Research in *Aedes* mosquito vectors at GHTM/IHMT

João Pinto

Unidade de Parasitologia Médica

Global Health & Tropical Medicine
• Academic institution of Universidade NOVA de Lisboa
• Postgraduate training, research and cooperation for health development in Tropical Medicine and Global Health
• Staff: 96 people

Regular Courses

12 short courses*, since 2007

- 5 PhD
  - Biomedical Sciences
  - Tropical Diseases & Global Health
  - Human Genetics ans Infectious Diseases
  - Tropical Medicine
  - International Health
- 6 MSc
  - Biomedical Sciences
  - Medical Parasitology
  - Medical Microbiology
  - Health Statistics
  - Public Health & Development
  - Tropical Health

* In vector biology, malaria and arboviruses
Research at IHMT

- **Global Health and Tropical Medicine**
  - New R&D centre since 2014, rated “excellent” by FCT evaluation

- **Vector-Borne Diseases & Pathogens**
  - Vector bioecology and population biology, molecular epidemiology, drug & insecticide resistance, host-pathogen interactions
  - Malaria, leishmaniasis, arboviruses, HAT and TTDs
  - 37 PhD members.
Aedes research at GHTM/IHMT

- Bioecology and vector monitoring
- Population genetics and evolutionary biology
- Mechanisms of insecticide resistance
- New tools for vector control
- Social studies (community awareness & practice)
The 2012 Madeira Dengue outbreak
Aedes aegypti in Madeira Island

Ae. aegypti evolution in Madeira between 2005 and 2012
Dengue outbreak in Madeira, 2012-2013

- 2168 notified cases, 128 hospitalizations, no fatalities
  - DENV-1 serotype
Dengue outbreak in Madeira

- Nearly all municipalities affected but most cases concentrated in the capital Funchal

**Figure 2**
Cumulative incidence of dengue cases by parish, outbreak on Madeira, Portugal, 3 October–25 November 2012.
Exported cases

- 82 dengue cases in 14 countries imported from Madeira

Rapid Communications

More reasons to dread rain on vacation? Dengue fever in 42 German and United Kingdom Madeira tourists during autumn 2012

C Frank (FrankC@rki.de)¹, M Höhle¹, K Stark¹, J Lawrence²
Vector Monitoring
Vector monitoring

- Infestation indexes (2012 outbreak)

<table>
<thead>
<tr>
<th></th>
<th>Funchal</th>
<th>Câmara de Lobos</th>
<th>St Cruz</th>
<th>All municipalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. houses surveyed</td>
<td>273</td>
<td>125</td>
<td>22</td>
<td>420</td>
</tr>
<tr>
<td>N. of containers inspected</td>
<td>1681</td>
<td>1298</td>
<td>431</td>
<td>3410</td>
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<tr>
<td>House Index ($HI’&gt;4$)</td>
<td>32.9</td>
<td>12.0</td>
<td>45.5</td>
<td>27.4</td>
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<tr>
<td>Container Index ($CI’&gt;3$)</td>
<td>12.8</td>
<td>3.8</td>
<td>3.5</td>
<td>8.2</td>
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</tbody>
</table>

- The problem of Abandoned/closed houses
Vector monitoring

• Storm drains as major breeding sites for *Ae. Aegypti*
  – Absolute breeding index: 28.1
Vector monitoring

- Seasonality pattern of *Aedes aegypti* 2010-2012 (ovitraps)
Vector competence

- Oral infections with CHIKV, DENV-2 and ZIKV (N=20 per exp.)
  - Infection rate (IR)
  - Disseminated infection rate (DIR)
  - Transmission efficiency (TE)

- Higher susceptibility and transmission efficiency to CHIKV and DENV-2
- Lower susceptibility and transmission efficiency to ZIKV
Origins
mtDNA sequencing

- Single haplotype for both COI and ND4 mtDNA genes
Microsatellites

Global genetic diversity of *Aedes aegypti*

ANDREA GLORIA-SORIA, DIEGO AYALA, AMBICADUTT BHEECARRY

• 12 loci analysed for 79 samples collected worldwide
  – Bayesian clustering analysis (STRUCTURE)

• Genetic ancestry closest to Brasil, Venezuela and Guadeloupe
  – Strong human mobility with Brasil and Venezuela.
  – Further analyses are ongoing
Insecticide resistance
WHO tests carried out in 2014

Aedes aegypti is resistant to the three insecticide classes tested
Multiple resistance mechanisms

**Knockdown resistance mutations**
- F1534C mutation is fixed (0.98-1.00)
- V1016I mutation increasing (0.07-0.17)

**Metabolic resistance (biochemical assays)**
- Overexpression of detoxification enzymes (esterases)
Multiple resistance mechanisms

Detox Chip

- 9 cytochrome P450 oxidases
  - Cyp9J32
  - Cyp9J28
  - Cyp6BB2
  - Cyp9M6

- 1 esterase
  - CCEae3a – temephos metabolizer

- 2 glutathion S-Transferases

- 8 cuticular proteins: resistance through lower insecticide penetration

- 3 hexamerines: involved in cellular trafficking, linked to insecticide resistance.
New tools for vector control
Alternative methods for larval control

4 pilot-studies

- Assessment of storm drains treatment efficacy with marine salt
- Evaluation of Vectobac G for larval control in flower pots dishes
- Evaluation of Vectobac G for cemeteries treatment
- Use of pyriproxyfen as an effective larvicide against *Ae. aegypti*
Auto-dissemination of pyriproxyfen (PPF) in Paúl do Mar

- **Paúl do Mar**: isolated area 40 km off Funchal, accessible by a 2 km tunnel

- *Aedes aegypti* was detected in 2012 and high densities were recorded in 2013
Auto-dissemination of PPF in Paúl do Mar

- Pilot-study supported by the FP7/EC DENFREE Consortium (Inst. Pasteur, Paris)

**Auto-dissemination process** - Mosquitoes resting on PPF-treated surfaces pick up particles on their legs and transport them to their larval habitats where the chemical prevents pupae from emerging as adults.
Susceptibility of local *Aedes aegypti* to PPF

- Susceptibility of local population to PPF
- No repellent effect in oviposition of *Ae. aegypti*
Auto-dissemination of PPF in Paúl do Mar

- Seven BG-Sentinel traps as PPF dissemination stations
  - Collection bags powdered with PPF (Sumilarv® 0,5G, 20-30 µm particles)
  - One hour On/Off cycles

- 37 artificial breeding sites (ABS)
  - 20 3rd instar larvae (strain Funchal)
  - Surveyed in 48h intervals. Pupae collected and reared in lab

- **Pre-treatment:** 10 Sept 2014 – 4 Oct 2014
- **Treatment 1:** 4 Oct 2014 – 17 Oct 2014
- **Treatment 2:** 20 Oct 2014 – 17 Nov 2014
Auto-dissemination of PPF in Paúl do Mar

Pre-treatment
Auto-dissemination of PPF in Paúl do Mar

Treatment 1
Auto-dissemination of PPF in Paúl do Mar

Treatment 2
More recently
• 19 partner institutions from 13 countries
  – Identify regions where resistance may challenge vector control
  – Improve resistance monitoring and implementing new vector control tools

• 5 commissioned reviews:
  • Aedes integrated management
  • New tools for vector control
  • Current status and mechanisms of insecticide resistance
  • Insecticide resistance management

• Working group to implement WIN/Africa
  • Leader: M. Coulibaly (Malaria Research & Training Center, Mali)
Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans

Catherine L. Moyes\(^1\), John Vontas\(^2\), Ademir J. Martins\(^4\), Lee Ching Ng\(^5\), Sin Ying Koou\(^5\), Isabelle Dusfour\(^6\), Kamaraju Raghavendra\(^7\), João Pinto\(^8\), Vincent Corbel\(^9\), Jean-Philippe David\(^10\), David Westman\(^1\)
56 partners from 21 countries/regions

Objectives
- Clarify the impact of Zika infection in pregnancy
- Understand ZIKV natural history in humans and the environment
- Establish a collaborative network in Latin America for to boost epidemics preparedness

<table>
<thead>
<tr>
<th>Work package</th>
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<tbody>
<tr>
<td>WP 1</td>
<td>Clinical Science</td>
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<tr>
<td>WP 2</td>
<td>Clinical biology &amp; immunology</td>
</tr>
<tr>
<td>WP 3</td>
<td>Virology and antivirals</td>
</tr>
<tr>
<td>WP 4</td>
<td>Pathophysiology &amp; animal models</td>
</tr>
<tr>
<td>WP 5</td>
<td>Zika virus animal reservoirs</td>
</tr>
<tr>
<td>WP 6</td>
<td>Vectors &amp; vector control</td>
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<tr>
<td>WP 7</td>
<td>Social sciences</td>
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<td>WP 8</td>
<td>Communication, Dissemination, and Evaluation (CoDE)</td>
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<tr>
<td>WP 9</td>
<td>Management of consortium and exploitation</td>
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</table>
A Global Alliance for Zika Virus Control and Prevention

- Work Package nº 6: Vectors and Vector Control
  - Identify Zika vectors in Latin America and Caribe
  - Studies on vector competence to ZIKV
  - Importance of coinfections in the mosquito
  - Insecticide resistance and new vector control tools
Team and acknowledgments

Carla A. Sousa
Leading researcher
casousa@ihmt.unl.pt

Gonçalo Seixas (PhD student)
- Ecology & Genetics
- Insecticide resistance

Gonçalo Alves (MSc student)
- Vector control tools

Bianca Pires (MSc student)
- Vector control tools

Madeira team

Ana Clara Silva (team leader)
Bela Viveiros (mosquito monitoring)
Margarida Clairouin (mosquito monitoring)
Luis Antunes (geography)

Manuel Biscoito (team leader)
Ysabel Margarita Gonçalves (mosquito monitoring)
Juan Silva (mosquito monitoring)

Funding

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA

ZIKAlliance

Jeff Powell
- Population genetics

Richard Paul
- Greg Devine
  - PPF experiments

Linda Grigoraki
- John Vontas
  - Metabolic resistance

David Weetman
- Metabolic resistance
  - (Detox chip)
Thank you!