Dengue Disease: Prospective Primary Dengue Study, St George’s University

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Lecture Objectives

• Review dengue and the dengue epidemic.
• Review objectives, methodology and importance of the “Dengue in a Travel Population: Prospective Cohort Study of Primary Dengue Infection”.

UPSTATE MEDICAL UNIVERSITY
Dengue Key Facts

• Mosquito-borne viral infection (*Aedes aegypti*), tropical and sub-tropical regions, urban areas at high risk.

• Global health problem half of the world's population is now at risk-390 million dengue infections per year, 96 million symptomatic.

• Dengue fever is a flu-like illness on 2\textsuperscript{nd} infection can lead to severe dengue.

• There is no specific treatment for dengue/ severe dengue, but early detection and access to proper medical care lowers fatality rates below 1\%.

• Vaccines are in clinical trials.
DENGUE ALERT!!!
Caribbean Island Visitors Warned Of Dengue Virus Risk

Jamaica declared dengue fever epidemic on January 3, 2019

Jamaica: 123 dengue fever cases and 2 deaths.

January 19th, 2019 – The Caribbean Public Health Agency (CARPHA) issued a warning to Caribbean countries saying ‘to brace for a severe outbreak of the dengue fever’ reported the *Jamaican Observer* on January 18, 2019.
Dengue Viruses

- Genus *Flavivirus* & Family *Flaviviridae*
- Four serotypes
  - DEN-1, -2, -3, and -4
  - Antigenically distinct
- Genetic diversity and phylogenetics
  - 3-5 DEN-1 genotypes
  - 5-6 DEN-2 genotypes
  - 4 DEN-3 genotypes
  - 2 DEN-4 genotypes
- Variations in virus virulence
  - RNA virus mutations
  - Recombination events
Global Distribution of Dengue

Mosquito-borne Virus (Arbovirus)

Aedes aegypti

Aedes albopictus
Dengue Life Cycle

Extrinsic
7-21 Days

Intrinsic
4-7 Days

Aedes mosquito

Humans

Aedes aegypti
Protecting Yourself From Dengue

• There is no vaccine or therapeutic.

• Personal Protective Measures:
  ➢ Insect repellent (DEET, Picaridin
  ➢ Long sleeves and pants.
  ➢ Screens on windows.
  ➢ Eliminating water sources in and around home ie flower pots, beer bottles, used tires.
  ➢ Eliminate mosquitoes in the home using insecticide.
  ➢ Maximize protection during daytime (feeding time for Aedes aegypti).
AFRIMS and Children’s Hospital, Bangkok, Thailand
Kamphaeng Phet Field Site

- Long-term relationship and field laboratory co-located with Kamphaeng Phet Provincial Hospital.
Dengue Serotypes at Bangkok Children's Hospital From 1973 to 2001

Reported Cases of Dengue Hemorrhagic Fever in Thailand from 1958 to 2014
Age-Specific Rates of DHF in Thailand 1985-2002

KEY

- 0 to 4 years
- 5 to 9 years
- 10 to 14 years
- 15 years or greater
Clinical Spectrum of Primary Dengue Infection

DF: Fever with headache, myalgia, arthralgia, rash, leukopenia.

Subclinical Infection
Clinical Spectrum of Secondary Dengue Infection

DSS: Rapid weak pulse, narrow pulse pressure, hypotension.

DHF: Grades I, II, III, IV. Bruising, bleeding, hemoconcentration, thrombocytopenia, DIC.

DF: Fever with headache, myalgia, arthralgia, rash, leukopenia.

Subclinical Infection
Pathogenesis of Severe Dengue

First Dengue (Primary) Infection

DF: Fever with headache, myalgia, arthralgia, rash, leukopenia.

Subclinical Infection

Second Dengue (Secondary) Infection

DF: Fever with headache, myalgia, arthralgia, rash, leukopenia.

DSS: Rapid weak pulse, narrow pulse pressure, hypotension.

Subclinical Infection
Petechiae on chest wall in child with DHF.

Subcutaneous hemorrhage in child with DHF.
Petechiae on soles of feet in child with DHF.

Showering of petechiae following blood pressure cuff inflation in child with DHF.
Melena

Gastric Bleeding
Shock Syndrome

• Shock syndrome is the severe form of viral hemorrhagic fever and results from:
  • intravascular volume depletion from plasma leakage into the third space and/or blood loss.
  • cardiovascular collapse.
Plasma leakage the hallmark for dengue hemorrhagic fever.
Clinical Treatment Of DHF

**DHF Suspected.**
Initial IV fluid, 5% Dextrose Ringer’s Lactate
6 ml/kg/hour

Follow-up HCT, vital signs, urine output

**Improvement**
(Urine output ↑, HCT ↓, pulse and pressure stable)
Initial IV fluid, 5% Dextrose Ringer’s Lactate
6 ml/kg/hour
Reduce rate to 5 ml/kg/hour

Continued improvement, reduce rate to 3 ml/kg/hour

With further improvement, stop IV fluid at 24 to 48 hours

**Clinical Deterioration Or No Improvement**
(HCT ↑, pulse ↓, pressure ↑20 mm Hg, urine output ↓)

With signs of shock, increase fluids to 15 ml/kg/hour

Continued signs of shock, increase fluids to 15 ml/kg/hour

Unstable vital signs, urine output, establish CVP or Swan-Ganz monitoring

**HCT, Vital Signs Stable, Diuresis**

**HCT↑**
Use of colloids

**CLINICAL IMPROVEMENT**

**HCT↓**
Blood transfusion
Dengue Translational Research Program

Clinical Trials: Vaccines, drugs, human infection model

Prospective Cohort Studies: Thailand Grenada

Laboratory Research: Role of NS1, Immunology, Proteomics
Dengue Translational Research Program

Clinical Trials: Vaccines, drugs, human infection model

Prospective Cohort Studies: Thailand, Grenada

Laboratory Research: Role of NS1, Immunology, Proteomics
Dengue Research

• Why do people get severely ill from dengue infection?

• What are the host and viral factors that result in severe hemorrhagic illness?
Clinical Spectrum of Dengue Infection

- **DSS**: Rapid weak pulse, narrow pulse pressure, hypotension.
- **DHF**: Bruising, bleeding, hemoconcentration, thrombocytopenia, DIC.
- **DF**: Fever with headache, myalgia, arthralgia, rash, leukopenia.
- **Subclinical Infection**
Clinical Course of Child with DHF: Pathogenesis Studies

7 year old male with acute secondary dengue-1, grade III DHF

- Positive tourniquet test
- Pleural effusion index 25.5

Fever (°C)

Pulse pressure (mm Hg)

White blood cell count

Liver

Hematocrit (%)

Platelet count

Clinical illness day

Fever day

WBC, Platelet, AST, ALT, Albumin
Magnitude of dengue-3 viremia by day of illness

Plasma D3V level (log D3V cDNA copies/ml)

Relationships between maximum plasma viremia and continuous measures of disease severity in secondary dengue-3 virus (D3V) infection.
Relationships between viremia, immune activation markers, and time of defervescence in secondary dengue-3 virus (D3V) infections.
Clinical Spectrum of Dengue Infection

DSS: Rapid weak pulse, narrow pulse pressure, hypotension.

DHF

DF: Fever with headache, myalgia, arthralgia, rash, leukopenia.

Subclinical Infection
Prospective Study of Dengue Virus Transmission and Disease in Primary School Children in Thailand
Study Design KPS I

- Five year, prospective, school-based study (grades 2-6), of 2200 children in Kamphaeng Phet Province.

Kamphaeng Phet
Bangkok
School-Absence Based Prospective Study

**Timeline**

- **January**: Venipuncture, bank serum and T-cells, plaque reduction, neutralization titers
- **June**: Venipuncture, HAI
- **August**: Venipuncture, HAI
- **November**: Venipuncture, HAI

**Active surveillance**: school absences, school illness, blood sampling for ill children (HAI, virus isolation, 2-week convalescent)
Study Objectives

• Determine the epidemiology of inapparent and symptomatic dengue virus infection.
• Determine the host and viral factors that influence dengue disease severity prior to the onset of disease.
• Increase our understanding of the spatial and temporal circulation of dengue serotypes.
• Develop a study model in which to study the efficacy of a dengue vaccine.
<table>
<thead>
<tr>
<th>Year</th>
<th>Total</th>
<th>Inapp</th>
<th>Symptomatic Not Hosp (DF)</th>
<th>Symptomatic Hosp. (DF+DHF)</th>
<th>Ratio Inapp:Symp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>7.9</td>
<td>4.3</td>
<td>2.9</td>
<td>0.70</td>
<td>1.2 : 1.0</td>
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<tr>
<td>1999</td>
<td>6.5</td>
<td>3.2</td>
<td>2.5</td>
<td>0.80</td>
<td>1.0 : 1.0</td>
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<td>2000</td>
<td>2.2</td>
<td>1.4</td>
<td>0.7</td>
<td>0.06</td>
<td>1.8 : 1.0</td>
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<tr>
<td>2001</td>
<td>14.9</td>
<td>7.6</td>
<td>4.6</td>
<td>2.70</td>
<td>1.1 : 1.0</td>
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<tr>
<td>2002</td>
<td>5.2</td>
<td>2.8</td>
<td>1.5</td>
<td>0.80</td>
<td>1.2 : 1.0</td>
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<tr>
<td>Avg./Year</td>
<td>7.3</td>
<td>3.9</td>
<td>2.4</td>
<td>1.00</td>
<td>1.2 : 1.0</td>
</tr>
</tbody>
</table>

* per 100 during the dengue season (June to November)
Dengue Incidence by School and Year

Incidence

% 25

1998 1999 2000 2001 2002

Schools

1 2 3 4 5 6 7 8 9 10 11 12
Dengue Serotype Circulation by School: All Years

- DEN-1
- DEN-2
- DEN-3
- DEN-4

Graph showing the circulation of different dengue serotypes over a range of years from 1 to 12.
Hospitalization Rates by School and Year

% of Cases Hospitalized

Schools

1 2 3 4 5 6 7 8 9 10 11 12

1998 1999 2000 2001 2002
Ratio of Inapparent to Symptomatic Dengue Illness by School and Year

[Graph showing the ratio of inapparent to symptomatic dengue illness across different schools and years (1998-2002).]
Ratio of Inapparent to Symptomatic Dengue Illness by School for All Years

Ratio

<table>
<thead>
<tr>
<th>Schools</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
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<td>2</td>
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<tr>
<td>11</td>
<td>1.5</td>
</tr>
<tr>
<td>12</td>
<td>2.5</td>
</tr>
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</table>
Primary Dengue is Poorly Studied

- First infection sets the immunologic stage for severity upon the second infection.
- Most primary dengue is subclinical therefore difficult to capture symptomatic patients.
- Most studies are done in endemic areas and first infection occurs in the first 5 years of life.
- “Travelers” study of flavivirus naïve individuals who live in an endemic dengue area at high risk for infection and offers an opportunity to study the immunology of primary infection.
Travelers Study

• Dengue is the most common cause of fever in returning travelers.

• Travelers study is difficult to do:
  ✓ Hard to capture population.
  ✓ Difficult surveillance.

• Efficacy trial of drugs/vaccines in travelers an essential indication.
Dengue in a Travel Population: Prospective Cohort Study of Primary Dengue Infection
Short Title: Grenada Surveillance

SUNY Principal Investigator: Timothy Endy, MD, MPH
SUNY Upstate Medical University
766 Irving Ave., 2203 WH
Syracuse, NY 13210

SGU Principal Investigator: Calum Macpherson, PhD
St. George’s University
True Blue, Grenada

Funders: Janssen Global Public Health, a division of Janssen Pharmaceutica N.V.
SUNY Upstate Medical University
St. George’s University

Study Location: St. George’s University,
True Blue, Grenada
### Synopsis

<table>
<thead>
<tr>
<th>Planned Trial Period</th>
<th>August 2018-May 2021</th>
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<tbody>
<tr>
<td><strong>Trial Participants</strong></td>
<td>Students and their partners, 18 years and older during their first two years at St. George’s University.</td>
</tr>
<tr>
<td><strong>Participant Duration</strong></td>
<td>Up to 2 years</td>
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<tr>
<td><strong>Planned Sample Size</strong></td>
<td>Up to 2400</td>
</tr>
<tr>
<td><strong>Trial Design</strong></td>
<td>Prospective Population-Based Study of Primary Dengue Virus Disease. Prospective cohort study of incoming students and their partners matriculated at St George’s University. Blood samples for research surveillance and assays will be drawn before surveillance; active surveillance during the year for acute dengue. Subjects who become febrile will also have acute and convalescent samples taken at time of first evaluation through 28 days later.</td>
</tr>
</tbody>
</table>
### Inclusion Criteria:

Subjects must meet all of the following criteria in order to be eligible for trial enrollment:

1. A male or female 18 years of age or older at the time of enrollment;
2. Written informed consent obtained from the subject;
3. In first term of their upper level education OR in the Foundations of Medicine term

### Exclusion Criteria:

A subject fulfilling *any* of the following criteria will be excluded from trial enrollment:

1. No signed informed consent;
2. Planned extended absence (> four weeks) away from Grenada during the school year (except during school breaks).
3. Subjects who the investigator believes will not comply with the requirements of the protocol (e.g. study compliance, return for follow-up visits)
<table>
<thead>
<tr>
<th>Objectives:</th>
<th>Primary:</th>
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<tbody>
<tr>
<td></td>
<td>determine the incidence of DENV infection in the student population</td>
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<tr>
<td></td>
<td>Secondary:</td>
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<tr>
<td></td>
<td>determine the feasibility to support a prophylactic dengue efficacy trial</td>
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<td></td>
<td>characterize DENV viral kinetics for febrile subjects</td>
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<td></td>
<td>determine the incidence of ZIKV infection</td>
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<td></td>
<td>determine the incidence of CHKV infection</td>
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<tr>
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<td>Exploratory:</td>
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<tr>
<td></td>
<td>characterize the immunology, virus genetics and pathogenesis of primary DENV infection</td>
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<td></td>
<td>Evaluate febrile illness that is DENV, CHKV or ZIKV negative</td>
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<tr>
<td>Endpoints:</td>
<td>Primary:</td>
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<td>-----------</td>
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<tr>
<td></td>
<td>- anti-DENV seroconversion as determined by ELISA and flow NT or identification of DENV by RT-PCR</td>
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<tr>
<td></td>
<td>Secondary:</td>
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<tr>
<td></td>
<td>- based on incidence of DENV infection, assess feasibility of performing an efficacy trial for a dengue prophylactic drug</td>
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<tr>
<td></td>
<td>- DENV viral load determination by RT-PCR for febrile subjects</td>
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<tr>
<td></td>
<td>- anti-ZIKV seroconversion as determined by ELISA</td>
</tr>
<tr>
<td></td>
<td>- anti-CHKV seroconversion as determined by ELISA</td>
</tr>
</tbody>
</table>

| Statistical Methods: | This is a descriptive study to assess the incidence of DENV infection in a traveler’s population. No formal statistical hypothesis tests will be conducted. Descriptive analysis will be based on the per-protocol analysis sets. Student t-test, linear regression and chi-square will be performed, depending on the data set being analyzed. |
## Cohort Surveillance

<table>
<thead>
<tr>
<th>Year</th>
<th>Cohort</th>
<th>Subjects</th>
<th>Surveillance Type</th>
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<tbody>
<tr>
<td>2018</td>
<td>Cohort 1</td>
<td>800</td>
<td>Active Surveillance</td>
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<td>2019</td>
<td>Cohort 2</td>
<td>800</td>
<td>Active Surveillance</td>
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<tr>
<td>2020</td>
<td>Cohort 3</td>
<td>800</td>
<td>Active Surveillance</td>
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<tr>
<td>2021</td>
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*Rainy Season*

*Upstate Medical University*
Summary

• The Grenada prospective dengue study is the first of its kind.
• Will provide essential understanding of the pathogenesis and immunology of dengue infection.
• Will refine diagnostic assays for dengue essential for diagnosing acute dengue and vaccine development.
• Will be a platform in which to test the effectiveness of drugs and vaccines in a travelers population.