THE ABUNDANCE AND INFECTION STATUS OF ANOPHELES MOSQUITOES IN LOUDOUN COUNTY, VIRGINIA

Andrew Lima—Clarke (Manassas, VA)
Priya Krishnan—ODU M.S. candidate (Richmond, VA)
Objectives

• To determine:
  1) the abundance and feeding status of Anopheles at three Loudoun County, VA resting box sites.
  2) the presence of human Plasmodium in Anopheles bloodmeals.
  3) the presence of avian Plasmodium and Haemoproteus in bloodfed Anopheles.
Why Loudoun County?

• *Plasmodium vivax* malaria in (2) Loudoun Co. teens in 2002
  – Diagnosed 8/23/02 and 8/25/02
  – Patients lived about ½ mile apart

• Subsequent surveillance
  – Multiple pools of *An. quadrimaculatus* and *An. punctipennis* positive for *P. vivax* 210
  – Evidence of local transmission
Collection Sites and Proximity to Local Transmission in 2002

6 miles
Site Selection

• Proximity to Potomac River
  – Near site of suspected 2002 transmission
• Dense canopy (>75%)
• Minimal understory
  – Look for closely planted trees or sewer lines
  – Pine barrens in northeast
• Little or no direct sunlight
  – Boxes face WEST
Features of Resting Boxes

• Useful ONLY for Cs. melanura and An. quadrimaculatus in eastern U.S.

• Cheap and easy to build
• Efficient collections
  – Only requires 1 daytime visit per week
    • “Passive”
• All feeding stages collected
  – Useful for virus/bloodmeal studies, vector dynamics
Methods

• 3 Collection Sites
  – All less than 1 km from Potomac River

• Early August – Early October
  – Epiweeks 31 thru 40

• 30 Resting Box Collections
  – 10 boxes per site
  – Visit all 3 sites 1 day/week
  – 3 trap nights per week during 10 weeks surveillance
  – 30 trap nights per season
Methods (Cont’d)

- RB collxns made in early afternoon
  - 12 to 4 pm
- Specimens transported to lab on dry ice
  - Cold chain maintained thru testing
  - Feeding status noted as:
    - Unfed
    - Bloodfed
    - Gravid
Feeding Status

• **UNFED**
  – No swelling of abdomen

• **BLOODFED**
  – Visible red or black blood inside abdomen

• **GRAVID**
  – Eggs visible with NO blood present
Species Commonly Collected from Resting Boxes in northern VA (2008 thru 2010)

- **An. quadrimaculatus**
  - Primary vector of *Plasmodium vivax* and *falciparum* in eastern U.S.
  - mammals, humans
  - Flight distance 1-3 miles?

- **An. punctipennis**
  - Secondary vector *Plasmodium spp.*
  - mammals, birds
Other species collected from resting boxes in Loudoun County, VA (2008 thru 2010)

• *Cx. erraticus*
  – birds, humans, reptile/amphibian?

• *Cx. territans*
  – reptiles, amphibians

• *Species collected in very low abundance:*
  – *Cx. pipiens* (13)
  – *Cx. restuans* (5)
  – *Cs. melanura* (3)
  – *Cq. perturbans* (2)
  – *Ae. vexans* (1)
  – *Ur. Sapphirina* (1)
Objective 1

To determine the abundance of resting *Anopheles* in Loudoun County, VA.
# Raw historical data

<table>
<thead>
<tr>
<th></th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting box collections</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Total # <em>Anopheles</em> collected</td>
<td>5431</td>
<td>1947</td>
<td>2514</td>
</tr>
<tr>
<td># of bloodfed <em>Anopheles</em> tested</td>
<td>1370</td>
<td>719</td>
<td>1271</td>
</tr>
<tr>
<td># pools submitted</td>
<td>189</td>
<td>81</td>
<td>143</td>
</tr>
</tbody>
</table>

- More than 50% of 2008 samples come from a single collection on 30 AUG 2008
  - Preceded by 3.5” of rain two weeks before
  - Largest rainfall event during the collection period for all 3 years
  - Followed by very dry, hot weather
Total # Anopheles collected from resting boxes, 2008 – 2010 (90 trap nights)

<table>
<thead>
<tr>
<th>Total # Anopheles</th>
<th>Total # bloodfed</th>
<th>Avg. # Anopheles/season</th>
<th>Avg. # Anopheles per site each epiweek</th>
<th>Avg. # Anopheles per resting box</th>
</tr>
</thead>
<tbody>
<tr>
<td>9,556</td>
<td>3,360</td>
<td>3,185</td>
<td>106</td>
<td>11</td>
</tr>
</tbody>
</table>
Abundance of major species collected in resting boxes, 2008 thru 2010

An. punctipennis
330, 3%

Cx. erraticus
451, 4%

Cx. territans
71, 1%

An. quadrimaculatus
9542, 92%
Feeding status of *Anopheles* collected by year (2008 – 2010)

<table>
<thead>
<tr>
<th></th>
<th>Unfed</th>
<th>Bloodfed</th>
<th>Gravid</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>1341</td>
<td>1370</td>
<td>2391</td>
</tr>
<tr>
<td>2009</td>
<td>946</td>
<td>719</td>
<td>278</td>
</tr>
<tr>
<td>2010</td>
<td>881</td>
<td>1271</td>
<td>359</td>
</tr>
<tr>
<td>3 year AVG.</td>
<td>1056</td>
<td>1120</td>
<td>1009</td>
</tr>
</tbody>
</table>
Feeding status of *Anopheles* collected from resting boxes (2008 – 2010)

- Gravid: 3028, 32%
- Bloodfed: 3360, 35%
- Unfed: 3168, 33%
Total number of *Anopheles* collected weekly across (3) resting box sites (2008 - 2010)

![Graph showing the total number of *Anopheles* collected weekly across three resting box sites from 2008 to 2010. The graph includes data for each year and a 3-year average.](image-url)
Mean weekly number of *Anopheles* (2008 - 2010)

-Late August thru mid-September generally yield the highest collections

-epiweeks 35 thru 38
# Summary

<table>
<thead>
<tr>
<th>Total abundance</th>
<th>Algonkian Regional Park</th>
<th>Youngs Cliff</th>
<th>Potomac Drive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anopheles quadrimaculatus</em></td>
<td>Significant difference 2008-2009, 2008-2010</td>
<td>No significant difference across 3 years</td>
<td></td>
</tr>
<tr>
<td><em>Anopheles punctipennis</em></td>
<td></td>
<td>Stable across the 3 years</td>
<td></td>
</tr>
</tbody>
</table>
Objective 2

To determine the presence of human *Plasmodium* in *Anopheles* mosquitoes in Loudoun county in Northern Virginia.
# Human and avian malaria parasites

<table>
<thead>
<tr>
<th>Disease</th>
<th>Hosts</th>
<th>Causative Agent</th>
<th>Primary mosquito vector(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>Humans</td>
<td>( P. \text{ vivax} ) ( P. \text{ falciparum} ) ( P. \text{ ovale} ) ( P. \text{ malariae} )</td>
<td>Anopheles spp.</td>
</tr>
<tr>
<td></td>
<td>Birds</td>
<td>( P. \text{ cathemerium} ) ( P. \text{ elongatum} ) ( P. \text{ gallinaceum} ) ( P. \text{ hermansi} ) ( P. \text{ relictum} )</td>
<td>Culex spp. \ Aedes spp. (rare?)</td>
</tr>
</tbody>
</table>
Background on Domestic Malaria

• Majority acquired abroad
  – Travel between U.S. and malaria-endemic countries

• Local transmission is uncommon
  – Presence of capable hosts, vectors, and ideal weather
Local Transmission of Malaria in Virginia

August 2002 - *Plasmodium vivax* malaria in Loudoun County in Northern Virginia.

- Locally acquired
- Patients lived about ½ a mile apart.
- Lack of risk factors for malaria e.g. International travel, blood transfusion.
- *An. quadrimaculatus* and *An. punctipennis* pools collected in Loudoun county tested positive for *P. vivax* 210 subtype.
DNA Extraction

• Head/thorax removed from *Anopheles*
• 2008 and 2009 - thorax and abdomen were ground
• 2010 - abdomens were ground.
• Total DNA extracted (DNA of mosquito and anything in the blood-meal).
No Amplicons with DNA of Mosquitoes Collected in 2008

- Extracted DNA tested by PCR using primers against the white gene of *Anopheles gambiae* according to the method of Rafferty et al. 2002 (modified).
- No amplicons.
- 2008 samples deemed not available for further molecular tests.
- Only 2009 and 2010 samples used.
Multiplex PCR to Test for Human *Plasmodium*

- 224 pools tested for human *Plasmodium*
  - 2008: N/A
  - 2009: 81 pools
  - 2010: 143 pools
No evidence of human *Plasmodium* in *Anopheles* at 3 resting box sites (2009 and 2010)

Lane 1- DNA marker
Lane 2 to lane 14 - DNA extracted from blood meals of *Anopheles* mosquitoes collected in 2009
Lane 15 - Positive control *Plasmodium vivax* DNA, expected size 300 bp
Lane 16 - Negative control
## Summary

<table>
<thead>
<tr>
<th>Year</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em></td>
<td>Not applicable</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>Not applicable</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. ovale</em></td>
<td>Not applicable</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. malariae</em></td>
<td>Not applicable</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Objective 3

- Determine the presence of avian *Plasmodium* and *Haemoproteus* in *Anopheles* mosquitoes from Loudoun County, VA
Haemosporidian Parasites

The order Haemosporidiae contains *Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp.

Mosquito - *Culex* spp.

Midges - *Culicoides* spp.

Black fly - *Simuliidae* spp.

*Plasmodium* spp.  
*Haemoproteus* spp.  
*Leucocytozoon* spp.
### Human and Avian Malaria Protozoans

<table>
<thead>
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<th>Hosts</th>
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<tr>
<td>Malaria</td>
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<td><em>P. vivax</em> <em>P. falciparum</em> <em>P. ovale</em> <em>P. malariae</em></td>
<td><em>Anopheles spp.</em></td>
</tr>
<tr>
<td></td>
<td>Birds</td>
<td><em>P. cathemerium</em> <em>P. elongatum</em> <em>P. gallinaceum</em> <em>P. hermansi</em> <em>P. relictum</em></td>
<td><em>Culex spp.</em> <em>Aedes spp. (rare?)</em></td>
</tr>
</tbody>
</table>

- Introduction of Avian malaria to geographically isolated areas (i.e. Hawaii, 1826) can devastate bird populations.
  - Lost resistance due to isolation.
Nested PCR to test for avian Haemosporidian parasites

• 224 pools were tested for presence of Haemosporidian parasites in the 1st PCR.
  – 2009: 81 pools
  – 2010: 143 pools
  • Pools that tested positive for presence Haemosporidian parasites tested for *Plasmodium*
    and *Haemoproteus* in the 2nd PCR.
Avian Malaria in *Anopheles*

- Natural infections in *Anopheles* mosquitoes not known.
- *Anopheles* mosquitoes collected by Catherine Wallace from Southern Virginia tested positive for Haemosporidian parasites
  - *Haemoproteus* and *Plasmodium*.
- Positive blood meal doesn’t infer infectivity
- Need to test salivary glands for sporozoites.
Evidence of Haemosporidian parasites in bloodfed Anopheles collected from 3 resting box sites (2009 and 2010)

<table>
<thead>
<tr>
<th>Haemosporidia</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>An. quadriramculatus</td>
<td>An. punctipennis</td>
</tr>
<tr>
<td>Algonkian Regional Park</td>
<td>10/20 = 50%</td>
<td>0/20 = 0%</td>
</tr>
<tr>
<td>Youngs Cliff</td>
<td>4/20 = 20%</td>
<td>2/20 = 10%</td>
</tr>
<tr>
<td>Potomac Drive</td>
<td>4/20 = 20%</td>
<td>0/20 = 0%</td>
</tr>
</tbody>
</table>
Evidence of *Plasmodium* and *Haemoproteus* Parasites in Mosquitoes Collected at the 3 resting box sites (2009)

<table>
<thead>
<tr>
<th>Sites</th>
<th><em>Haemoproteus</em></th>
<th><em>Plasmodium</em></th>
<th><em>Haemoproteus</em> and <em>Plasmodium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>An. quadrinaculatus</td>
<td>An. punctipennis</td>
<td>An. quadrinaculatus</td>
</tr>
<tr>
<td>Algonkian Regional Park</td>
<td>1 (5%)</td>
<td>0</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Youngs Cliff</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Potomac Drive</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- 4 unknown Haemosporidia infections (20%)—Suspected *Leucocytozoon*.
- 2 (10%) at Youngs Cliff, 2 (10%) at Algonkian Regional Park
Conclusions (Abundance)

• *An. quadrimaculatus* collected in large numbers during all 3 years of the study
  
  • Significant difference between 2008/2009 and 2008/2010 at Algonkian Regional Park
    • 3.5” rainfall on 15 AUG followed two weeks later by record #s
  
  • Peaked in late August thru early September
  
  • Effective “passive” technique for collecting resting mosquitoes of any feeding status
    • 1:1:1 ratio (Unfed:Bloodfed:Gravid)
Conclusions (Human malaria)

• 2008 samples did not amplify
  – Degradation?

• No evidence of human *Plasmodium* in the *Anopheles* pools collected in 2009 and 2010 at all 3 sites.
  – False positives from past years could be a result of picking up on avian *Plasmodium*. 
Conclusions (Avian malaria)

• Evidence of haemosporidian parasites in *Anopheles* pools in 2009 in all 3 sites.
  • 20/81 (25%) of *Anopheles* pools infected with avian *Plasmodium* and *Haemoproteus* at Algokian Regional Park; most being *Anopheles quadrimaculatus*.

• No evidence of Haemosporidian parasites in *Anopheles* pools in 2010.
  • Why not? Preparation of samples?
Future Studies

• Extend sampling period to encompass entire mosquito season
  – Examine trends in bloodfed specimens by time of year collected
  – Determine true peak of activity for *Anopheles*.
    • Population dynamics between *quads* and *puncs*

• Pool **abdomens** of bloodfed mosquitoes **individually**
  – Host bloodmeal analysis
  – Test for Haemosporidia
    • “nested” PCR on positive mosquitoes to determine genus/species of protozoan
      – Infected vs Infective
        » Examine salivary glands for evidence of sporozoites
Other recent resting box studies

• Komar 1995 (SE Massachusetts)
  – Nestable fiber pots
    • melanura, quads
• Odiere 2007 (Kenya)
  – Clay pots
    • gambiae, quinqs
• Bentley 2009
  – LED resting boxes
    • Unlit attracted more bloodfeds
    • Red/IR LEDs attracted more quads overall

• Kweka 2009 (Tanzania)
  – Host odor-baited resting boxes (cow sweat/urine)
    • gambiae s.s., arabiensis
    • Alternative to HLC?

• Need for additional studies
  – Integrate baits with control?
    • non-repellent insecticides
    • Entomopathogenic fungi
  – Mark/recapture
    • Flight range analysis
Thanks due to...

- VMCA Officers and Members
- Clarke
- VCU Dept. of Biology (Richmond)
  - Dr. Ghislaine Mayer
  - Dr. Kevin Caillouet
  - Priya Krishnan
  - Caty Wallace
- Property Owners
- Loudoun County Health Department
QUESTIONS???