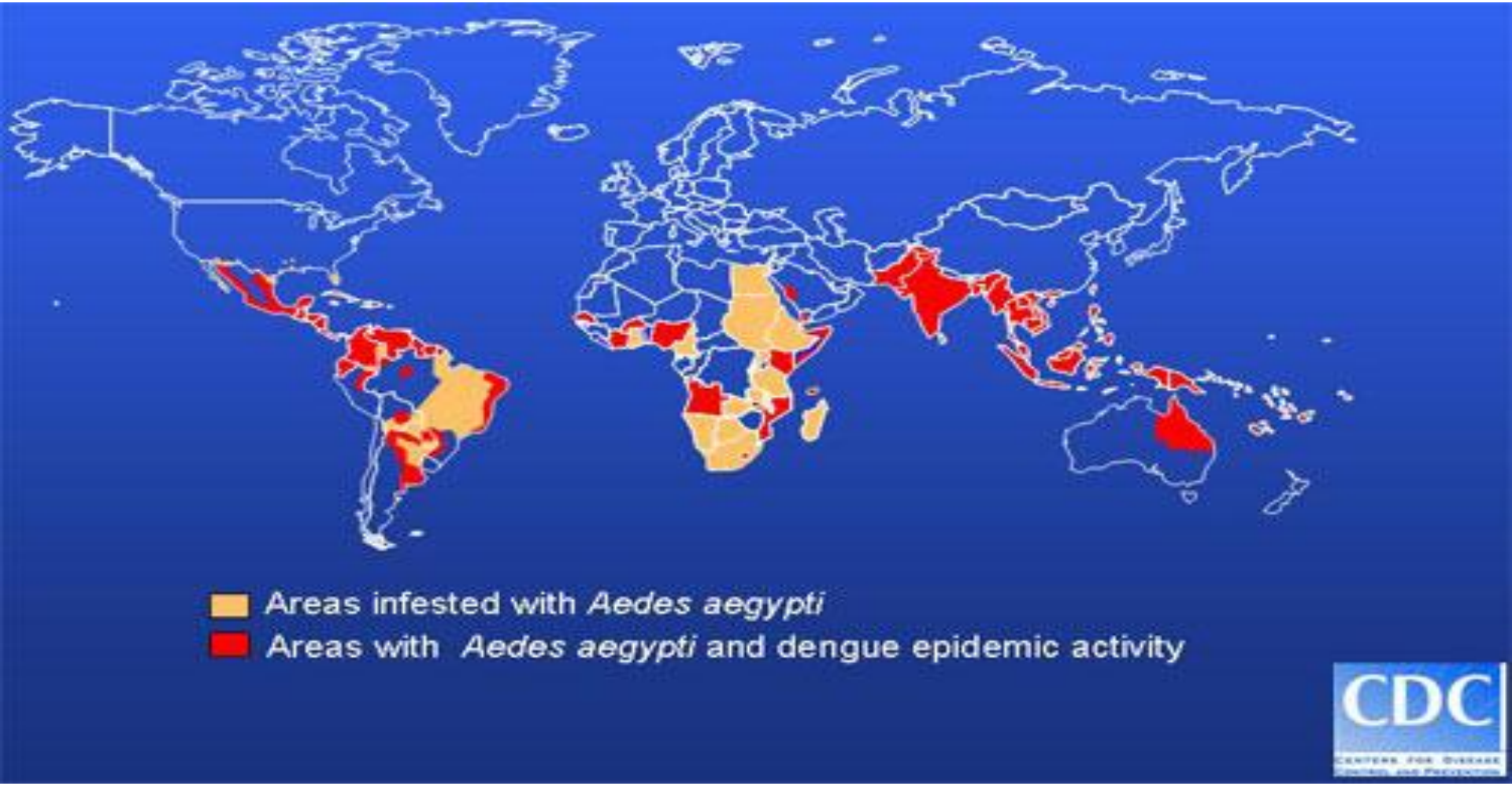
- Leading arboviral (mosquito-borne) infection
- Major health problem in the subtropics and tropics (~35^{o}N and ~35^{o} S)
  - Southeast Asia, India, Middle East, Caribbean, Central and South America, Australia, South and Central Pacific
  - Transmission in ~ 100 countries
  - Recent suspected dengue outbreaks Yemen, Pakistan, Saudi Arabia, Sudan, Cape Verde



Epidemiology Assessment (2)

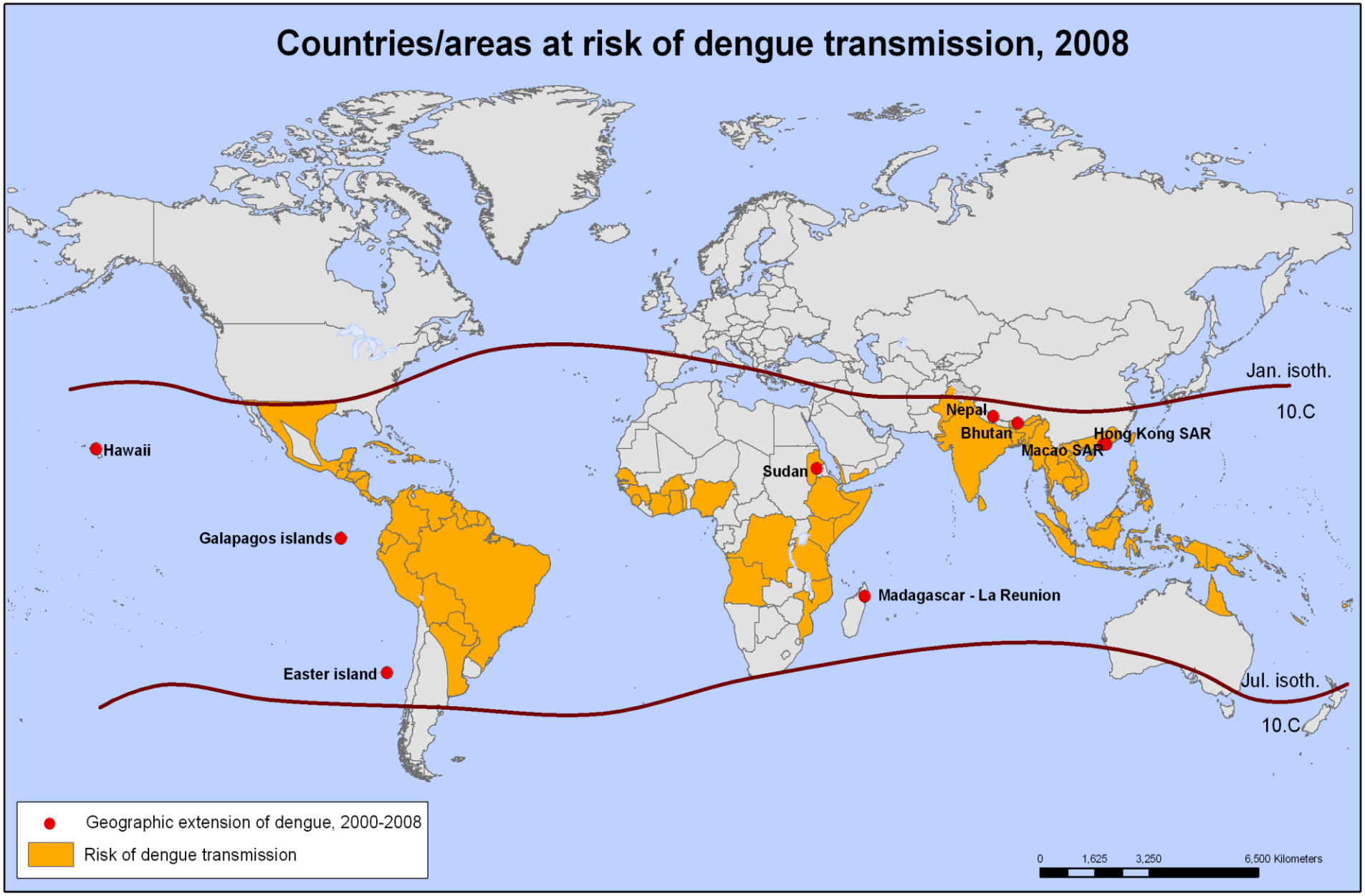
- 2.5 billion people at risk for infection
- 50-100 million infections annually
- ~500,000 cases of DHF annually
- Up to 25,000 deaths annually
- Significant Economic Burden
  - SE Asia: 1,300 disability-adjusted life years
  - Similar to TB, other childhood and tropical diseases

World distribution of dengue viruses and their mosquito vector, *Aedes aegypti*, in 2005



The tropical zone of the world between 350N and 350S latitude and area not over 1,000 ft. above sea level is the usual habitat, the areas are marked by monsoon-rains.

Countries/areas at risk of dengue transmission, 2008



World Health Organization

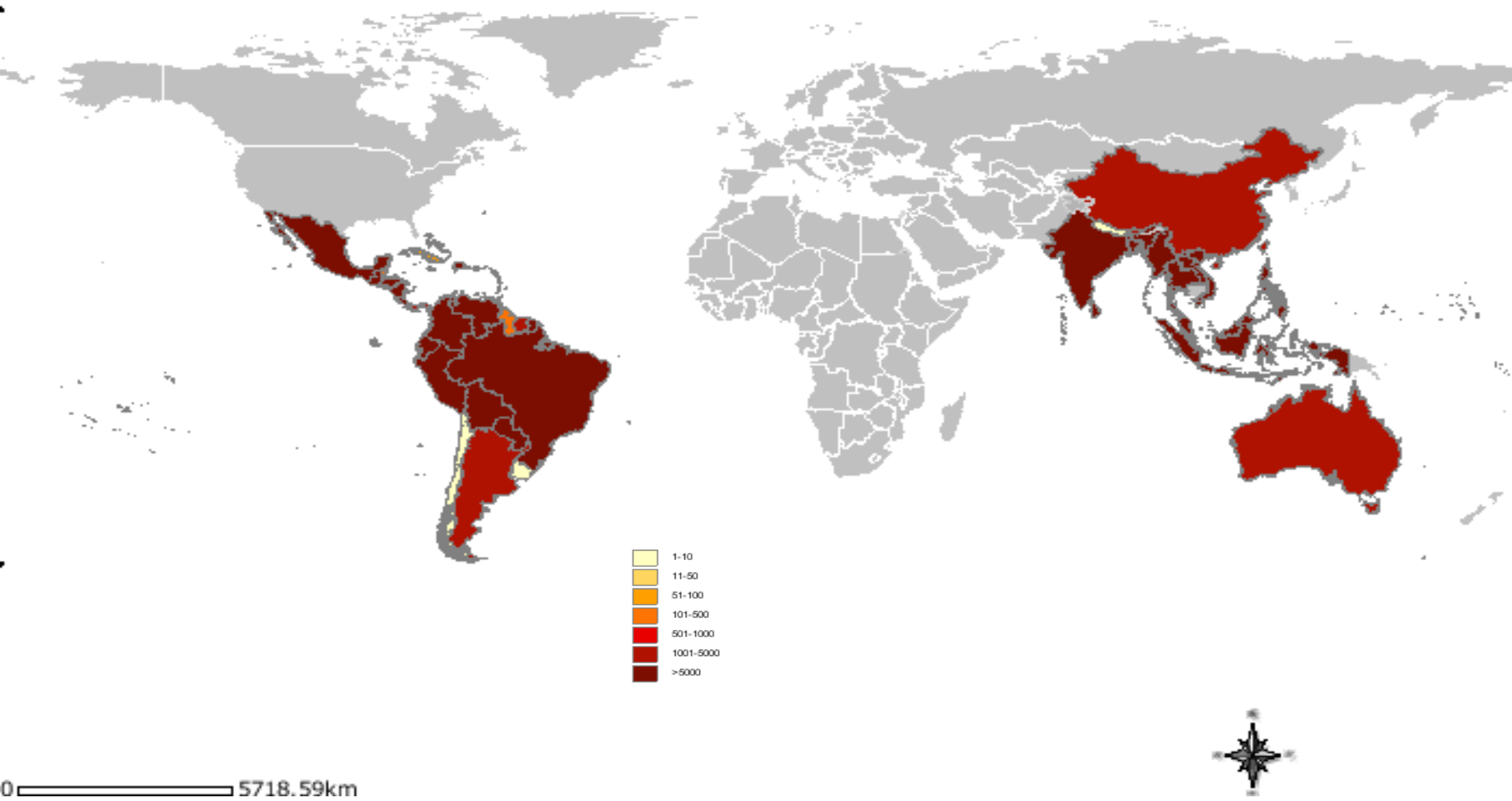
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

© WHO 2008. All rights reserved

Data Source: DengueNet, World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)

http://www.cdc.gov.

Total Dengue Cases and Deaths, 2003-2008



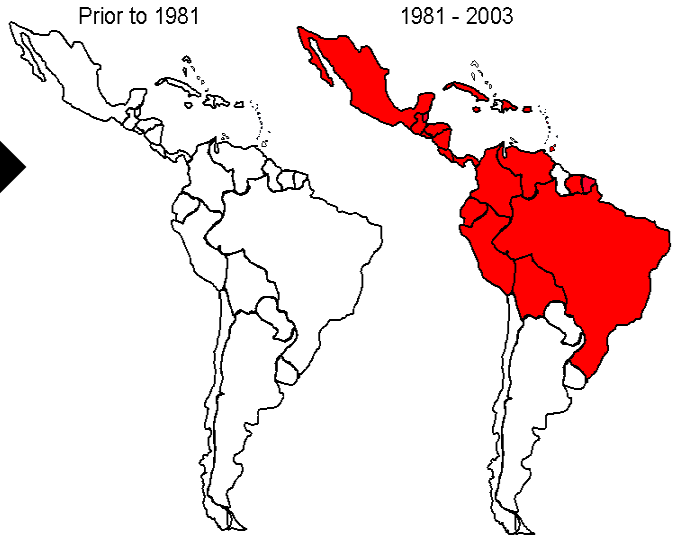
Source: DengueNet

The Global Resurgence of Dengue

- Unprecedented global population growth
- Unplanned and uncontrolled urbanization
- Lack of effective mosquito vector control
- Globalization of trade
- More man-made breeding grounds (waste)
- Increased international air travel
- Decay in public health infrastructure

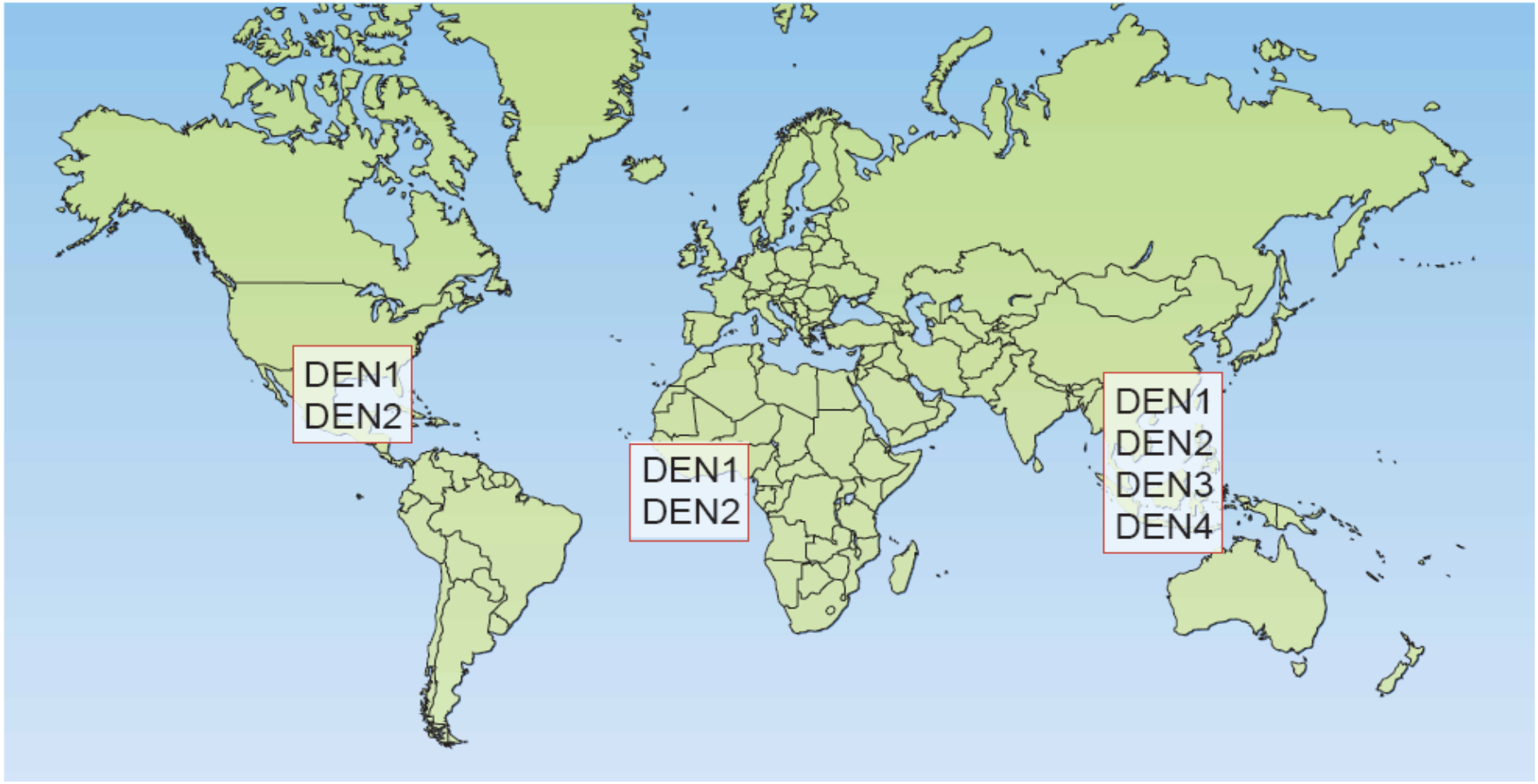
Aedes aegypti in the Americas (1970), at the end of the mosquito eradication program, & in 2002

American Countries with laboratory confirmed dengue hemorrhagic fever, prior to 1981 and from 1981 to 2003

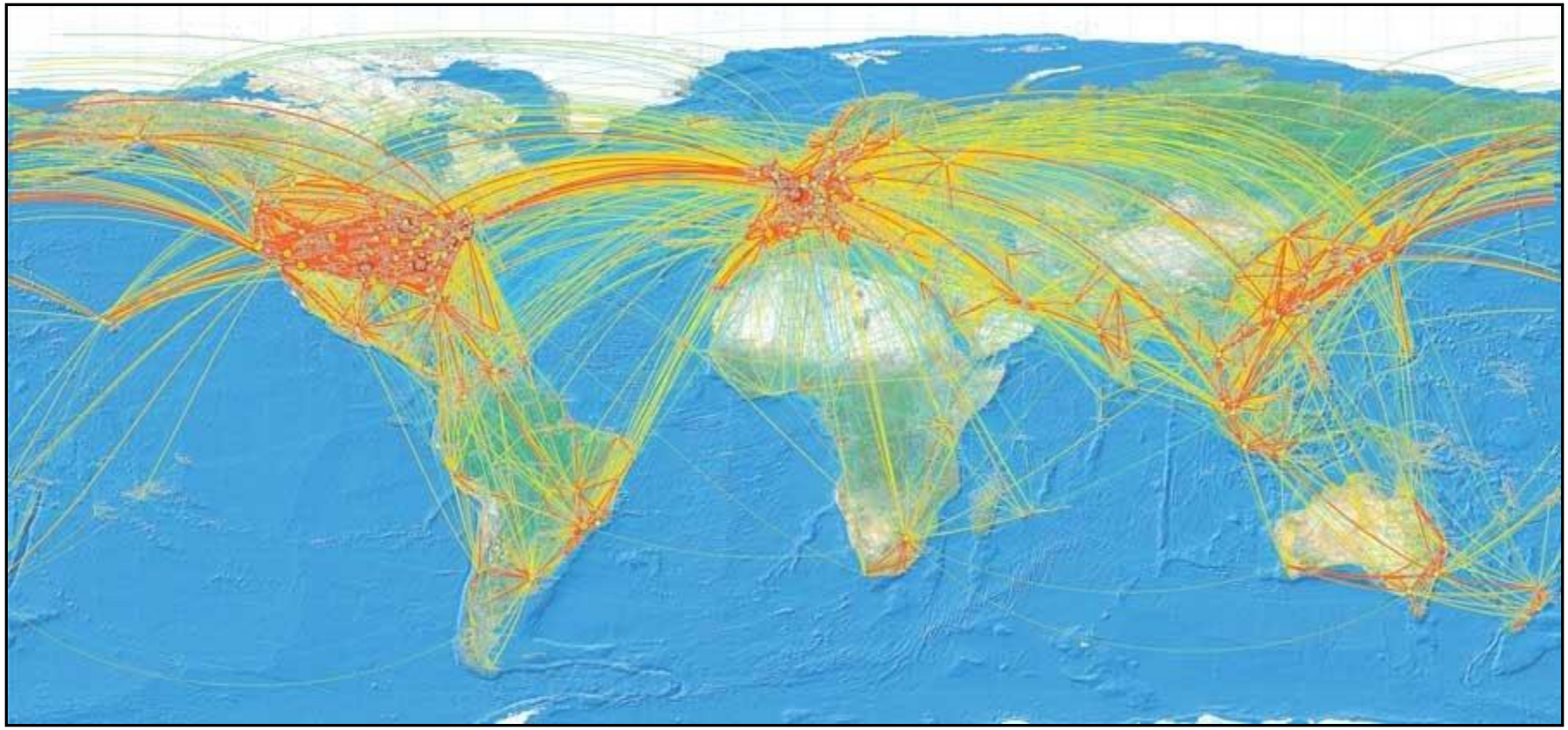


Source: WHO/PAHO/CDC, Aug. 2004

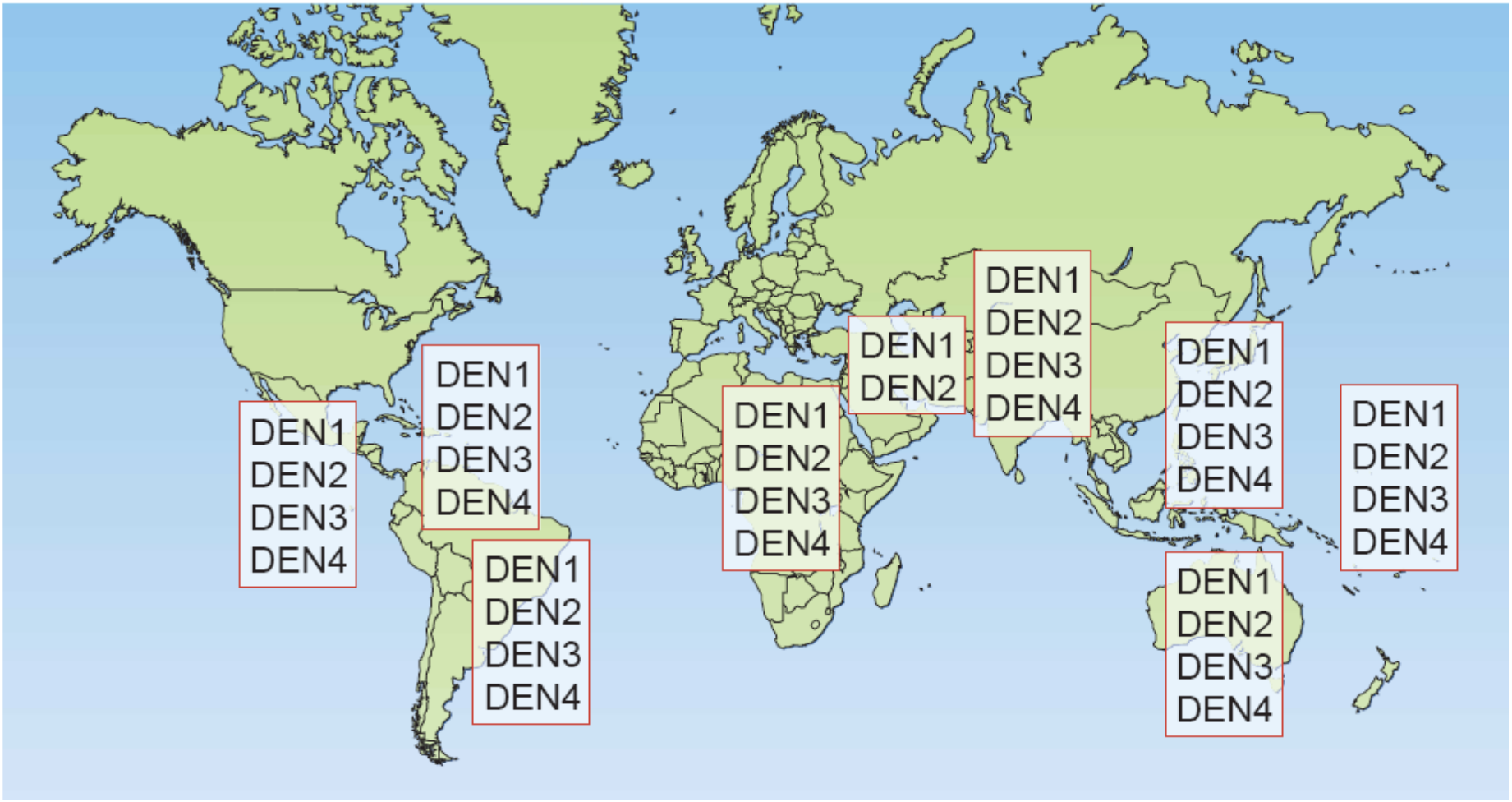
Global distribution of dengue virus serotypes, 1970



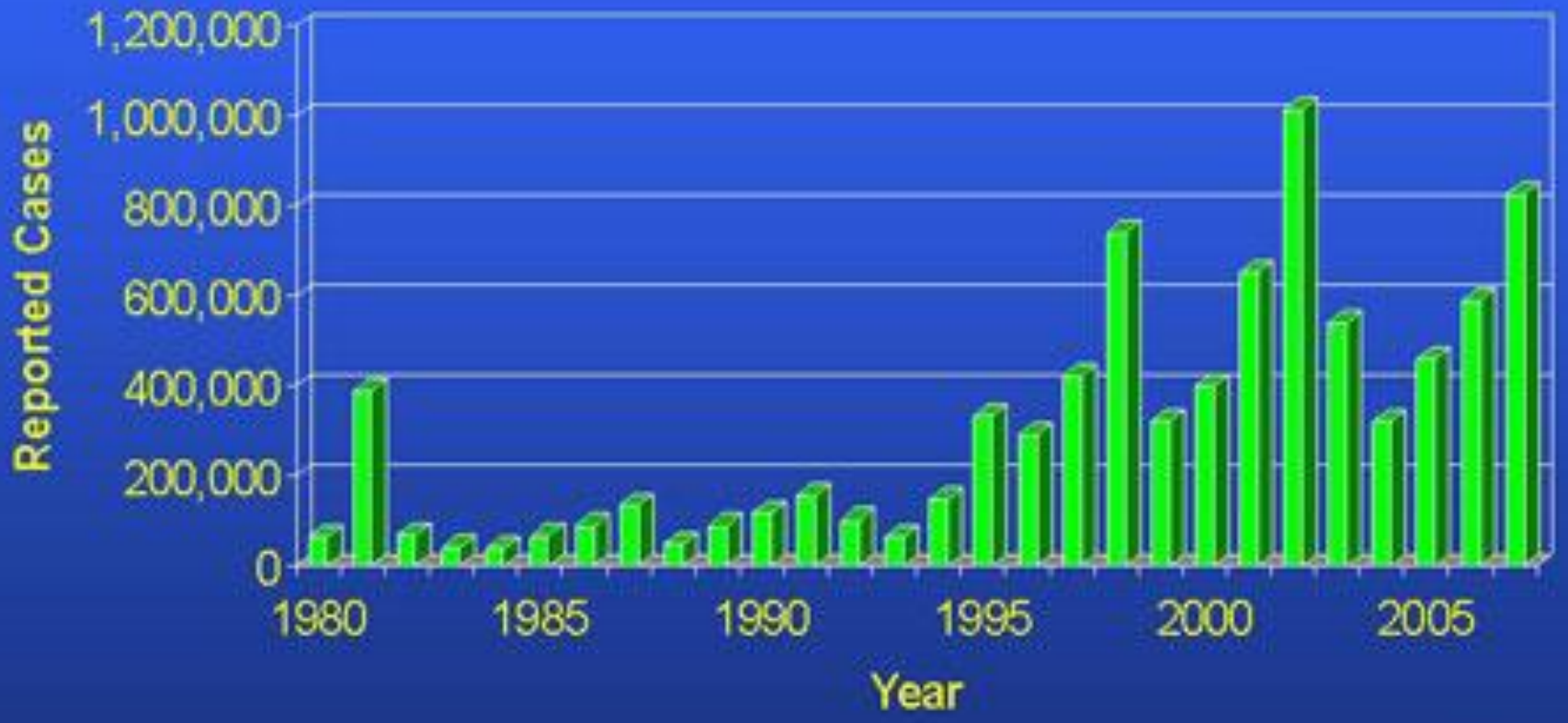
Air Traffic Global Flight Patterns



Global distribution of dengue virus serotypes, 2004



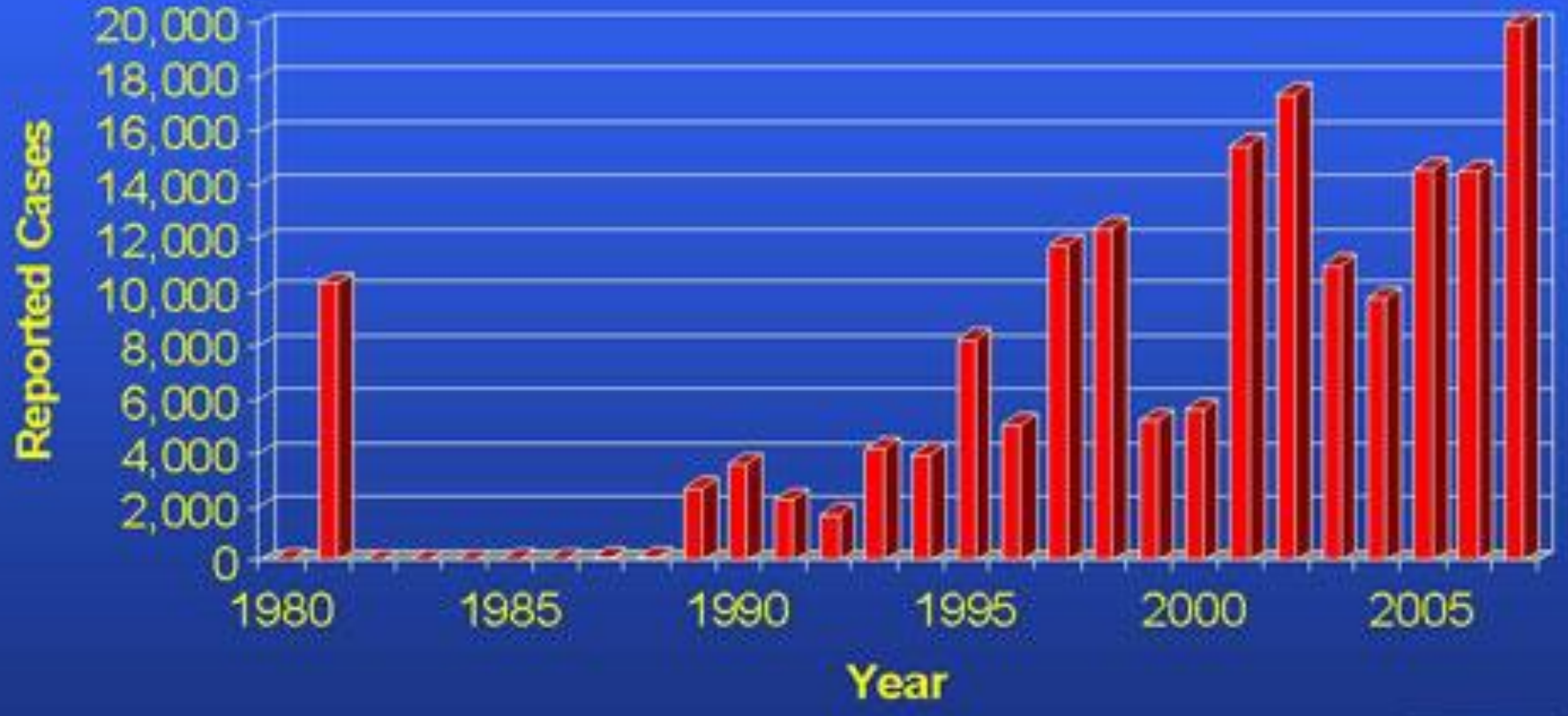
Dengue in the Americas 1980 – 2007 *



* Data: PAHO (Nov. 30, 2007)

CDC

Dengue Hemorrhagic Fever in the Americas 1980 – 2007 *



* Data: PAHO (Nov. 30, 2007)

CDC

Presence of DEN-3 in the Americas, 1994 -2007

1994-1995
1997-2007
Not reported

SOURCE: Pan American Health Organization – Nov. 30, 2007

CDC

Departamento de Salud Secretaría Auxiliar de Salud Ambiental
Programa de Higienización del Ambiente Físico Inmediato
Directora: Dra. Cano
Casos Sospechosos de Dengue Semanal, Año 2010

1	97	97	**27**	516	5,808
2	172	269	**28**	495	6,303
3	151	420	**29**	789	7,092
4	135	555	**30**	734	7,826
5	168	723	**31**	662	8,488
6	193	916	**32**	887	9,375
7	192	1,108	**33**	1,053	10,428
8	180	1,288	**34**	928	11,356
9	244	1,532	**35**		
10	193	1,725	**36**		
11	178	1,903	**37**		
12	173	2,076	**38**		
13	192	2,268	**39**		
14	198	2,466	**40**		
15	218	2,684	**41**		
16	151	2,835	**42**		
17	156	2,991	**43**		
18	172	3,163	**44**		
19	156	3,319	**45**		
20	176	3,495	**46**		
21	192	3,687	**47**		
22	235	3,922	**48**		
23	242	4,164	**49**		
24	330	4,494	**50**		
25	384	4,878	**51**		
26	414	5,292	**52**		

Dengue in the USA

- Some historical dengue outbreaks in the USA
  - 1780: Philadelphia, PA
  - 1826-8: Savannah, GA
  - 1850-1: Charleston, SC, Savannah, GA, New Orleans, LA, Mobile, AL, Galveston, TX, Augusta, GA
  - 1922: Texas, Savannah, GA
  - 1934: Florida
  - 1945: New Orleans

http://www.topnews.in/number-dengue-cases-delhi-reaches-913-2238269

Dengue in the USA (2)

- Recent indigenous transmission
  - Texas:
    - 1980: 23 cases, first locally acquired since 1945
    - 1980-1999: 64 cases (lab-documented)
    - 2005: DEN-2 epidemic in Brownsville; estimated incidence of recent dengue infection (4% of population)
  - Hawaii:
    - 2001-2002: 122 cases (first since 1944)
  - Florida (Key West):
    - 2009-2011: 93 cases (as of 17 May 2011)
    - 6 cases to date in 2011: Miami-Dade (2), Palm beach (2), Martin (1), Hillsborough (1)[1] Counties

[1]Anil L, Stanek D, Blackmore C, Stark L, Mock V.
http://www.doh.state.fl.us/Environment/medicine/arboviral/pdfs/2011/2011Week42ArbovirusReport_10-22-2011.pdf

It is here!

Los Angeles Times | ARTICLE COLLECTIONS

Dengue fever outbreak feared in Key West [Updated]
July 14, 2010|By Thomas H. Maugh II, Los Angeles Times
Federal officials said Tuesday that they fear an outbreak of dengue fever in Florida after a survey of Key West residents found that at least 5% had been infected or exposed to the virus. With the exception of a handful of isolated cases along the Texas-Mexico border, there had previously been no cases in the continental United States since 1946 and no outbreak in Florida since 1934.

WebMD
Better information. Better health.

5% of Key West Population Infected in 2009; New Case Suggests Ongoing Outbreak
By Daniel J. DeNoon
WebMD Health News
Reviewed by Laura J. Martin, MD

May 20, 2010 - An "extended, outbreak" of dengue fever is ongoing in Key West, Fla., where some 5% of residents were infected last fall.
The latest case of the mosquito-borne disease was in mid-April. It's not yet clear whether the April case is a continuation of the 2009 outbreak or a new outbreak from a different dengue strain.
Although only 28 cases have been definitively identified, blood tests conducted in September 2009 detected evidence of recent infection in 5.4% of 240 randomly selected residents.
"The best estimate from the survey is that about 5% of the population of Key West was infected in 2009 with dengue," dengue expert Christopher J. Gregory, MD, of the CDC's Epidemic Intelligence Service, tells WebMD.

Key West Dengue

- RT-PCR done on 1,178 pools of *Ae. aegypti* mosquitoes collected from Monroe County, FL from 27 January-17 December 2010
- DENV-1
- KW sequence grouped as a member of a large clade of recent DV from Central America
  - Nicaragua, 2006, 2008
- Unknown time of introduction into FL

Graham AS, Pruszynski CA, Hribar LJ et al., 2011. Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 17, No. 11, November 2011

Dengue Impact on U.S. Military Operations

- Philippines
- World War II
- Vietnam

Hospitalized US military personnel, Philippines

Dengue Impact on recent U.S. Military Operations

- Somalia
  - Operation Restore Hope (1993)
  - 58/289 (20%) hospitalized febrile troops had laboratory-confirmed DF
  - 69/289 (24%) suspected DF cases
- Haiti
  - Operation Uphold Democracy (1994)
  - 31/103 (30%) hospitalized febrile troops had DF
- Defense Medical Surveillance System (DMSS)
  - 1997-2006: 26 DF cases hospitalized, 170 ambulatory

USASOC Study

- Seroprevalence study
- USASOC personnel deployed to dengue-endemic areas in Latin America
  - At least 30 days, from 2006-2008
  - 500 specimens
  - DoD Serum Repository
  - Sandwich ELISA
- 11.0% seroprevalence rate

Caci JB, Lyons AG, Tack DM; ASTMH Abstract # 90, 2010

Pathogenesis

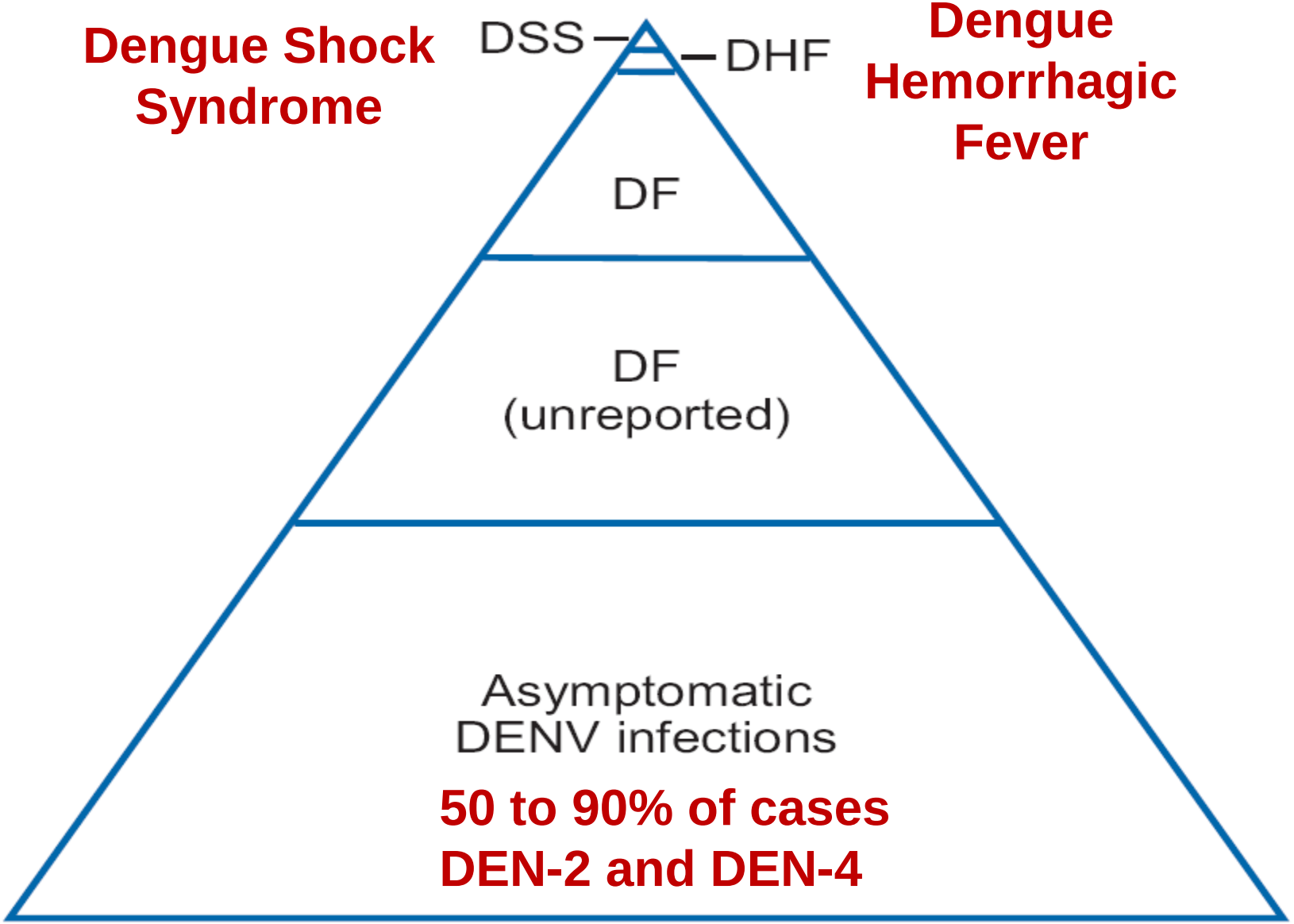
- Multiple theories regarding pathogenesis but none accepted
- Lack of a reliable animal model
- Complicated host and viral interactions
  - Different responses in adults and infants
- Antibody dependent viral enhancement
  - Upregulation of infection
  - Increased cytokine activity
- Unknown etiology of capillary leak syndrome characterized by DSS

Pathogenesis (2)

- No evidence of direct viral infection of endothelial cells[1]
- Transient disruption in the function of the endothelial glycocalyx layer
  - A molecular sieve
  - Hypoalbuminemia, proteinuria
  - DENV and NS1 adhere to heparan sulfate
  - Increased urinary heparan sulfate excretion seen in kids with severe dengue[2]

[1]Leong AS et al. Semin Diagn Pathol 2007;24:227-36
[2]Avirutnan P et al. PLoS Pathog 2007;3(11):e183.

Clinical Manifestations

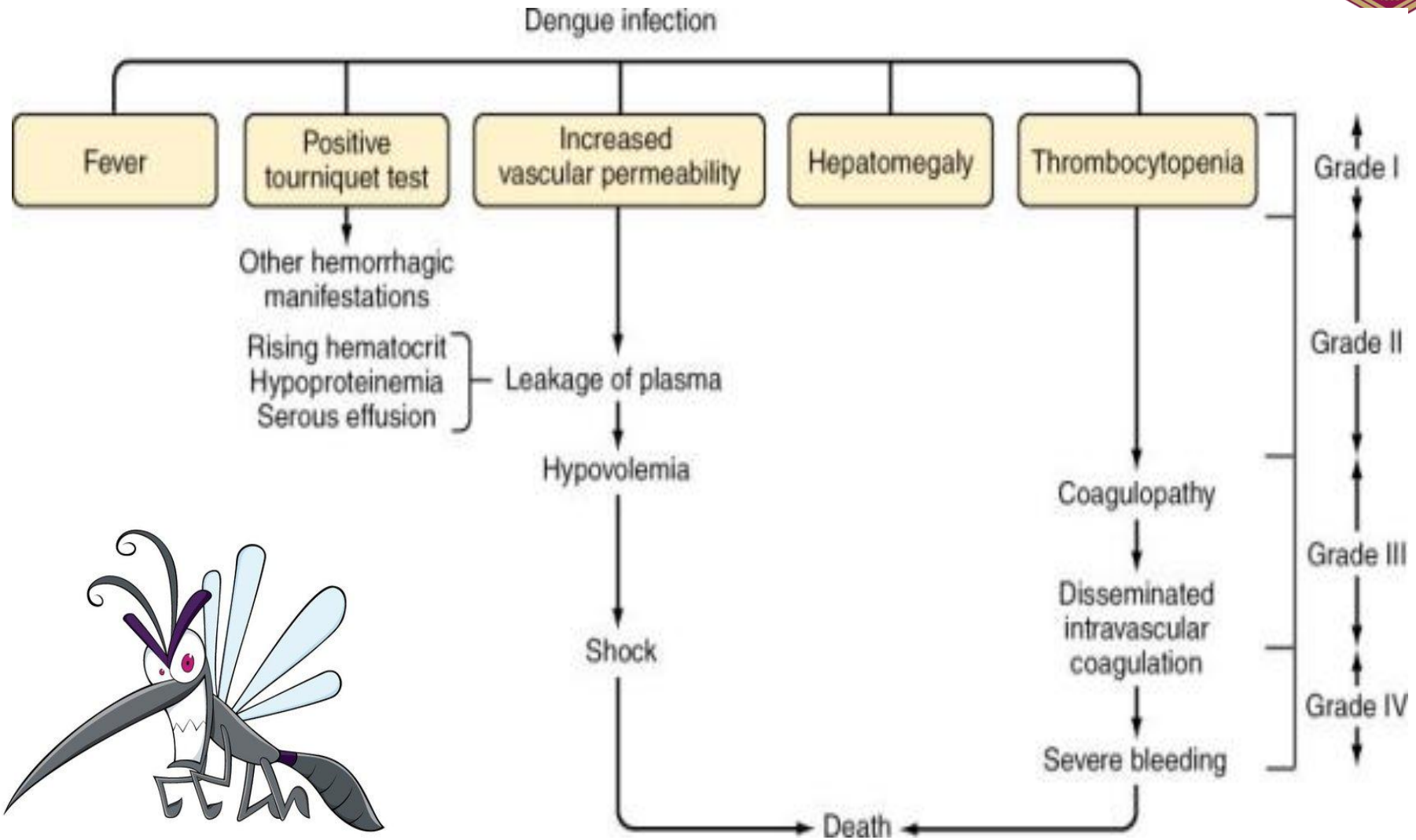


Kyle J Annu Rev Microbiol 2008. 62:71-92.

Clinical Dengue

- Spectrum of clinical illness
  - Primarily defined in Thai cohorts
  - Asymptomatic infection **50%**
  - Undifferentiated fever
  - Dengue fever **45%**
  - Dengue hemorrhagic fever (DHF)/ Dengue shock syndrome (DSS) **<5%**
    - Case fatality rate for DHF <1% with proper medical management; >50% without.

Old Classification of Dengue



http://lukemaciel.deviantart.com/art/Mosquito-da-Dengue-87552543

Old Definition of Dengue Hemorrhagic Fever

- Fever lasting 2-7 days
- Tendency to hemorrhage
  - Positive tourniquet test (TT)
  - Spontaneous bleeding
- Platelet count $<100X10^9$ per litre
- Evidence of plasma leak
  - Increasing hematocrit
  - Pleural effusions

New Classification

- Dengue Fever (DF)
  - Classical DF, recovers without major sequelae
- Severe Dengue
  - Plasma leakage resulting in shock
  - Accumulation of serosal fluid
  - Severe bleeding
  - Severe organ impairment

Dengue: Guidelines for Treatment, Prevention and Control. Geneva, WHO, 2009

Most Dengue infections are…

- Asymptomatic, or
- Mild symptomatic illness
  - Undifferentiated fever
  - +/- Rash

Dengue Fever (DF)

- Incubation period 3-7 days, illness lasts ~7 days

- A range of clinical manifestations
  - Three phases
  - Febrile
  - Critical
  - Recovery

- Epi: Travel/residence in urban areas of tropics/sub-tropics

http://professoralilianbiologia.blogspot.com/2008/04/lilian-cia.html

DF (Day 1, Febrile Phase)

- Abrupt onset high fever (≥38.5^{o} C)
  - 5-7 days fever (biphasic)
- Rash
  - Early flushlike rash may be replaced by a macular/morbilliform rash. Late petechiae
- Chills, vomiting
- Arthralgias, myalgias
- Severe headache
- Eye, Retro-orbital pain
- Lumbosacral pain

http://www.cartunista.com.br/romario_dengue.html

DF (Day 2, Febrile Phase)

- Severe muscle, joint pain
- Nausea, vomiting
- Lassitude, prostration
- Respiratory symptoms
- Epistaxis, gum bleeding, petechiae
  - Classic DF with some hemorrhage is NOT DHF
- PE:
  - Fever
  - Generalized rash (may be replaced by macular/morbilliform later on). Petechial late
  - Relative bradycardia
  - Generalized lymphadenopathy
  - Petechial hemorrhages
- After 3-7 days, no sequelae

Rash

http://www.itg.be/itg/DistanceLearning/LectureNotesVandenEndenE/imagehtml/ppages/CD_1038_061c.htm. Used with permission

Case 3

- 27 y/o AD USMC from Puerto Rico presented with 2 days of increasing fever (>38.5 C), severe headache, rash, arthralgias, myalgias, while on deployment in the Philippines. After 5 days of illness, his fever suddenly resolved. Should you:
- 1) Discharge
- 2) Draw labs and observe

Symptomatic Dengue Clinical Syndromes

Dengue Fever (DF)

Dengue Hemorrhagic Fever (DHF)

Dengue Shock Syndrome (DSS)

Critical Phase

- Small proportion of patients
  - Children, young adults
- Occurs at time of defervescence
  - Around days 4-7 of illness
- Systemic vascular leak syndrome
  - Increasing hematocrit
  - Hypoproteinemia
  - Pleural effusions
  - Ascites

Severe Dengue

- Severe plasma leakage
  - Shock (DSS)
  - Serosal fluid accumulation with respiratory distress
- Severe bleeding
  - Clinically evident
- Multi-organ involvement
  - Liver: AST/ALT >1000
  - CNS: Impaired consciousness, seizures, encephalopathy
  - CV and other

DHF

http://www.pattayagogos.com/news08a.htm
http://denguehemorrhagicfever.tumblr.com/

Dengue Hemorrhagic Fever (DHF)

- Onset as per classical dengue
- Damage to blood and lymph vessels
- Defervescence followed (2-5 days) by
  - Ascites, abdominal pain
  - Pleural effusion
  - Hemorrhagic manifestations (gum bleeding, phlebotomy bleeding) which may progress to shock
  - Central cyanosis
  - Diaphoresis
- Epi: Exposure in dengue endemic region with possible previous dengue infection

DHF (2)

- PE:
  - Restlessness
  - Abdominal pain
  - Hemorrhage
    - Petechiae
    - Spontaneous ecchymoses
    - Bleeding: GI, GU, phlebotomy
  - Tender hepatomegally (75%), splenomegally
  - Pleural* effusions (80%) perirenal effusions (77%), hepatic, splenic, pericardial, peritoneal effusions* (variable%)
  - Shock
    - Rapid, weak pulse
    - Pulse pressure <20 mmHg
    - Unobtainable BP

Lab

- Positive tourniquet test (or hemorrhagic manifestations)
- Thrombocytopenia (<100,000)*
- Increase in aPTT, decrease in fibrinogen
- Plasma leakage
  - Hemoconcentration (Hct. inc. >20%)*
  - Pleural effusion/ascites
  - Petechiae
- Hepatorenal shutdown with shock
- Viral isolation from acute serum
- Convalescent IgM (+)
- Peak proteinuria**
  - 0.56 v. 0.08 g/d (P<0.001), onset 1 day after defervescence (-2 to 3 days)

**Vasanwala FF et al. BMC Infect. Dis., 2011, Aug 5, 11(1): 212.

Dengue Shock Syndrome (DSS)

- Fluid leak outside of blood vessels
- Lasts 1-2 days
- Massive hemorrhage
- Shock, peripheral vascular collapse
  - Hypoperfusion c/b myocardial dysfunction: metabolic acidosis and MOF
- Cyanosis, massive pleural effusions, ascites
- Narrowing pulse pressures (<20 mmHg)
- Can be fatal (50% in underserved populations; 1% in established centers)

Subcutaneous hemorrhage in DHF

Risk Factors for DHF/DSS

- Pre-existing immunity from previous infection (heterogenous subtype)
- Diabetics, asthmatics, other chronic diseases
- DENV type
  - DENV-1,3 > 2,4
- Increased time between infections
- Under age 15
  - Increased capillary fragility
- HLA type and race*
  - Caucasian>AA
  - HLA Class-1 alleles
- Female sex
- AB blood group
- Promotor variant of DC-SIGN receptor
- Single-nucleotide polymorphism in TNF gene

*De la C Sierra B, Kouri G, Guzman MG. Arch. Virol., 2007, 152(3) 533-42. Epub 2006 Nov. 16.

Factors that reduce the risk of severe dengue

- Race
- Second or third degree malnutrition
- Polymorphisms in the Fc-gamma and Vitamin D receptor genes

Criteria For Dengue +/- Warning Signs

- Probable case:
  - Resident/travel to dengue endemic area and 2 of the following:
  - Nausea, vomiting
  - Rash
  - Aches and pains
  - + TT
  - Leukopenia
  - Any warning sign

Warning Signs

- Continual/increasing abdominal pain/tenderness
- Persistent vomiting
- Clinical fluid accumulation (serosal)
- Mucosal bleeding
- Lethargy, restlessness
- Tender hepatomegaly (>2 cm), ascites
- Lab: increase in Hct. concurrent with rapid decrease in platelets*
- Sudden reduction in temperature

Recovery Phase

- Altered vascular permeability syndrome resolves
  - After 48-72 hours
- Rapid improvement in patient's symptoms
- Rash
  - Mild maculopapular to severe, pruritic lesions (leukocytoclastic vasculitis)
  - Resolves with desquamation (1-2 weeks)
- Profound fatigue for several weeks

Case 4

- 30 y/o AD Sailor who recently returned from a TDY to Thailand 4 days ago. Has had 2 days of fever, excruciating HA, eye pain, severe myalgias, arthralgias, sweats and rash. You suspect dengue.
- How to diagnose?
- How to treat?

Lab

- Marked leukopenia
- Thrombocytopenia
- Moderate elevation of AST/ALT
- Viral isolation to Day 5 only
- Negative malaria smears
- Dengue IgM (+) on Day 6 serum
  - Takes 5 days to manifest
- PCR available
- Convalescent: 4-fold rise in IgG may be required

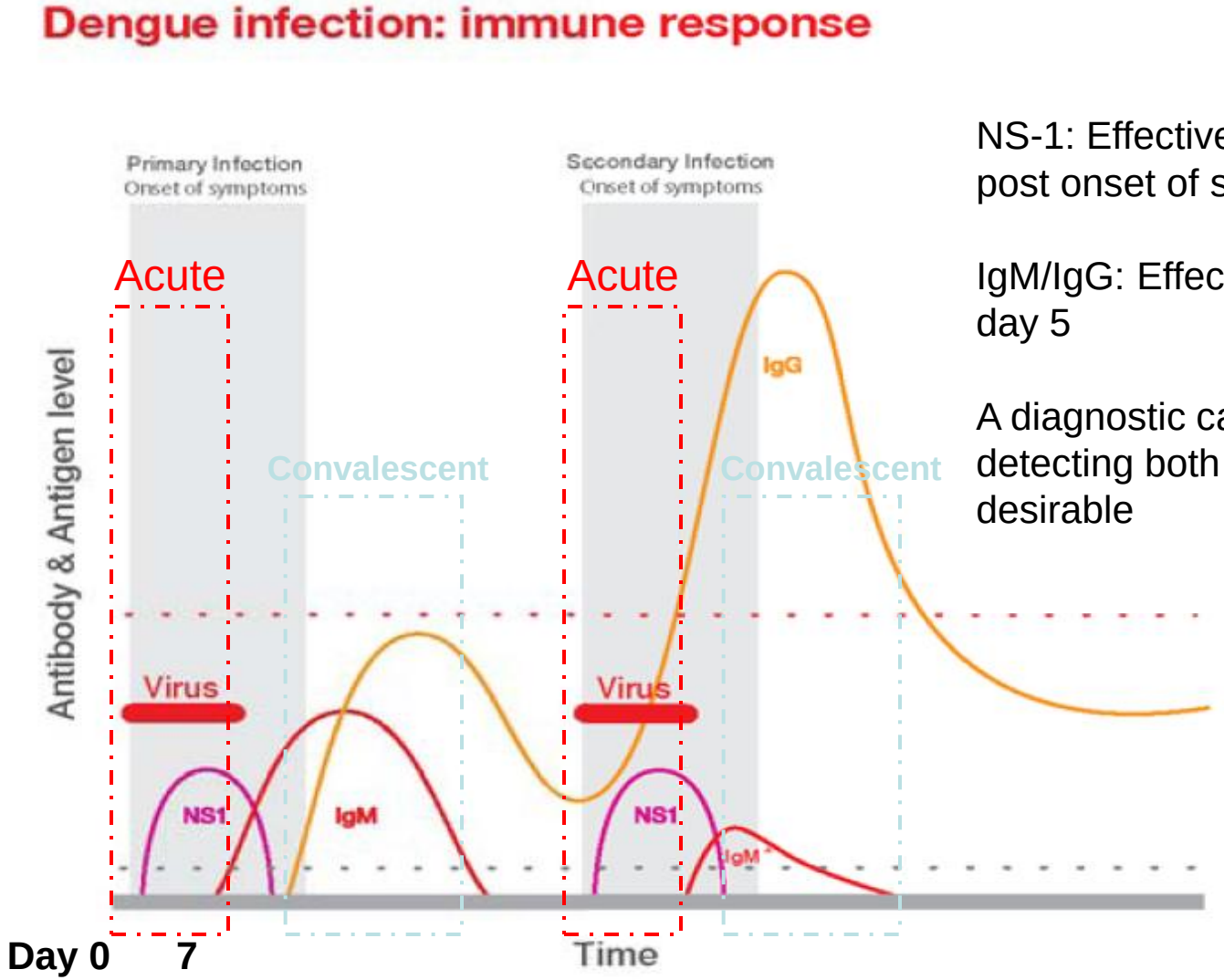
Lab (2)

- Antibody specificity increases over time
- Most readily available diagnostic tests
  - ELISA (serology)
    - Cross-reactive; not specific
    - MAC-ELISA, IgG ELISA
    - IgM/IgG (>1.2, 1.4?) not defined
  - PRNT, microneutralization (serology)
    - More specificity
    - Research, vaccine work
  - Viral Isolation
    - Most specific

Lab (3)

- Nucleic Acid Amplification (NAAT)
  - RT-PCR
  - Real Time RT-PCR
  - NASBA
  - None commercialized to date
  - None standardized
- Antigen Detection
  - NS1
  - Antigen capture ELISA, lateral flow antigen detection, NS1 IgM, IgG responses.
  - Do not differentiate between the different serotypes

Immune Response to Dengue



NS-1: Effective days 1-5 post onset of symptoms

IgM/IgG: Effective after day 5

A diagnostic capable of detecting both is desirable

Slide courtesy of Dr. Subhamoy Pal

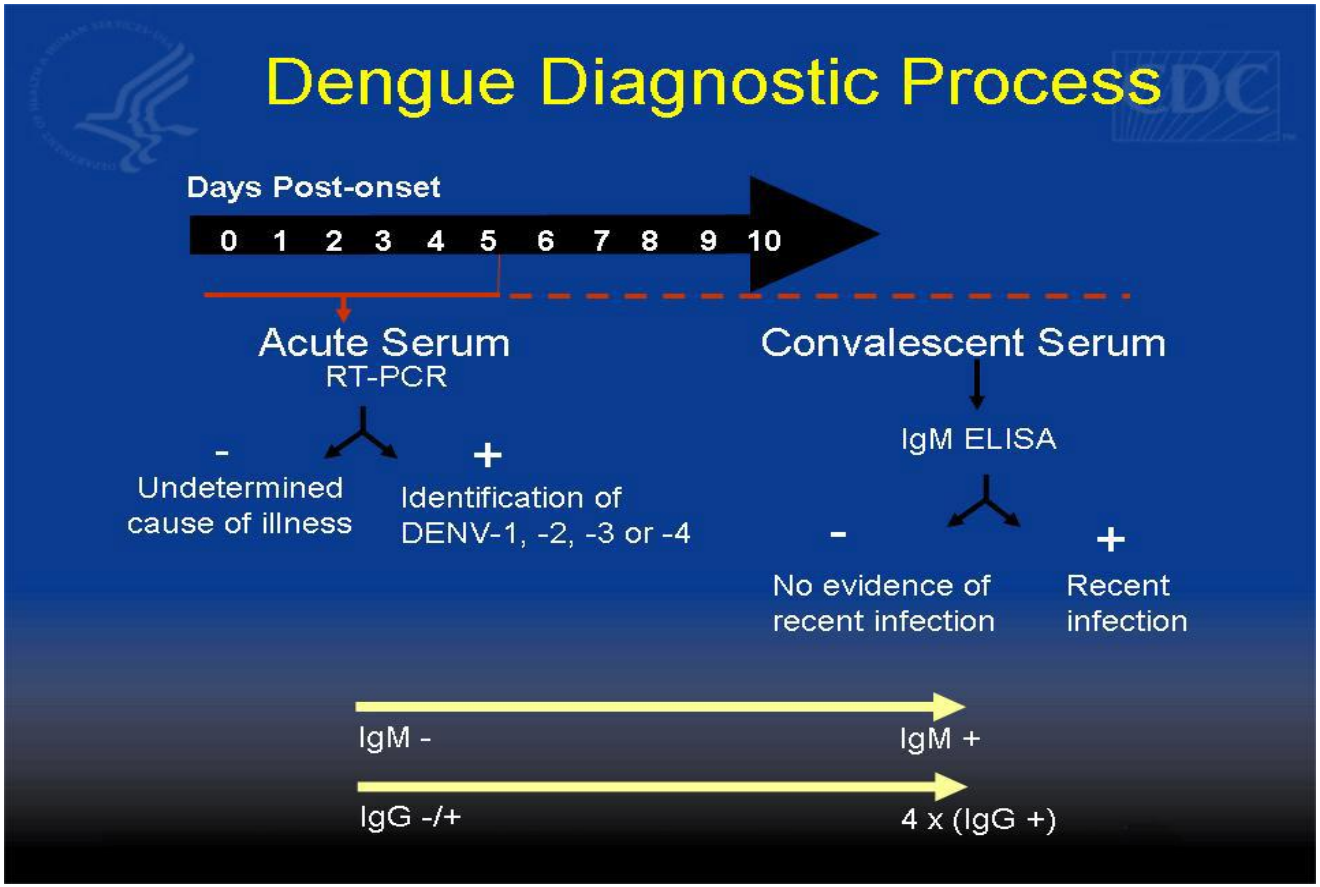
Lab (4)

- Primary infection
  - IgM first to appear, at end of 3-5 day fever period (~50%), day 6-10 (93-99%), peak (2 weeks), undetectable by 2-3 mos.
  - IgG appears at end of first week of illness, persists for >year
  - RT-PCR can provide a same-day diagnosis with a similar sensitivity to culture
- Secondary infection
  - IgM typically LOWER titer than primary infection; false negatives have occurred
  - IgG typically HIGHER titer than primary infection; may x-react with other flaviviruses (JE, YF, WN)

Criteria for Confirmed and Probable Dengue Infection

- Confirmed
  - Viral isolation
  - Genome detection
  - Antigen detection
  - IgM/IgG seroconversion
- Probable
  - IgM positive
  - Elevated IgG titer (> 1,280 by HAI)

Diagnosis



http://www.cdc.gov/dengue/clinicalLab/diagnosticProcess.html

Tests Used for the Lab Diagnosis of Primary Dengue Infection

Test	Diagnostic Window	Sample Required	Sample Storage	Turnaround Time
Viremia (Culture)	Acute Phase	1 mL	-80°C	2 weeks
RT-PCR	Acute Phase	140 µL	Refrigerate if <6 hrs, if >, -20°C	1-2 days
IgM ELISA	Day 4 to –Day 90 post infection	1 mL	Frozen or refrigerated	1-2 days
IgG ELISA	Day 14 to > 1 year post-infection	1 mL	Frozen or refrigerated	1-2 days
PanBio duoCassette	Day 4 to Day	10 µL	Refrigerate if <6 hrs, if >, -20°C	1-2 days
Serum Neutralization (PRNT)	1 week to >1 year post-infection	1 mL	Frozen or refrigerated	1 week

SST or red top tube
Virus isolation in cell culture and detection by IFA
Used with IgG ELISA to differentiate primary from secondary infection
Used with IgM ELISA to differentiate primary from secondary infection

Rapid Diagnostic Tests (RDT's)

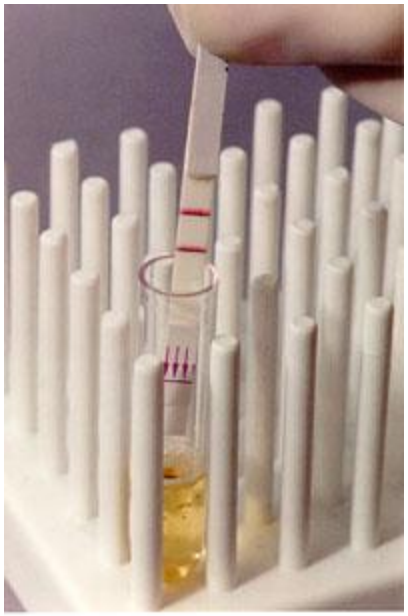
Important for:

- Quick diagnosis (lab results take time and require labs)
- In resource-limited settings
- Alerts a unit to ID threats
- Helpful for triage during outbreaks
- Curtail geographic spread of infectious diseases
- **Stability operations and infrastructure building**

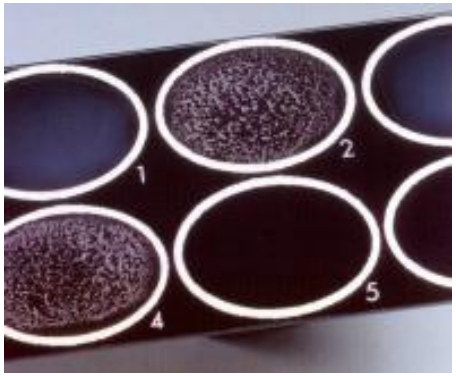
Worldwide demand for better diagnostics to manage treatment and prevention

Slide courtesy of Dr. Subhamoy Pal

Current Rapid Diagnostic Technologies

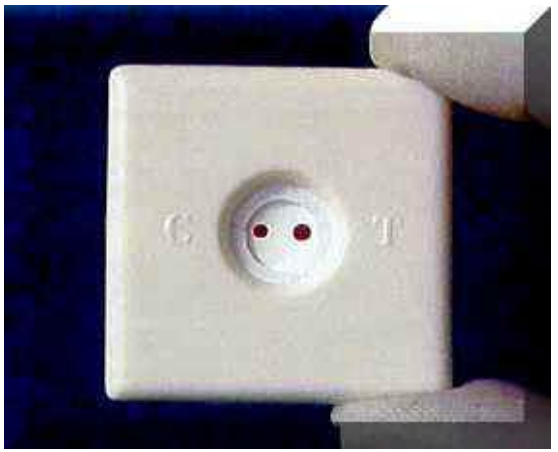


Lateral Flow



Agglutination

Solid Phase



Flow through

Isothermal Nucleic Acid Tests

Slide courtesy of Dr. Subhamoy Pal

Product Introduction

#1: IgG/IgM Dengue Duo Cassette

10µL of serum, plasma, or whole blood

15 minutes (time to result)

Wu et. al. CDLI 2000, pp 106-109

CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Jan. 2000, p. 106–110 Vol. 7, No. 1
1071-412X/00/$04.00+0
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Comparison of Two Rapid Diagnostic Assays for Detection of Immunoglobulin M Antibodies to Dengue Virus

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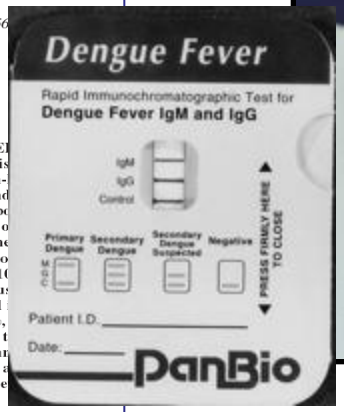
Received 25 June 1999/Returned for modification 4 August 1999/Accepted 18 October 1999

Two easy-to-use commercial diagnostic assays, a dipstick enzyme-linked immunosorbent assay (EI (Integrated Diagnostics, Baltimore, Md.) and an immunochromatographic card assay (PanBio, Bris Australia) were evaluated for detection of immunoglobulin M (IgM) antibody to dengue virus with an in- IgM antibody capture microplate ELISA as a reference assay. The dipstick ELISA was based on the ind ELISA format using dengue 2 virus as the only antigen and enzyme-labeled goat anti-human IgM antibo the detector. The total assay time was 75 min. The immunochromatographic card assay was based o antibody capture format and separately measured both anti-dengue virus IgM and IgG in the same Colloidal-gold-labeled anti-dengue virus monoclonal antibody bound with dengue virus 1 to 4 antigen co was the detector, and anti-human IgM and IgG were the capture antibodies. The total assay time was <10 Sera from 164 individuals classified as either anti-dengue virus IgM positive (94) or anti-dengue virus negative (70) in the reference microplate ELISA with a dengue virus 1 to 4 antigen cocktail were tested two commercial assays. The dipstick ELISA missed 7 of 94 positive samples, for a sensitivity of 92.6%, the immunochromatographic card assay missed two positive samples, for a sensitivity of 97.9%. Of t negative samples, four were false positive by the dipstick ELISA and two were false positive in the imm chromatographic card assay, resulting in specificities of 94.3 and 97.1%, respectively. Both commercial a provide sensitive and specific detection of anti-dengue virus IgM antibody and could prove useful in se where the microplate ELISA is impractical.



Slide courtesy of Dr. Subhamoy Pal

Product Introduction cont'd

#2: NS-1/IgG/IgM Dengue Duo Cassette

120µL of serum or plasma

15 minutes (time to result)

Osorio et al. Virology Journal 2010, 7:361

RESEARCH Open Access

Comparison of the diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue infection

Lyda Osorio[1*], Meleny Ramirez[1], Anilza Bonelo[2], Luis A Villar[3], Beatriz Parra[2]

Abstract

Background: We compared the diagnostic accuracy and reproducibility of commercially available NS1-based dengue tests and explored factors influencing their sensitivities.

Methods: Paired analysis of 310 samples previously characterized as positive (n = 218) and negative (n = 92) for viral isolation and/or RT-PCR and/or IgM seroconversion. Masked samples were tested by two observers with Platelia™ Dengue NS1 Ag, second generation Pan-E™ Dengue Early ELISA, SD Dengue NS1 Ag ELISA, Dengue NS1 Ag STRIP™, and SD BIOLINE™ Dengue Duo (NS1/IgM/IgG).

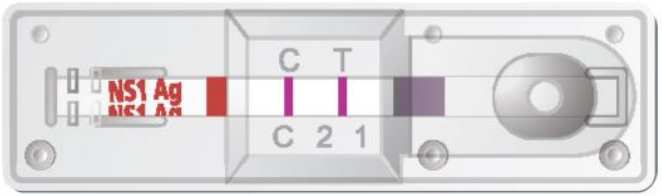
Results: SD BIOLINE™ NS1/IgM/IgG had the highest sensitivity (80.7% 95%CI 75-85.7) with likelihood ratios of 7.4 (95%CI 4.1-13.8) and 0.21 (95%CI 0.16-0.28). The ELISA-format tests showed comparable sensitivities; all below 75%. STRIP™ and SD NS1 had even lower sensitivities (<65%). The sensitivities significantly decreased in samples taken after 3 days of fever onset, in secondary infections, viral serotypes 2 and 4, and severe dengue. Adding IgM or IgG to SD NS1 increased its sensitivity in all these situations.

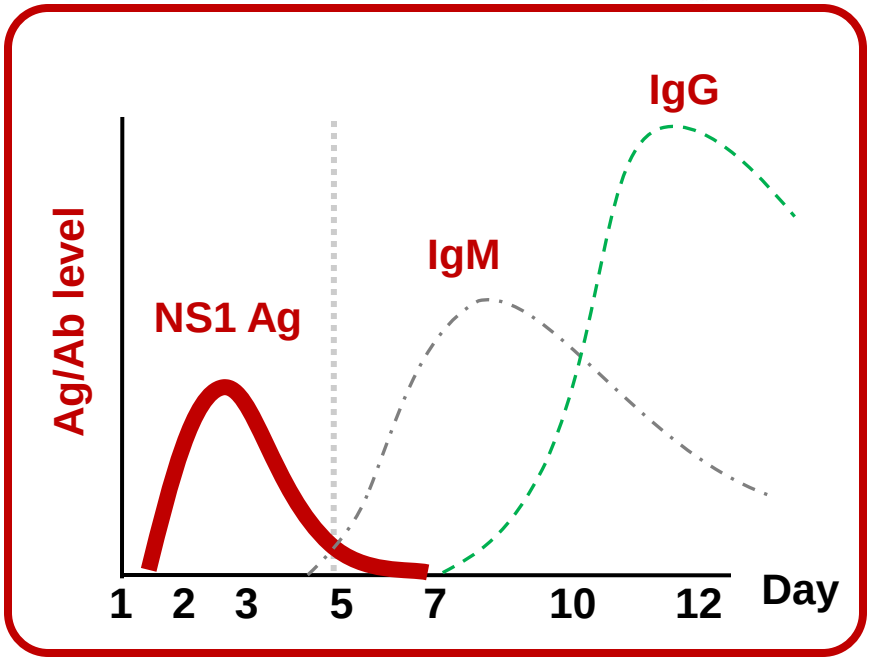
Slide courtesy of Dr. Subhamoy Pal

Standard Diagnostics Dengue Duo (NS-1) RDT

NS1 Ag

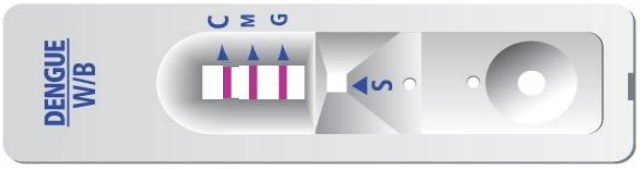
3 drops (110 µl) of plasma or serum for early acute phase samples (day 1 ~5)

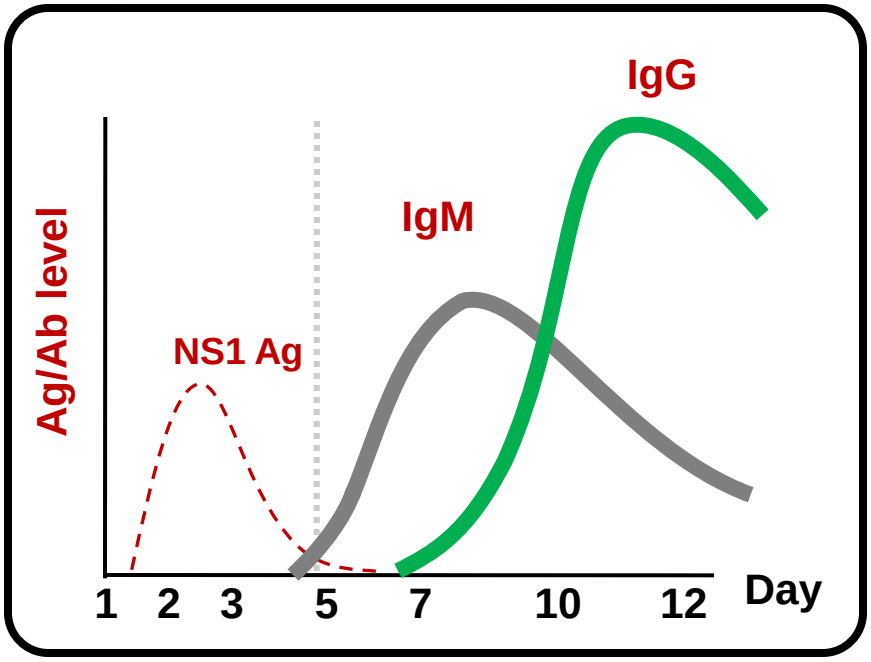




IgG/IgM Ab

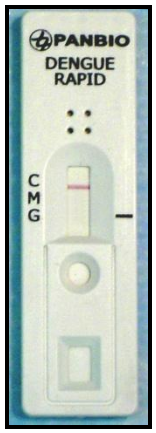
10 µl of plasma or serum for early convalescence phase samples (after day 5 ~ 14)

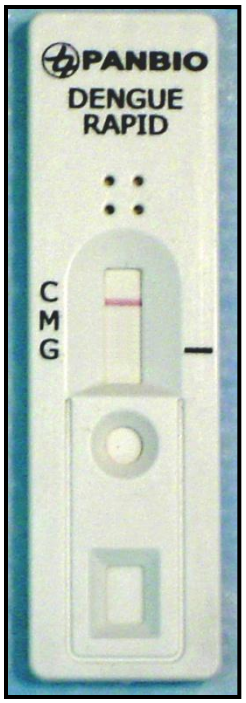




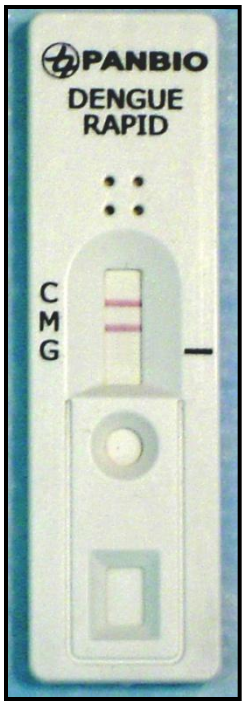
Slide courtesy of Dr. Subhamoy Pal

Interpretation

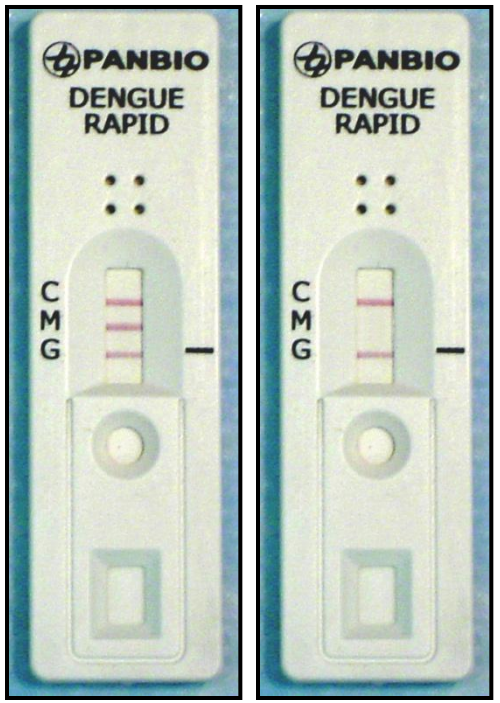




Negative



Primary



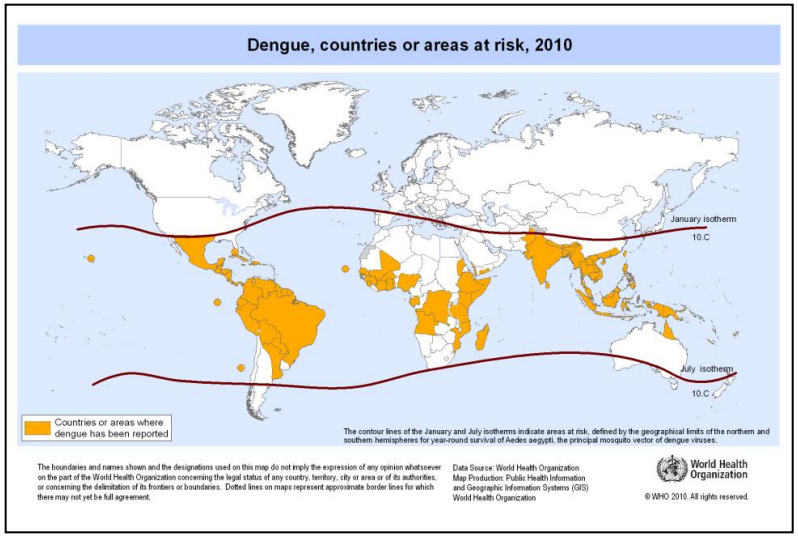
Secondary

Slide courtesy of Dr. Subhamoy Pal

Why make a primary/secondary determination?

- The majority (>90%) of DHF/DSS cases are secondary infections
  - One 20 year longitudinal study suggests that among all DHF/DSS cases 9% are primary and 91% are secondary (Nisalak, A., et al., Am J Trop Med Hyg, 2003. 68(2): p. 191-202)
- Overall, 2-4% of secondary infections proceed to severe dengue. Other risk factors also need to be considered (Guzman, M.G., et al.,*1997.* Am J Epidemiol, 2000. 152(9): p. 793-9; discussion 804)

Positive predictive value of secondary infection leading to DHF varies by region and attack rate.



Slide courtesy of Dr. Subhamoy Pal

Commercially Available NS-1 Products

- Rapid Tests
  - Bio-Rad Strip™
  - SD (Focus) BIOLINE Dengue NS1 Assay
  - SD (Focus) BIOLINE Dengue Duo IgM/IgG/NS1 Assay
  - Panbio Dengue Early Rapid
- ELISA format
  - Panbio Dengue Early PanE (2nd Generation)
  - SD NS-1 Dengue Ag ELISA
  - Bio-Rad Platelia™ Dengue NS1 Ag
    (Evaluated by NMRC)

Slide courtesy of Dr. Subhamoy Pal

Assay specifications

Parameters	SD Rapid	Biorad Rapid	Panbio Rapid	BioRad ELISA	Panbio ELISA
Number of steps	1	2	3	5	7
Blood matrices	EDTA-treated blood, plasma, sera	Plasma, sera	Sera	Plasma, sera	Sera
Assay Time	15-20 minutes	15-30 minutes	15 minutes	140 minutes	160 minutes
Volume necessary	105 uL	50 uL	50 uL	50 uL	75 uL
Format	Cassette	Dipstick	Dipstick	96-well	96-well
Extra materials required	No	Tubes, pipette	Pipette	Pipette, incubator, plate reader	Pipette, incubator, plate reader
Storage	Room Temp.	2-8°C	2-8°C	2-8°C	2-8°C

Slide courtesy of Dr. Subhamoy Pal

Summary of data

Panbio Dengue IgM/IgG Duo Cassette RDT

- Down-selected from among several Dengue RDT's
- Meets KSA and Attributes of draft CDD
- Marketed overseas with record of stability
- Ideal after day 5 post-onset of symptoms

SD NS-1 Cassette RDT's developed recently

- Available NS-1 RDT's comprehensively evaluated
- Meets KSA and Attributes of draft CDD
- Required for early diagnosis of dengue between day 0-7 post-onset of symptoms

Together, the two RDT's can enable dengue diagnosis through all stages of infection to fulfill capability gap.

Slide courtesy of Dr. Subhamoy Pal

Advantages and limitations of different dengue diagnostic tests

Diagnostic tests	Advantages	limitations
Viral isolation and identification	• Confirmed infection • Specific • Identifies serotypes	• Requires acute sample (0–5 days post onset) • Requires expertise and appropriate facilities • Takes more than 1 week • Does not differentiate between primary and secondary infection • Expensive
RNA detection	• Confirmed infection • Sensitive and specific • Identifies serotype and genotype • Results in 24–48 hours	• Potential false-positives owing to contamination • Requires acute sample (0–5 days post onset) • Requires expertise and expensive laboratory equipment • Does not differentiate between primary and secondary infection

Advantages and limitations of different dengue diagnostic tests: Serology

Diagnostic Tests	Advantages	Limitations
IgM or IgG seroconversion	• Confirmed infection • Least expensive • Easy to perform	• IgM levels can be low in secondary infections • Confirmation requires two or more serum samples • Can differentiate between primary and secondary infection*
IgM detection (single sample)	• Identifies probable dengue cases • Useful for surveillance, tracking outbreaks and monitoring effectiveness of interventions	• IgM levels can be low in secondary infections

*Primary infection: IgM-positive and IgG-negative (if samples are taken before day 8–10); secondary infection: IgG should be higher than 1,280 haemagglutination inhibition in convalescent serum.

Advantages and limitations of different dengue diagnostic tests: Antigen Detection

Diagnostic Test	Advantages	Limitations
Clinical specimens (for example, using blood in an NS1 assay)	• Confirmed infection • Easy to perform • Less expensive than virus isolation or RNA detection	• Not as sensitive as virus isolation or RNA detection
Tissues from fatal cases (for immunohistochemistry, for example)	• Confirmed infection	• Not as sensitive as virus isolation or RNA detection • Requires expertise in pathology

Tourniquet Test (TT)

- Positive in up to 50% of patients with classical dengue and almost all with DHF
- Non-specific
- Procedure:
  - Inflate BP cuff halfway between systolic and diastolic BP for 5 minutes
  - Release
  - Count # petechiae in a quarter-sized patch below the cuff
  - >20 is positive

Sample Prep

- Collect 2 separate red gel separator tubes (“tiger-tops”)
  - Gently invert 5 times
  - Allow blood to clot min. 30 min (vertical)
  - Centrifuge at full speed (1100-1399 G) for 10 min
  - Pipette off serum into separate cryovials
  - Refrigerate or ice bath (2-8°C, ELISA/PRNT)
  - RT-PCR: store @ 2-8°C for up to 6 hours (immediate RNA extraction possible) otherwise, store @ -20°C for up to 14 days. Limit to one freeze-thaw cycle.
  - Isolation: store @ -80°C until ready for transport

Rx

- Symptomatic, supportive
  - CAREFUL fluid management
- Acetaminophen
  - NO Aspirin (Reye's)
- Oral Fluid Replacement
  - If can take PO, no complications, mentally with it
- Serial monitoring of (to trigger IV therapy)
  - HR, BP, Skin perfusion, Urine output, Hct (>20%)
- Development of any warning sign
  - Hospitalization, close observation
  - Judicious use of IV fluids on poor PO, rapidly increasing Hct.

Rx (2)

- Shock
  - PROMPT fluid resusctiation
  - Isotonic crystalloid (initially)/colloid (for those presenting in profound shock, no response to crystalloid) solutions
  - Keep to minimum required to support CV stability
  - Plasma, cryoprecipitates, whole blood (care to not fluid overload)
  - PREVENTIVE transfusions should be avoided
- Desmopressin? IV gamma globulin? Steroids? Drugs (chloroquine, balapiravir, statins)? No evidence for efficacy
- Beware pulmonary edema: may need PPV
  - DHF-DSS is the 3rd most common cause of ARDS in hospitalized children in Malaysia

Dengue Prevention

- Prevention:
  - There is no prophylactic drug for dengue
  - There is no licensed vaccine (yet) to prevent dengue
  - Reduce risk by use of personal protective measures (DEET, permethrin-treated uniforms, screened windows, mosquito netting) and local vector control (eliminate breeding sites, insecticides)
  - New approaches to vector control
    - Genetically altered male mosquitoes
    - Embryonic introduction of wolbachia into *A. aegypti*

Prevention

http://www.rafaela.gov.ar/es/popupDenge2.html

http://wahootours.hyperboards.com/action/view_topic/topic_id/5675

And now for something completely different…

- 31 y/o female recently returned from Singapore…
- Fever (39.5°C), nausea, myalgias, back pain, HA, bilateral conjunctivitis, severe bilateral arthralgias (shoulders, knees, ankles, elbows, wrists, fingers).
- Lab: Lymphopenia (0.6 G/L), AST 177 UI/L, ALT 116 UI/L, LDH 780 UI/L, Nl Bili, CRP 64 mg/L.
- Course: developed chronic distal arthritis and tenosynovitis, swelling of the joints without fluid accumulation.

Chikungunya

Summary

- Dengue is a significant threat to the US military and civilian populations in endemic areas.
  - Recognize atypical presentations: maintain healthy suspicion
  - May not have high case fatality rates, but illness will significantly affect mission(s)
- Vaccine development is underway and is challenging
  - WRAIR is a leader in developing dengue vaccines
  - Several candidate vaccines are in the pipeline

Dengue and Hemorrhagic Fever
A Potential Threat to Public Health in the United States

"Most individuals in the United States are as little concerned about dengue fever as they were a decade ago about West Nile fever. That situation could change if dengue continues its expansion as one of the world's most aggressive reemerging infections."

Morens D and Fauci A. JAMA 2008. 299:214-216.

Viral Disease Branch

Walter Reed Army Institute of Research

Division of Viral Diseases