SEROTYPE AND PHYLOGENETIC ANALYSIS OF DENGUE VIRUS – JOHOR 2012 - 2014

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INTRODUCTION
OBJECTIVE
METHODOLOGY
RESULT
LIMITATION
CONCLUSION
REFERENCES
ACKNOWLEDGEMENT
**DENGUE**

- A mosquito-borne disease
- ½ world population at risk of infection.
- A major public health challenge globally as well as in Malaysia,
- with escalating incidence rate.

### Dengue Cases Year 2012 - 2014

<table>
<thead>
<tr>
<th></th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johor</td>
<td>1860</td>
<td>4843</td>
<td>6234</td>
</tr>
<tr>
<td>Malaysia</td>
<td>21 900</td>
<td>43 346</td>
<td>108 698</td>
</tr>
</tbody>
</table>
DENGUE

Key contributing factors:

- Densely populated urban city
- Highly mobile population conducive to disease spread
- Environment that supports vector proliferation

- Dengue Control - Integrated vector management strategy with a robust surveillance system:
  - Human case surveillance (e-Dengue)
  - Entomological surveillance using GIS
**DENGUE VIRUS**

- DENV1, DENV2, DENV3, DENV4
- Infection of one DenV does not provide immunity against the other
- Sequential infection: Increase virus replication & severity of disease

**Sequencing of DENV-RNA**
- Verified strain variation within serotype
- Virus be classified into genetically distinct groups within serotypes called genotype

Source: 2011 *Nature Education*
DENGUE VIRUS SEROTYPE SURVEILLANCE

- Starts 2011
- Monitoring and characterising circulating dengue viruses in different states
- Uses the sentinel surveillance approach
- 9 Sentinel in Johor:
  - Hospital Sultanah Aminah, Johor Bahru
  - Hospital Sultan Ismail, Johor Bahru
  - Hospital Pakar Sultanah Fatimah, Muar
  - Hospital Kluang, Kluang
  - Mahmoodiah Policlinic, Johor Bahru
  - Sultan Ismail Health Clinic, Johor Bahru
  - Pasir Gudang Health Clinic, Johor Bahru
  - Mengkibol Health Clinic, Kluang
  - Batu Pahat Health Clinic
OBJECTIVE

1. Analyse trend of circulating dengue virus serotype in Johor
2. Assess relation between dengue virus serotype and disease severity
3. Assess dengue virus genotype
• Descriptive study design
• Secondary data for virus surveillance
  – Johor Public Health Laboratory (DVSS 2012-2014)
• Data for reported dengue cases:
  – Secondary data from year 2012-2014 from Johor State Department of Health, Vector unit.
• Data Analysis - Microsoft Excel
• Molecular technique:
  – DENV RNA extraction from serum
  – Serotyping (RT-PCR)
  – Genotyping
• Sequencing & Phylogenetic analysis: IHA, Singapore / Software DNA baser & MEGA 6
Johor Public Health Lab

RNA extraction
↓
DENV serotyping
↓
DENV genotyping
↓
Sequencing & Phylogenetic analysis by IHA, Singapore

Interpretation

Johor State Department of Health

Vector Unit

Dengue case report

Dengue outbreak report
### Dengue Incidences in Johor 2012-2014

<table>
<thead>
<tr>
<th>Year</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. Cases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(IR)</td>
<td>1860</td>
<td>4843</td>
<td>6234</td>
</tr>
<tr>
<td></td>
<td>(48.3)</td>
<td>(139.6)</td>
<td>(179.9)</td>
</tr>
<tr>
<td><strong>No. outbreak</strong></td>
<td>131</td>
<td>562</td>
<td>633</td>
</tr>
<tr>
<td><strong>No. Death</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(CFR)</td>
<td>1</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(0.49)</td>
<td>(0.40)</td>
</tr>
</tbody>
</table>

- IR: incidence rate
- CFR: case fatality rate.
Comparison of Dengue Incidence with Dengue Serotype

showed a shift from

- 2012: DENV3 (mean percent 32.6%) / DENV4 (mean percent 19.5%)
- 2013: DENV2 in 2013 (mean percent 64.6%),
- 2014: DENV1 in 2014 (mean percent 40.0%).
DENV-1
E gene phylogeny (UNITEDengue)

- Majority: Genotype I (n=26) & Genotype III (n=9)
- More closely related to DENV from Malaysia instead of Singapore

Phylogenetic Analysis

DENV1

- Majority: Genotype I (n=26) & Genotype III (n=9)
- More closely related to DENV from Malaysia instead of Singapore
**DENV2**

- Cosmopolitan genotype (n=52)
  - Clade 1
  - Clade 1b

- More closely related to DENV from Malaysia instead of Singapore
DENV3

- Genotype III (n=18)
- More closely related to DENV from Malaysia instead of Singapore
DENV-4
E gene phylogeny (UNITEDengue)

Red: Singapore
Blue: Malaysia
Green: Johor (Set3 to 6)

**Common strain in Malaysia**

1 new case - Johor

**DENV4**

- Genotype II (n=4)

* Total of 4 new sequences added

**GII**

- New strain detected in SG since April 2013 (15)
- A closely related sporadic case reported from KL

**GI**

- Uncommon in SG
<table>
<thead>
<tr>
<th>Year</th>
<th>Serotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>DENV1</td>
<td>G1</td>
</tr>
<tr>
<td></td>
<td>DENV2</td>
<td>Cosmo Clade 1, Cosmo Clade 3</td>
</tr>
<tr>
<td></td>
<td>DENV3</td>
<td>G1, G3</td>
</tr>
<tr>
<td></td>
<td>DENV4</td>
<td>G2</td>
</tr>
<tr>
<td>2013</td>
<td>DENV1</td>
<td>G1, G1a</td>
</tr>
<tr>
<td></td>
<td>DENV2</td>
<td>Cosmo Clade 1, Cosmo Clade 1b</td>
</tr>
<tr>
<td></td>
<td>DENV3</td>
<td>G3</td>
</tr>
<tr>
<td></td>
<td>DENV4</td>
<td>G1, G2</td>
</tr>
<tr>
<td>2014</td>
<td>DENV1</td>
<td>G1a</td>
</tr>
<tr>
<td></td>
<td>DENV2</td>
<td>Cosmo Clade 1b</td>
</tr>
</tbody>
</table>
Incomplete clinical information on the request forms - prevents further analysis into clinical manifestation and disease severity of dengue infection

Results are not real time and subsequently cannot contribute towards predicting disease burden and severity
CONCLUSION

- Yearly shift of dominant serotype contributes to the increase of disease incidence.
- While the association of DENV2 dominance with higher reported fatality is of concern, the reason behind the association remain unclear.
- It could be inherent in the strain, or due to secondary infections following previous infections by DENV-3.
- Though being a neighboring state with Singapore, Johor showed a different genotype predominance from Singapore & are more closely related to local DENV genotype.
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ACKNOWLEDGEMENT

• Johor State Department of Health
• Institute Health Agency, Singapore
• National Public Health Laboratory
The End

THANK YOU
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>JOHOR</td>
<td>63</td>
<td>11,441</td>
</tr>
<tr>
<td>KEDAH</td>
<td>5</td>
<td>712</td>
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<tr>
<td>KELANTAN</td>
<td>13</td>
<td>2,131</td>
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<tr>
<td>MELAKA</td>
<td>6</td>
<td>1,693</td>
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<tr>
<td>NEGERI SEMBILAN</td>
<td>2</td>
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<tr>
<td>PAHANG</td>
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<tr>
<td>PERAK</td>
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<td>7,759</td>
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<tr>
<td>PERLIS</td>
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<td>171</td>
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<td>PULAU PINANG</td>
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<tr>
<td>SARAWAK</td>
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<td>SELANGOR</td>
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<td>49,712</td>
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<tr>
<td>TERENGGANU</td>
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<td>885</td>
</tr>
<tr>
<td>WP KUALA LUMPUR</td>
<td>30</td>
<td>6,055</td>
</tr>
<tr>
<td>WP LABUAN</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>WP PUTRAJAYA</td>
<td>0</td>
<td>183</td>
</tr>
<tr>
<td><strong>MALAYSIA</strong></td>
<td><strong>280</strong></td>
<td><strong>92,105</strong></td>
</tr>
</tbody>
</table>

*Sumber Daripada Bilik Gerakan Denggi Kebangsaan CPRC, Kementerian Kesihatan Malaysia (KKM)*