WHOPES methods to test insecticide susceptibility of 4 *Aedes aegypti* field populations in Cambodia

Sébastien Boyer, Institute Pasteur Cambodia
Sergio Lopes, Malaria Consortium
• Dengue is endemic in Cambodia

• No vaccine yet!

• The best control method = Vector control

• Actual control: larval and adult insecticides

• Question: methods are still efficient?
Questions

Do resistance to insecticide exists in the field for the main dengue vector?

Is there larval resistance to temephos in endemic areas?

Is there adult resistance to the two main insecticides?

- Deltamethrin (fumigation)
- Permethrin (nets, household aerosols, indoor/outdoor thermal fogging)
Methods (1/5)

WHO methodology [1]

Sampling and larvae collections in the field

Rearing F1 generation for bioassays

World Health Organization (WHO) Pesticide (WHOPES)

Use of standardized WHOPES methods

WHOPES methods

Adults bioassays

Larvae bioassays

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Methods (2/5)

Field collection of immature stages

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Methods (3/5)

Mosquito rearing

- Rearing for F1 generation
- F1 generation eggs
- Rearing F1 for bioassays tests
- Larvae ready!
- Adults ready!

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WHO & WHOPES methods to test insecticide susceptibility of 4 Aedes aegypti field populations in Cambodia

**Methods (4/5)**

Larval bioassays

<table>
<thead>
<tr>
<th>Serial Temephos concentrations used (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.004</td>
</tr>
<tr>
<td>0.01</td>
</tr>
<tr>
<td>0.02</td>
</tr>
<tr>
<td>0.03</td>
</tr>
<tr>
<td>0.05</td>
</tr>
<tr>
<td>0.2</td>
</tr>
</tbody>
</table>

WHO & WHOPES protocol standard

Tested insecticide: temephos (Abate ©)

- 25 larvae / cup
- 4 cups (=4 replicates) / concentration
- 7 concentrations (including 0 for control)

700 3rd-instar larvae per population

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WHO & WHOPES methods to test insecticide susceptibility of 4 Aedes aegypti field populations in Cambodia

Methods (5/5)

Adult bioassays

WHO & WHOPES protocol standard

Tested insecticides: deltamethrin (0.03%) and permethrin (0.25%)

- 25 adults / tube
- 4 tubes (=4 replicates)
- One WHO discriminating dose per insecticide

100 adult per insecticide
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Methods (5/5)

Adult bioassays

WHO & WHOPES protocol standard

Tested insecticides: deltamethrin (0.03%) and permethrin (0.25%)

- 25 adults / tube
- 4 tubes (=4 replicates)
- One WHO discriminating dose per insecticide

100 adult per insecticide + 100 adult for synergist
Data analysis

Larval bioassays

Determination of LD50 and LD90 with log probit analysis
(performed with R)

\[
RR \text{ ratio} = \frac{LD50 \text{ field population}}{LD50 \text{ sensitive strain}}
\]

Adult bioassays

Mean comparison
Results

Larval bioassays

Sensitive strain (*Aedes aegypti* USDA strain)
Results

Larval bioassays

Sensitive strain (*Aedes aegypti* USDA strain)

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Results

Larval bioassays

Sensitive strain (*Aedes aegypti* USDA strain)

![Graph showing concentration vs. mortality for Temephos against susceptible strain of *Aedes aegypti*.]
Results

Larval susceptibility to Temephos

<table>
<thead>
<tr>
<th>Populations</th>
<th>Urban</th>
<th>Rural</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD50</td>
<td>LD90</td>
</tr>
<tr>
<td>Phnom Penh</td>
<td>0.020</td>
<td>0.028</td>
</tr>
<tr>
<td>Siem Reap</td>
<td>0.014</td>
<td>0.020</td>
</tr>
<tr>
<td>Kampong Cham</td>
<td>0.031</td>
<td>0.052</td>
</tr>
<tr>
<td>Battambang</td>
<td>0.125</td>
<td>0.221</td>
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</tbody>
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*Insufficient F1 larvae to conduct testing

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WHOPES methods to test insecticide susceptibility of 4 Aedes aegypti field populations in Cambodia

Results
Larval susceptibility to Temephos

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<tr>
<th>Populations</th>
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*Insufficient F1 larvae to conduct testing

Sensitive strain: LD50 = 0.0037 mg/L & LD90 = 0.0047 mg/L

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WHOPES methods to test insecticide susceptibility of 4 Aedes aegypti field populations in Cambodia

Results

Larval susceptibility to Temephos

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<td>5.3</td>
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<tr>
<td>Siem Reap</td>
<td>3.8</td>
<td>-</td>
</tr>
<tr>
<td>Kampong Cham</td>
<td>8.4</td>
<td>13.0</td>
</tr>
<tr>
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<td>33.6</td>
<td>11.2</td>
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Values of resistance ratio (RR) greater than 5 is an indication of resistance and values less than or equal to 5 are considered as susceptible (WHO 2016)
WHOPES methods to test insecticide susceptibility of 4 Aedes aegypti field populations in Cambodia

## Results

### Larval susceptibility to Temephos

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<td>Phnom Penh</td>
<td>Resistant</td>
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<tr>
<td>Siem Reap</td>
<td>Susceptible</td>
<td>-</td>
</tr>
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<td>Resistant</td>
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Values of resistance ratio (RR) greater than 5 is an indication of resistance and values less than or equal to 5 are considered as susceptible (WHO 2016)

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Results

Adult susceptibility to two pyrethroids

USDA strain

% mortality

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Deltamethrin</th>
<th>Permethrin</th>
</tr>
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<tbody>
<tr>
<td>without PBO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBO</td>
<td></td>
<td></td>
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WHOPES methods to test insecticide susceptibility of 4 Aedes aegypti field populations in Cambodia

Results

Adult susceptibility to two pyrethroids

USDA strain

% mortality

- blue: without PBO
- orange: PBO

Results:

- Adult susceptibility to two pyrethroids
- Control
- Deltamethrin
- Permethrin

Negative control: essential to control the conditions of the test
Results

Adult susceptibility to two pyrethroids

USDA strain

% mortality

0 25 50 75 100

- without PBO
- PBO

Control  Deltamethrin  Permethrin

98-100% of mortality = sensitive population
WHOPES methods to test insecticide susceptibility of 4 Aedes aegypti field populations in Cambodia

Results

Adult susceptibility to two pyrethroids

USDA strain

% mortality

98-100% of mortality = sensitive population

90-98% of mortality: apparition of resistance to monitor

0-90% mortality: Resistant population

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Results

Adult susceptibility to two pyrethroids by Province

Kampong Cham

Kampong Cham urban population

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<thead>
<tr>
<th>% mortality</th>
<th>Control</th>
<th>Deltamethrin</th>
<th>Permethrin</th>
<th>without PBO</th>
<th>PBO</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
<td>75</td>
<td>25</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Kampong Cham rural population

<table>
<thead>
<tr>
<th>% mortality</th>
<th>Control</th>
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<th>Permethrin</th>
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Resistance to Permethrin
Resistance to Deltamethrin
Results

Adult susceptibility to two pyrethroids by Province

Battambang

<table>
<thead>
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<th>% mortality</th>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Permethrin</td>
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Resistance to Permethrin
Resistance to Deltamethrin

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Results

Adult susceptibility to two pyrethroids by Province

Siem Reap

Siem Reap urban population

Siem Reap rural population

Resistance to Permethrin
Resistance to Deltamethrin

Resistance to Permethrin
Resistance to Deltamethrin

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Results

Adult susceptibility to two pyrethroids by Province

Phnom Penh

Phnom Penh urban population

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Phnom Penh rural population

<table>
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Resistance to Permethrin
Resistance to Deltamethrin
WHOPES methods to test insecticide susceptibility of 4 Aedes aegypti field populations in Cambodia

Results

Adult bioassays

Synthesis

Permethrin

Deltamethrin

Strong resistance

Resistance

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Main conclusions

Do resistance to insecticide exists in the field for the main dengue vector?

Is there larval resistance to temephos in endemic areas?

Is there adult resistance to the two main insecticides?

Deltamethrin (fumigation)

Permethrin (nets)
Main conclusions

Do resistance to insecticide exists in the field for the main dengue vector?

- Is there larval resistance to temephos in endemic areas? **YES**
- Is there adult resistance to the two main insecticides? **YES**

- Deltamethrin (fumigation)
- Permethrin (nets)
WHOPES methods to test insecticide susceptibility of 4 Aedes aegypti field populations in Cambodia

Perspectives after this work

Public Health

Need a change in vector control methods

Mapping insecticide resistance in Cambodia = same tests in all province

Testing resistance to other insecticides: *Bacillus thuringiensis* ser. *israelensis*, *Bacillus sphaericus*, spinosad, pyriproxifen, methoprene...

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