Advances in Malaria Research: In the Lab and the Field
Groundbreaking science in the fight against one of the world’s deadliest diseases

Web Summit
November 12, 2009
12:30 – 2:15pm
Questions?

Submit to Twitter

#JHMal09

(include your affiliation in the message)

No Twitter account? Use webcast page:

http://www.jhsph.edu/malariasummit2009
40% of world population lives in endemic areas
300 - 500 million cases annually
1.5 – 3 million deaths annually
Our Mission

Founded in 2001, the Johns Hopkins Malaria Research Institute (JHMRI), is dedicated to the search for basic science breakthroughs to attack the complex life cycle of malaria.

- Creating new strategies for blocking malaria transmission
- Exploring new approaches to vaccines
- Attracting new scientists to malaria research
- Developing new diagnostic techniques
- Creating the next-generation of antimalarial drugs
- Mapping the mosquito and disease in endemic areas
What is Malaria?

Single-cell parasites of the protist kingdom
Protozoans: animal-like (4 phyla)
Algae: plant-like (6 phyla)
Slime molds: fungus-like (2 phyla)

Malaria is transmitted by Anopheles mosquitoes

Four species commonly infect humans:
- *Plasmodium falciparum*
- *Plasmodium vivax*
- *Plasmodium ovale*
- *Plasmodium malariae*
Malaria Life Cycle

Malaria: Breaking the Cycle
Malaria: A Moving Target

Drug resistance
  Chloroquine
  Resistance to all approved antimalaria drugs

Insecticide resistance
  DDT
  Resistance to every chemical class of insecticide

Emerging infections
  *Plasmodium knowlesi*
Questions?

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http://www.jhsph.edu/malariasummit2009
Mosquito resistance to the malaria parasite *Plasmodium*

- *Plasmodium’s* development in the mosquito
- Mosquito immunity to *Plasmodium*
- The mosquito’s natural intestinal bacteria can influence transmission of *Plasmodium*

www.dimopoulosgroup.org
Initiation is triggered in the mosquito gut by:

- Temperature drop of 5°C
- Xanthurenic acid concentration increase
- pH: 7.4 – 8.3
- >20mM bicarbonate
Plasmodium’s development in the mosquito
The ookinete-stage *Plasmodium*

- Blood meal
- Peritrophic matrix
- Ectoperitrophic space
- Midgut epithelium
- Basal laminae
- Hemocoel

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Plasmodium’s development in the mosquito

The oocyst -stage *Plasmodium*

Day 4

Day 6

Day 8

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Plasmodium’s development in the mosquito
The sporozoite -stage *Plasmodium*
Bottlenecks of *Plasmodium* development in *Anopheles*

Contractions and expansions of parasite populations

Sinden *et al.*, Insect Biochem. & Mol. Biol. 2004
CONCLUSIONS:

• *Plasmodium* has to complete complex developmental processes in the mosquito in order to complete its life cycle.

• Critical bottlenecks exist and result in contractions of parasite populations in the mosquito vector.
Mosquito immunity to *Plasmodium*

**Mosquito innate immune system**

- **Pattern Recognition Receptors**
  - Signal Amplification Pathways (serine proteases)
  - Phagocytosis
  - Signal Transduction Pathways (Toll, IMD)
  - Melanization
  - Effector Genes (AMP)

**Pathogen Recognition**
Mosquito immunity to *Plasmodium*
Assaying *Anopheles* defenses to *Plasmodium* in the midgut

Dong et al., *PLoS Pathogens* 2006
Many of the discovered genes had anti-*Plasmodium* function

Inactivation of these genes in the mosquito resulted in a lesser resistance to infection

Dong *et al.*, *PLoS Pathogens* 2006
CONCLUSION:

The *Anopheles* mosquito uses multiple factors of its immune system to fight against malaria parasite infection.
Implication of immune pathways in anti-Plasmodium defense

Positive regulators

Negative regulators
Implication of immune pathways in anti-\textit{Plasmodium} defense
Anopheles defenses to Plasmodium in the midgut
Implication of Imd and Toll pathways in anti-*Plasmodium* defense

Garver *et al.*, *PLoS Pathogens* 2009
Implication of the Imd pathway in anti-Plasmodium defense

EFFECTORS?

Known anti-Plasmodium factors

FBN9
LRRD7
TEP1

Dong & Dimopoulos JBC 2009
Garver et al., PLoS Pathogens 2009
Transient Imd pathway activation has insignificant impact on longevity

Garver et al., *PLoS Pathogens* 2009
CONCLUSIONS:

• The Imd pathway is a more potent regulator of anti-\textit{P. falciparum} defense in multiple mosquito vector species.

• The transient activation of the Imd pathway does not cause a significant fitness cost at laboratory conditions.

Garver et al., \textit{PLoS Pathogens} 2009
Can the Imd pathway be used for malaria control?

Active against *Plasmodium* in multiple vector species

<table>
<thead>
<tr>
<th>Species</th>
<th>CTRL</th>
<th>IMD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. gambiae/P. falciparum</td>
<td>Uninfected</td>
<td>Infected</td>
<td>0.001</td>
</tr>
<tr>
<td>A. albimanus/P. falciparum</td>
<td>Uninfected</td>
<td>Infected</td>
<td>0.001</td>
</tr>
<tr>
<td>A. stephensi/P. falciparum</td>
<td>Uninfected</td>
<td>Infected</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Activation has insignificant impact on vector fitness

<table>
<thead>
<tr>
<th>Trait</th>
<th>CTRL</th>
<th>IMD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longevity</td>
<td>Sucrose fed</td>
<td>Blood fed</td>
<td>P.f. Infected</td>
</tr>
<tr>
<td>% Survival</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Days</td>
<td>0</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

Can be used at multiple locations and vectors.

Spread of transgene Rel2 in a natural population.

Control multiple anti-*Plasmodium* factors

*Plasmodium* cannot develop resistance.
Plasmodium resistant GM mosquitoes based on the IMD/Rel2 system

Midgut & fatbody specific promoter driven Rel2

- Imd
- CYTOPLASM
- Caspar
- REL2
- NUCLEUS

Gene activity over time:
- Blood Ingestion
- 15hr
- 30hr

Promoters:
- Fat body promoter
- Gut promoter

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Plasmodium resistant GM mosquitoes based on the Rel2 system

Genetically modified mosquito lines

Gut promoter -driven Rel2

Fatbody promoter -driven Rel2

*\(p<0.0001\)

**A. stephensi** transgenic lines (generation 3)

<table>
<thead>
<tr>
<th>Lines</th>
<th>WT</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
<th>#5</th>
<th>#6</th>
<th>#7</th>
<th>#9</th>
<th>#13</th>
<th>#14</th>
<th>#15</th>
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<tbody>
<tr>
<td>nr</td>
<td>102</td>
<td>66</td>
<td>94</td>
<td>76</td>
<td>56</td>
<td>35</td>
<td>77</td>
<td>100</td>
<td>66</td>
<td>85</td>
<td>70</td>
<td>88</td>
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<tr>
<td>prevalence</td>
<td>93.1%</td>
<td>87.9%</td>
<td>81.7%</td>
<td>63.2%</td>
<td>87.5%</td>
<td>71.4%</td>
<td>74.0%</td>
<td>82.0%</td>
<td>77.3%</td>
<td>68.2%</td>
<td>81.4%</td>
<td>30.7%</td>
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<tr>
<td>mean</td>
<td>9.8</td>
<td>6.3</td>
<td>1.8</td>
<td>2.5</td>
<td>3.9</td>
<td>2.7</td>
<td>2.7</td>
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<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.5</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>% (mean)</td>
<td>100.0%</td>
<td>64.1%</td>
<td>18.9%</td>
<td>25.6%</td>
<td>39.7%</td>
<td>27.2%</td>
<td>28.1%</td>
<td>40.2%</td>
<td>27.8%</td>
<td>28.8%</td>
<td>40.6%</td>
<td>6.3%</td>
</tr>
</tbody>
</table>
The mosquito’s natural intestinal bacteria can influence transmission of *Plasmodium*

~2,000-fold expansion of mosquito gut bacteria after a blood meal

*Richman et al.*, 1997
The microbial flora stimulates anti-\textit{Plasmodium} activity

Reduction of the bacteria flora render mosquitoes more susceptible to \textit{Plasmodium} infection

\textbf{A}

\textbf{B}

\textbf{Dong et al., PLoS Pathogens 2009}
The microbial flora stimulates immune gene expression

The mosquito’s natural bacteria flora activate the immune system

Dong et al., PLoS Pathogens 2009
Anti-Plasmodium genes control midgut bacteria flora

The presence of the microbial flora activates immune genes that control the proliferation of the flora and Plasmodium infection.

Dong et al., PLoS Pathogens 2006
Dong et al., PLoS Pathogens 2009
CONCLUSION:

• The microbial flora is a regulator of mosquito susceptibility to *Plasmodium*
What happens in the field?

JHMRI field laboratory, Macha - southern Zambia
An *E. cloacae*–like bacteria is able to completely inhibit *Plasmodium falciparum* development in the mosquito gut.

- PBS: ~10^9
- HIA: ~10^8
- HIA10: ~10^7
- HIA100: ~10^7

** = p<0.001
*  = p<0.01

- compared to PBS
www.dimopoulousgroup.org

• Ruth Aguilar
• Chris Cirimotich
• April Clayton
• Shuchismita Das
• Yuemei Dong
• Lindsey Garver
• Fabio Manfredini
• Musapa Mulenga
• Jose Ramirez
• Shuzhen Sim
• Jayme Souza-Neto
• Emma Warr
• Zhiyong Xi

• CDC Arbovirus Diseases Branch
• Johns Hopkins Malaria Research Institute Core Facilities
• Bruce Christensen group
• Anne Durbin (JHU, Virology)
• Vish Nene (array development)
• Ken Olson (cell lines)

SUPPORT
• NIH/NIAID R01AI061576, R01AI059492, RO1AI078997
• WHO/TDR
• NSF & ASM
• Ellison Medical Foundation
• Johns Hopkins Malaria Research Institute

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1. Background to Malaria Institute at Macha (MIAM)

Born by Macha Mission Hospital …
Research to fight malaria and find new drugs (1989 -)

MHMRI registered with GOZ 1994
“..malaria research: drug trials, prevention and control.”

1997 MMRI registered non-profit, USA
Vector research, incidence studies
New office block & laboratory space (2001)

2003 MOU (Macha Mission Hospital, MMRI, GOZ, JHMRI)
“to develop a malaria field research and training centre”
-new lab. & office facilities, birth of MIAM

MIAM official opening, Jan. 2005
2. Brief Overview of Current Research at MIAM

<table>
<thead>
<tr>
<th>Area</th>
<th>FNDP</th>
<th>MDG</th>
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<tbody>
<tr>
<td>Malaria Epidemiology, transmission, drug resistance</td>
<td>Pgme 2, BHCP, Pgme 3, Mal prevent. contrl.</td>
<td>6</td>
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<tr>
<td></td>
<td>Non-invasive diagnostics</td>
<td></td>
</tr>
<tr>
<td>Malaria elimination</td>
<td>Pgme 3</td>
<td>1, 4, 5, 6</td>
</tr>
<tr>
<td>Surveillance systems</td>
<td>Pgme 3</td>
<td>1, 4, 5, 6</td>
</tr>
<tr>
<td>Entomology</td>
<td>Pgme 2, 3</td>
<td>6</td>
</tr>
<tr>
<td>HIV/TB ART, drug resistance, treatment, prevention</td>
<td>Pgme 4, HIV, AIDS, STIs, Pgme 5, TB contr.</td>
<td>1, 4, 5, 6</td>
</tr>
</tbody>
</table>

MGDs
* Goal 1: Eradicate extreme poverty and hunger
* Goal 2: Achieve universal primary education
* Goal 3: Promote gender equality and empower women
* Goal 4: Reduce child mortality
* Goal 5: Improve maternal health
* Goal 6: Combat HIV/AIDS, malaria and other diseases
* Goal 7: Ensure environmental sustainability
* Goal 8: Develop a Global Partnership for Development

FNDP (Zambia Fifth National Development Plan)
* Programme 2: Basic health care package (BHCP)
* Programme 3: Malaria prevention and control
* Programme 4: HIV, AIDS, STI
* Programme 5: TB control
Development of Saliva Test for Malaria

Background

- New hope for curbing the malaria scourge
  - RBM, public-private partnerships
  - Global scale-up of effective malaria intervention ACT’s, ITN’s, IRS
  - Possible interruption of transmission

- Formidable disease resilience necessitates:
  - efficient epidemiological surveillance
  - resurgence/drug resistance
  - accurate screening for asymptomatic reservoirs essential
Current *P. falciparum* screening constraints

Necessitates drawing blood
- Finger-prick
- Venipuncture

Limitations
- requires use of needles or sharps in remote settings
  - Adequately trained personnel – not readily available
    - VHW level
    - Home level
  - Biohazard, accidental infection risk
- certain communities: blood taboos, beliefs etc
- Repeated testing - drug/vaccine efficacy trials/monitoring
- Low access to potential reservoirs for research/control programmes

Important need for non-invasive detection
Why saliva screening?

- Non-invasive, simpler, safer
- Greater community participation/co-operation
- Greater access to subclinical reservoirs for research/control programmes
- Strengthen research/surveillance
- Reduced sample collection cost/workload
**DNA Detection:**

*Is the infection in saliva the same as in blood sample?*

*Plasmodium falciparum* MSP2 amplicon from saliva (173Qs), urine (173Qu, 173u) and blood (173b) samples of patient 173. Qs and Qu denote saliva and urine samples extracted by Qiagen commercial kit, while uD denotes whole urine sample extracted by the Chelex method. Qs-, Qu-, uD- and b-, denote amplicon from corresponding extracts of saliva, urine and blood provided by thick film negative healthy control. 3D7, amplicon from positive control laboratory standard. Extraction of urine replicate sample 173u was performed on 20.02.06, while Qiagen extractions were carried out on 09.02.06. Identical MSP2 alleles are apparent in amplicon from urine, saliva and blood samples of patient 173, as compared to 3D7 lab standard.
DNA Detection:

Is the infection in saliva the same as in blood sample?

Figure 1 Amplicon from saliva, urine and blood extracts of field samples 216, 34 and 210. Matching MSP2 alleles are seen in saliva (Qs) and blood (b) amplicon from each individual. In contrast, between-patient polymorphic differences are evident, reflecting diverse infections. Urine samples from these patients did not amplify, except that of 210 (210Qu). Qs, Qu denote Qiagen saliva and urine extracts, respectively, by crude lysate approach; Cu denotes Qiagen urine extracts, by cultured animal cells protocol; Du denotes Chelex direct extraction on whole urine. Qs-, Cu-, b- were corresponding extracts of saliva, urine and blood samples from healthy negative control, while 3D7 was positive control laboratory standard.
Is *P. falciparum* DNA detection in saliva repeatable?

- Yes, by range of genomic primers and extraction methods, machines...
  - MSP2 – chr 2
  - PfDHFR – chr 4
  - PfDHPS – chr 8
  - Pfmdr1 – chr 5
  - Pfcrt – chr 7
  - TA81 – chr 5
  - 18S rRNA gene (chr 1, 5, 7, 11)

- Yes, extracted at different time points with different methods
- Yes, other investigators, using Real-time platform:
What are the determinants of amplicon yield?

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>SUMMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction method</td>
<td>Higher amplicon with Qiagen than Chelex</td>
</tr>
<tr>
<td></td>
<td>Urine: 2.24X higher</td>
</tr>
<tr>
<td></td>
<td>Saliva: 2.25X higher</td>
</tr>
<tr>
<td>Sample type</td>
<td>Saliva extracts:</td>
</tr>
<tr>
<td></td>
<td>1.6 fold higher amplicon yield than urine</td>
</tr>
<tr>
<td>Parasite density</td>
<td>For each unit increase in log parasite density:</td>
</tr>
<tr>
<td></td>
<td>1.82-fold increase in amplicon yield</td>
</tr>
<tr>
<td>Primer set</td>
<td>U1-U4 (370bp, 229bp) 18.5X more likely to amplify than FC27 (750bp, 290-420bp)</td>
</tr>
</tbody>
</table>
Conclusions

- *P. falciparum* infection detectable using saliva samples
- Infection detected in saliva identical to that found in blood
- Sound prospects of oral-based malaria testing

Questions/Future Work

- Peak season surveys and assay optimizations
- What is the source of *P. falciparum* material in the saliva?
- Does saliva secretion gland matter?
- NYU collaboration
Mapping Mosquito Migrations

Gregory E. Glass, PhD
Director, Environmental Surveillance Core
JHMRI
Environmental Surveillance

http://terra.nasa.gov/About/

NASA Terra
Environmental Surveillance Core JHMRI:

Provides

- Study designs for research
- Environmental Information (satellite data/ground studies/surveys)
- Develop analytical methods
- Identify where and when people are at risk

Web-based service

General access to PI’s study
ESC: Merges Environmental & Health Data

- **GIS:** Spatially explicit study integration for multiple researchers
- Census
- Integrate Investigator data sets
- Field study selection
- Training
Heterogeneity in Transmission of Malaria – an Important Key in Control/Elimination

The Risk of a Mosquito-Borne Infection in a Heterogeneous Environment

David L. Smith, Jonathan Dushoff, F. Ellis McKenzie
1 Fogarty International Center, National Institutes of Health, Bethesda, Maryland, United States of America. 2 Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey, United States of America

Spatial variation in household risk for infection Macha, 2007/2008

Ignore it at your peril
Variation in Exposure to Vectors: Detecting Potential Larval Sites

**Challenges:** Sites can small, difficult to detect and ephemeral
Identifying Potential Breeding Sites

Why is Something So Simple So Hard to Do?

- Challenge to find all the breeding sites on the ground
- Can’t have dedicated satellite resources for breeding site detection

Our Approach:

- Apply physical models to identify potential breeding sites
- Evaluate model with observed data
- Evaluate health consequences for population
Hydrologic Models Generated from DEM

Models used to estimate:
Pattern of water flow/ accumulation
  Topographic wetness
  Topographic position
  (scale dependent)

Incorporate other information
Soils, land use
Human residence
Larval sites

Can we find the vectors?
Vectors Breed in Very Specific Parts of the Landscape

96% sites with An. arabiensis in two landforms representing 30% of area

122/124 sites have water between seasons; only 4 ‘new’ sites

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRTM Slope</td>
<td>0.3</td>
<td>0.01</td>
</tr>
<tr>
<td>TPI 500 m</td>
<td>0.74</td>
<td>0.01</td>
</tr>
<tr>
<td>greenness</td>
<td>320</td>
<td>0.02</td>
</tr>
<tr>
<td>moisture</td>
<td>0.001</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Relate Abundance & Distribution to Housing

GIS relates where people live with respect to breeding sites of vectors

If people live ‘too close’ they should be at increased risk for attack by adult vectors

What is too close?
Identify other regions that have similar environmental conditions
Targeting Households for Treatment with Limited Resources

Households near breeding sites have many more infected people

Knowing where to look provides approach for targeted intervention
Future Directions

Extend relationship between infection, breeding sites and environment

Incorporate dynamic environmental changes in hunt for mosquitoes

Does targeted treatment of larval sites reduce adult mosquitoes (& human disease) in high risk areas.

Evaluate impact of bednets/drugs in reducing human infection in high risk areas

Eliminate local malaria transmission within 3 years