ESTABLISHING A VECTOR ECOLOGY SITE TO UNDERSTAND TICK-BORNE DISEASES IN THE SOUTHEASTERN UNITED STATES

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HELP, my people are getting ate up by ticks. Some are even getting sick with Lyme disease and Rocky Mountain Spotted fever. You've got to do something.

Hmmm... that's very interesting, you know....

LIFECYCLE & TRANSMISSION
Gravid Female Lays Eggs
Larvae Seek a Host
Nymphs Overwinter
Adults Seek Host
Eggs Hatch
Molt
Transovarial
Transstadial
Transseasonal
Molt
Molt
Mate
Nymphs Overwinter
COMPARED TO OTHER ARTHROPODS
TICKS ARE SOME OF THE BEST VECTORS

- Live a long time (~2-3yrs)
- Generalist
- Use host for dispersal
- THICK exoskeleton
- Blood Feeding:
  - ALL active life stages
  - Long active blood meals
  - Sneaky (anti-coagulants)
  - Penetrate epidermis & can reach circulatory system
  - LOTS of eggs (~1000s)
- Host Finding

HOST FINDING
(QUESTING)

Eyes- see ‘shapes’
Haller’s organ

OTHER POTENTIAL REASONS

- Landscape changes
- Difficulty of diagnosis
- Identification of ‘new’ agents & spread of ‘old’ agents
- Warming weather trends
- Identication of ‘new’ agents & spread of ‘old’ agents
SPOTTED FEVER GROUP RICKETTIOSIS
(SFGR = RICKETTSIA)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Disease</th>
<th>Symptoms</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rickettsia rickettsii</td>
<td>Rocky Mtn. Spotted fever</td>
<td>Rash all over w/in 3-5 days</td>
<td>Doxycycline is first line of treatment</td>
</tr>
<tr>
<td>Rickettsia parkeri</td>
<td>American Boutonneuse fever</td>
<td>Variable rash</td>
<td></td>
</tr>
<tr>
<td>Rickettsia amblyommii</td>
<td>Non-pathogenic</td>
<td>No rash</td>
<td></td>
</tr>
</tbody>
</table>

PROBLEM: SAME DIAGNOSTIC TEST TO IDENTIFY HUMAN ANTIBODY

Identification of ‘new’ agents & spread of ‘old’ agents

Difficulty of diagnosis

EHRLICHIOSIS
(EHRLICHA SPECIES)

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<th>Bacteria</th>
<th>Disease</th>
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<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>Ehrlichia chaffeensis</td>
<td>Ehrlichiosis</td>
<td>Variable rash</td>
<td>Doxycycline is first line of treatment</td>
</tr>
<tr>
<td>Ehrlichia ewingii</td>
<td>Ehrlichiosis</td>
<td>Variable rash</td>
<td></td>
</tr>
<tr>
<td>Ehrlichia muris</td>
<td>Ehrlichiosis</td>
<td></td>
<td></td>
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</tbody>
</table>

PROBLEM: SAME DIAGNOSTIC TEST TO IDENTIFY HUMAN ANTIBODY

Identification of ‘new’ agents & spread of ‘old’ agents

Difficulty of diagnosis

Areas at Risk for Lyme Disease (Borrelia burgdorferi)

“In Tennessee, you’re not likely to get bitten by a blacklegged tick. And of the few blacklegged ticks sampled during the five-year survey, none was found to be infected with the bacteria that causes Lyme disease.”

For every 100 ticks collected at deer check stations or in the woods during the study, only one or two turned out to be a blacklegged tick. And of the few blacklegged ticks sampled during the five-year survey, none was found to be infected with the bacteria that causes Lyme disease.”

PROBLEM: SAME DIAGNOSTIC TEST TO IDENTIFY HUMAN ANTIBODY

Identification of ‘new’ agents & spread of ‘old’ agents

Difficulty of diagnosis
COMMON TN DISEASES
Rickettiosis: 19-63 diagnosed cases / million
Ehrlichiosis: 3.3-26 diagnosed cases / million
www.CDC.gov

RMSF & SFGR: COMPLEX PUZZLE
2.4% total cases, SW TN accounts for 26% of fatalities
Adjemian et al. 2009

Can we explain the incidence of tick-borne diseases in Tennessee?

1 - Ixodes scapularis
2- most abundant tick: Amblyomma americanum
Led to more questions...
OBJECTIVE

To determine if *Ixodes scapularis* is a vector of concern in Tennessee cases of human tick-borne diseases

Mays et al. submitted to *Parasites and Vectors*
MATERIALS & METHODS
Ixodes scapularis collected from deer were screened for Anaplasma, Babesia, Borrelia, Ehrlichia, & Rickettsia
2011: 17 ticks from 6 deer
2012: 30 ticks from 9 deer

Results: Babesia spp.
2011
0/17 (0%) ticks positive
Babesia spp. absent
2012
0/30 (0%) ticks positive

Results: Borrelia spp.
2011
0/17 (0%) ticks positive
Borrelia spp. Lyme disease Absent
2012
0/30 (0%) ticks positive
Results: *Ehrlichia/Anaplasma* spp.

2011
1/17 (6%) positive:
  - *Anaplasma phagocytophilum*
    - 99% Homologous (EF647585)

2012
3/30 (10%) positive:
  - *Panola Mountain Ehrlich*
    - 100% Homologous (HQ65)
  - *Ehrlichia ewingii*
    - 100% Homologous (AF19)

Results: *Rickettsia* spp.

2011
10/17 (59%) positive:
  - *Rickettsia amblyommii* - 1
    - 99% Homologous (JF694090)
  - *Rickettsia sp.* - 2
    - >98% Homologous (HM446484)
  - *Ixodes scapularis endosymbiont* - 7
    - >98% Homologous (EF689735)

2012
17/30 (57%) positive:
  - *Ixodes scapularis endosymbiont* - 17

Can we explain the incidence of tick-borne diseases in Tennessee?

1. *Ixodes scapularis*
2. Most abundant tick: *Amblyomma americanum*

“So not Lyme, but pathogenic *Anaplasma*, what about those Lone Star ticks?”
CHARACTERIZE THE PATHOGENS WITHIN THE MOST ABUNDANT TICK: AMBLYOMMA AMERICANUM

Obj. 1: Identify *Ehrlichia* & *Rickettsia* species
Obj. 2: Investigate seasonal prevalence
Obj. 3: Locate sites with ticks positive for a bacteria

MATERIALS & METHODS

100 Sites Sampled
Months Sampled: May-September

Tick Collection

Screened 942 adult Lone Star ticks for *Rickettsia* and *Ehrlichia* bacteria

RESULTS: BACTERIA IDENTIFICATION

17 / 926 (1.8%) *Ehrlichia* Pos.
- Anaplasma cadillac (2)
- Panola Mts. *Ehrlichia* (2)
- *E. chaffeensis* (1)
- *E. ewingii* (12)
  (ALL ARE PATHOGENIC!)

353 / 926 (38.3%) SFGR Pos.
All Sequenced were *R. amblyommi* (NON-PATHOGENIC)
RESULTS: SEASONAL PREVALENCE

RESULTS: SPATIAL DISTRIBUTION
Bacteria Positive Sites: Randomly Distributed
Ehrlichia Sites: Pines and Upland Hardwood
Rickettsia Sites: Bottomland Hardwood

Can we explain the incidence of tick-borne diseases in Tennessee?

1. *Ixodes scapularis* - The most abundant tick:
A. *Amblyomma americanum*
B. *A. maculatum* & pathogenic *Ehrlichia*

2. Role of other ticks, hosts, habitats...

3. But wait, we found
A. *A. maculatum* & a TON of *D. variabilis*. And look at all of these *Ehrlichia*!
Hendrick's Other Ticks

**Tick Collection**
9,475 ticks over 6 months

- **Amblyomma americanum**
- **Amblyomma maculatum**
- **Dermacentor variabilis**

### Nymphs
- May: 0
- June: 500
- July: 1000
- Aug: 1500
- Sept: 2000

### Adults
- May: 2000
- June: 4000
- July: 6000
- Aug: 8000
- Sept: 0

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**Dermacentor variabilis**

- **Hendrick's Thesis**
  - Questing ticks from 100 sites during 2012
  - Two collection methods: Dry Ice, Drag
  - *Dermacentor variabilis* collected at 99 sites

- **Screened 2545 ticks**
  - 0 (!) positive for *Anaplasma* or *Ehrlichia*
  - 93/2545 (3.7%!) positive for *Rickettsia*

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**ROCKY MOUNTAIN SPOTTED FEVER**

- 3.7% positive for a SFGR
- Sequencing arrived at 8am today...
  - ~R. montanensis
  - ~R. montana
- Unclear pathogenicity

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http://www.cdc.gov/rmsf/stats/
**AMBLYOMMA MACULATUM**

- Hendrick’s Thesis
  - Questing ticks from 100 sites during 2012
  - Two collection methods: Dry Ice, Drag
  - Gulf Coast ticks collected at 10 sites

**SPECIFYING HABITAT, HOST, AND PATHOGEN ASSOCIATIONS OF THE GULF COAST TICK, AMBLYOMMA MACULATUM**

Sarah E. Mays

**TRAPPING METHODS**

<table>
<thead>
<tr>
<th>Method</th>
<th>488</th>
<th>547</th>
<th>455</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO2 Trap</td>
<td>121</td>
<td>280</td>
<td>379</td>
<td>280</td>
</tr>
<tr>
<td>Drag</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flag</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=2102, F=3.36, df=5, p<0.0069
MAYS THESIS RESEARCH
Specifying Habitat, Host, and Pathogen Associations of the Gulf Coast Tick, *Amblyomma maculatum*

80 sites – different habitats! Different months!

TICK DISTRIBUTION

AMBLYOMMA MACULATUM
One Health Concern

Human Disease

Canine Disease

Livestock Disease
Hosts & Habitats

Habitat Wise Tails:
AR: “ON Cattle”
FL: “High-noon on tall grass”
WV: “Wherever you would find water moccasins”
MD: “Near land fills”

AN ASSESSMENT OF HOST-
TICK ASSOCIATIONS FOR
SMALL MAMMALS IN
WESTERN TENNESSEE

HABITAT & HOSTS AT AMES(?)

150 traps per night for 3 sets of 3 consecutive nights
CHECKING FOR TICKS

Checking Under Arms

Checking the Body

Checking the Facial Region

ENGORGED FEMALE TICK ON WHITE-FOOTED DEERMOUSE

TICKS ON HOSTS

Hispid Cotton Rat (Sigmodon hispidus) [73]
White-Footed Deermouse (Peromyscus leucopus) [160]
North American Deermouse (Peromyscus maniculatus) [34]
Cotton Deermouse (Peromyscus gossypinus) [1]
Woodland Vole (Microtus pinetorum) [16]
Marsh Rice Rat (Oryzomys palustris) [1]
Golden Mouse (Ochrotomys nuttalli) [7]
House Mouse (Mus musculus) [3]

Lone Star tick (Amblyomma americanum) [0]
Gulf Coast tick (Amblyomma maculatum) [7]
American dog tick (Dermacentor variabilis) [455]
Blacklegged tick (Ixodes scapularis) [96]

GREATEST ABUNDANCE OF TICKS ON HOSTS IN PINE HABITATS!

2 from White-footed deermouse & 8 from Hispid Cotton rat
Can we explain the incidence of tick-borne diseases in Tennessee?

1. *Ixodes scapularis*
2. Most abundant tick: *Amblyomma americanum*
3. Role of other ticks, hosts, and habitats...

"Agreed."

"But wait, we need to start looking into these different habitats!"

Focus...

**RESULTS THUS FAR**

<table>
<thead>
<tr>
<th>Tick Species</th>
<th>No. Tested</th>
<th>Rickettsia Pos. (%)</th>
<th>Ehrlichia Pos. (%)</th>
<th>Co-infection Pos. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amblyomma americanum</em></td>
<td>926</td>
<td>345 (37.3%)</td>
<td>9 (1.0%)</td>
<td>8 (0.8%)</td>
</tr>
<tr>
<td><em>Dermacentor variabilis</em></td>
<td>2545</td>
<td>93 (3.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Ixodes scapularis</em></td>
<td>47</td>
<td>23 (48.9%)</td>
<td>0 (0%)</td>
<td>4 (9%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>3518</strong></td>
<td><strong>461 (13.1%)</strong></td>
<td><strong>9 (0.3%)</strong></td>
<td><strong>12 (0.3%)</strong></td>
</tr>
</tbody>
</table>

All *Rickettsia* NON-PATHOGENIC (TBD?)

Many pathogenic *Ehrlichia* species:
- *Anaplasmaphagocytophilum*
THE TWIST

Vector-Borne and Zoonotic Diseases

Changning Tick Distributions

Next Questions...
Ticks use hosts for protein (blood) & dispersal.

Pathogens use hosts for reproduction, development & dispersal.

**ACKNOWLEDGEMENTS**

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- James Morrow (AMES)
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- Lauren Maestas (CAV)
- Michael Kennedy (Univ. Memphis)
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- Dave Paulsen (UT Med. Vet.)
- Stephen Kania (UT Vet. Med.)

*Many unnamed heroes*