Prevalence of Bacterial Pathogens in Ixodid Ticks in Chatham County, North Carolina

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Larval lone star tick bites leave a dermatitis. The bites are extremely pruritic. Scratching the bites typically results in development of secondary infections.
Tick bites occasionally result in transmission of pathogens. 60% of all human diseases are zoonoses. Additionally, 80% of pathogens with a high potential for bioterrorism are zoonotic.

For Lyme disease, the erythema migrans rash, occurs at the site of the tick bite.

For RMSF, the rash occurs on the wrists and ankles and spreads to the trunk of the body.
Humans are incidentally bitten by ticks.

Ticks have evolved a parasitic relationship with wildlife.

All ticks require a blood meal to complete their life cycle.

- Growth – molt from stage-to-stage
- Reproduction – production of eggs.
A large number of tick species with worldwide distribution.

- **Class Acarina**
  - Order Parasitiformes
    - **Family Argasidae** – soft ticks (5 genera)
    - **Family Nuttelliellidae** – hard ticks (1 genus)
    - **Family Ixodidae** – hard ticks (7 genera)
      - Genus *Dermacentor* – 30 species
      - *Amblyomma* – 102 species
      - *Ixodes* – 235 species
      - *Rhipicephalus* – 75 species
Common ticks in North Carolina

**Figure 2.** Male and female American dog tick.

**Figure 3.** Male and female brown dog tick.

**Figure 4.** Male and female lone star tick.

**Figure 5.** Male and female black-legged tick.
Reportable tick-borne diseases in NC

- **Spotted fever group rickettsioses**, including RMSF
  - Pathogen: *Rickettsia* spp.
  - Vector: *Dermacentor variabilis*, *Amblyomma maculatum*, *Amblyomma americanum* (?)

- **Lyme disease**
  - *Borrelia burgdorferi* s. l.
  - *Ixodes scapularis*

- **Ehrlichia chaffeensis** infection
  - Formerly human monocytic ehrlichiosis
  - *Amblyomma americanum*

- **Anaplasma phagocytophilum** infection
  - Formerly human granulocytic anaplasmosis
  - *Ixodes scapularis*, *A. americanum* (?)
Reported cases of ehrlichiosis caused by infection with *Ehrlichia chaffeensis*, reported to CDC in 2001-2002.
Anaplasmosis and Ehrlichiosis
caused by gram-negative bacteria that are grouped by the type of leukocytes that they invade.

**Anaplasma phagocytophilum** the pathogen that causes **human granulocytic anaplasmosis** (HGA) primarily infects granulocytes (neutrophils and rarely eosinophils). *(Morulae in cytoplasm of neutrophil)*

**Ehrlichia chaffeensis** the pathogen that causes **human monocytic ehrlichiosis** (HGE) primarily infects mononuclear leukocytes (predominantly monocytes and macrophages), but may also be seen occasionally in the granulocytes of some patients with severe disease. *(Morulae in cytoplasm of monocyte)*
White-tailed deer are a preferred host of LS and BL ticks.

- Deer are reservoir hosts for the HME and possibly HGA agent.
  - Antibody surveys
  - Tick infection studies
  - Culture
  - PCR
Borrelia burgdorferi s. l. is a complex of 14 genospecies worldwide:

- *B. burgdorferi* s. s., *B. bissetti*, *B. andersoni* (well established)
- *B. carolinensis* (Rudenko et al. 2009a)
- *B. americana* (Rudenko et al. 2009b)
- *B. kurtenbachii* (Margos et al. 2010)
Prototypical Lyme borreliosis vector: *Ixodes scapularis* (black-legged tick)

- A member of the *I. ricinus* complex.
- Distributed geographically in the upper midwestern, northeastern and throughout the southern US.

The nymph is primarily responsible for Lyme disease spirochete transmission.

*Peromyscus* mice are reservoirs for the LD spirochete.
Life cycle of *I. scapularis* in the NE

- In LD endemic areas, *I. scapularis* has a 2-year life cycle in which the seasonal activity of nymphs precedes that of larvae.
- Spring-summer occurrence of LD is correlated with nymph activity.
- In the northeast and upper midwest US, *I. scapularis* is an enzootic vector as well as a bridge vector for *B. burgdorferi* to people.
Incidence* rates for Lyme disease cases reported to CDC

<table>
<thead>
<tr>
<th>State</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maryland</td>
<td>22.1</td>
<td>22.2</td>
<td>45.8</td>
<td>31.0</td>
<td>25.7</td>
</tr>
<tr>
<td>Virginia</td>
<td>3.6</td>
<td>4.7</td>
<td>12.4</td>
<td>11.4</td>
<td>8.9</td>
</tr>
<tr>
<td>North Carolina</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>South Carolina</td>
<td>0.4</td>
<td>0.5</td>
<td>0.7</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Georgia</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Florida</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*incidence=confirmed cases per 100,000 persons, calculated using July 1st population estimates for each year

Source: http://www.cdc.gov/ncidod/dvbid/lyme/ld_IncidenceRatesbyState20052009.htm

Is Lyme disease expanding southward?
Fig. 1. Effort expended to live-capture reptiles and rodents from 1 April to 30 September, 1991.

Fig. 2. Number of animals examined for ticks, and number of animals found to be tick-infested.
Rocky Mountain spotted fever vectors

American Dog Tick
*Dermacentor variabilis*

Rocky Mountain Wood Tick
*Dermacentor andersoni*
Rickettsia rickettsii - the pathogen

Gram positive coccobaccillus. An obligate intracellular pathogen. Invades and kills endothelial cells of capillaries and other blood vessels, resulting in a leakage of blood into surrounding tissues and pulmonary and renal failures.
Spotted Fevers - Rashes
Spotted fever group rickettsiae that are known or suspected pathogens

<table>
<thead>
<tr>
<th>Disease</th>
<th>Rickettsia</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rocky Mountain Spotted Fever</td>
<td>R. rickettsii</td>
<td>1899</td>
</tr>
<tr>
<td>Mediterranean Spotted Fever</td>
<td>R. conorii</td>
<td>1910</td>
</tr>
<tr>
<td>North Asian Tick Typhus</td>
<td>R. sibirica</td>
<td>1934</td>
</tr>
<tr>
<td>Queensland Tick Typhus</td>
<td>R. australis</td>
<td>1946</td>
</tr>
<tr>
<td>Israeli Spotted Fever</td>
<td>R. conorii israelensis</td>
<td>1949</td>
</tr>
<tr>
<td>Oriental Spotted Fever</td>
<td>R. japonica</td>
<td>1984</td>
</tr>
<tr>
<td>Astrakhan Fever</td>
<td>R. conorii astrakhan</td>
<td>1988</td>
</tr>
<tr>
<td>African Tick-bite Fever</td>
<td>R. africae</td>
<td>1990</td>
</tr>
<tr>
<td>Thai Tick Typhus</td>
<td>R. honei</td>
<td>1991</td>
</tr>
<tr>
<td>&quot;Lymphangitis associated Rickettsiosis&quot;</td>
<td>R. sibirica mongolitimonae</td>
<td>1996</td>
</tr>
<tr>
<td>&quot;Not Named&quot;</td>
<td>R. heilongjiangensis</td>
<td>1996</td>
</tr>
<tr>
<td>Tick-borne Disease (&quot;DEBONEL&quot;)</td>
<td>R. slovaca</td>
<td>1997</td>
</tr>
<tr>
<td>&quot;Not Named&quot;</td>
<td>R. aeschlimanni</td>
<td>2002</td>
</tr>
<tr>
<td>&quot;Not Named&quot;</td>
<td>R. parkeri</td>
<td>2004</td>
</tr>
<tr>
<td>&quot;Not Named&quot;</td>
<td>R. massiliae</td>
<td>2005</td>
</tr>
<tr>
<td>&quot;Not Named&quot;</td>
<td>R. marmionii</td>
<td>2005</td>
</tr>
<tr>
<td>Presumptive</td>
<td>R. helvatica</td>
<td></td>
</tr>
<tr>
<td>Not formally named</td>
<td>R. canadensis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;R. amblyommii&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;R. texiana&quot;</td>
<td></td>
</tr>
<tr>
<td>Rickettsial Pox</td>
<td>R. akari</td>
<td>Mites</td>
</tr>
<tr>
<td>Cat Flea Rickettsiosis</td>
<td>R. felis</td>
<td>Fleas</td>
</tr>
</tbody>
</table>
Average Incidence 2000-2005

- 0.01-2.6
- 2.7-7.4
- 7.5-14.6
- 14.7-32.9
- 33+
Top Reporting States: Cases

![Bar chart showing the number of cases from 2001 to 2005 for MO, AR, TN, OK, and NC. The chart highlights the increase in cases over the years, with NC consistently showing the highest number of cases.](chart.png)
Spotted Fever Group Rickettsioses, including RMSF in NC
Annually, NC reports high incidence rates

In 2006, NC reported 42% of all RMSF cases in US (862 of 2052).
Record Increases

Are these really RMSF cases?
RMSF reported in NC

- >95% of cases are not laboratory confirmed:
  - Based on exposure, clinical symptoms and positive single acute serologic specimen.
  - Difficulty obtaining convalescent serology
- Collaborative eco-epidemiology study
  - Disease Epidemiology (NC Division of Epidemiology)
  - Human Serology (NC Lab of Public Health; Rickettsial Zoonoses Branch, CDC)
  - Deer Serology (SE Cooperative Wildlife Disease Study, UGA; Rickettsial Zoonoses Branch, CDC)
  - Tick Survey (Entomology, NC State Univ., Public Health Pest Mgt., NC DENR)
  - Tick Infection (SE Cooperative Wildlife Disease Study, UGA)
Study Questions

- What is the true disease burden from RMSF?
- What is the relative abundance of the American dog tick in peri-domestic environments?
- Is a novel tick vector emerging in the landscape?
- What is the prevalence of *R. rickettsii* in ticks?
- Are new spotted fever group rickettsiae emerging as human pathogens?
Enhanced surveillance for tick-borne illness and tick survey project conducted in 2005

- Chatham County, NC
  - Citizens and healthcare providers are concerned about
    - tick infestations
    - tick-borne infections (33 cases of RMSF vs. 14.2 cases per county statewide from 2000 to 2004)
  - Rural population in a woodland setting: in 2000, 27.9 persons per km$^2$ vs. 63.8 persons per km$^2$ statewide.
  - Typical piedmont county undergoing suburbanization: from 2000 to 2004 increase in population of 15.6% vs 6.1% statewide)
Methods

Enhanced surveillance for tick-borne illness

- Setting: Chatham County, outpatient clinics
- Time period: June 15 to August 15, 2005
- Patients: Four physician practices recruited
to report suspected tick-borne infections;
serology to detect *Rickettsia, Ehrlichia, Anaplasma*, and *Borrelia* infections conducted by NC State Lab and CDC

- Case classification (CDC)
  - **Probable**: clinically compatible illness and single positive serology
  - **Confirmed**: clinically compatible illness and $\geq 4$-fold change in titer of paired serologic samples
Tick survey

- Home sites ($n = 32$) in Chatham County sampled from April 9 to July 24, 2005.
  - Ticks collected by dragging were preserved in ethanol in the field.
  - Identified to species and life stage, and counted into pools for molecular analyses.
Molecular analyses

- Genomic DNA extracted from tick pools.
  - Ticks from 16 of 32 sites tested
  - Sequence analysis
  - To identify *Rickettsia, Ehrlichia, Anaplasma*, and *Borrelia*
People developing a tick-borne illness would likely have been bitten by lone star ticks.

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Number collected (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
</tr>
<tr>
<td><em>Amblyomma americanum</em></td>
<td>578 (8.9)</td>
</tr>
<tr>
<td><em>Dermacentor variabilis</em></td>
<td>17 (0.3)</td>
</tr>
<tr>
<td><em>Ixodes scapularis</em></td>
<td>0</td>
</tr>
</tbody>
</table>

Highest attack rates would be expected from lone star ticks, which residents often refer to as “deer ticks”.

Numbers and life stages of tick species collected by flagging vegetation at 26 sites in Chatham County, NC in 2006.

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Females</th>
<th>Males</th>
<th>Nymphs</th>
<th>All stages</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amblyomma americanum</em></td>
<td>260</td>
<td>342</td>
<td>3,093</td>
<td>3,695</td>
</tr>
<tr>
<td><em>Dermacentor variabilis</em></td>
<td>15</td>
<td>21</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td><em>Ixodes scapularis</em></td>
<td>4</td>
<td>0</td>
<td>15</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 2. Results of PCR analyses of ticks collected in Chatham County for selected pathogens.

<table>
<thead>
<tr>
<th>Tick Species</th>
<th>No. positive pools/ no. pools (no. ticks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ehrlichia chaffeensis</td>
</tr>
<tr>
<td>Amblyomma americanum</td>
<td>1/30 (308)</td>
</tr>
<tr>
<td>Dermacentor variabilis</td>
<td>1/21 (23)</td>
</tr>
<tr>
<td>Ixodes scapularis</td>
<td>0/1 (1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2/52 (332)</td>
</tr>
</tbody>
</table>

52 (12%) 439 of tick pools were tested for pathogens. Ticks testing positive were collected at 16 different home sites in 2005.

- **Ehrlichia chaffeensis**
  - 1 pool of 10 *A. americanum* males
  - 1 pool of 1 *D. variabilis* female

- **Anaplasma phagocytophilum**
  - 1 pool of 3 *A. americanum* males

- **Rickettsia** spp.
  - “*R. amblyommi***” (7 different home sites)
    - 11 pools containing 104 *A. americanum* ticks
    - 2 pools containing 2 *D. variabilis* ticks
  - *R. rickettsii* or *montanensis*
    - 2 pools containing 4 *D. variabilis* ticks
  - *R. rickettsii* or *felis*
    - 1 pool containing 1 *D. variabilis* female tick
  - **Rickettsia** spp.
    - 4 pools containing 6 *A. americanum* ticks

Table 3. Results of qPCR analyses for pathogens in *Amblyomma americanum* ticks collected at 26 residential sites in Chatham County, North Carolina in spring 2006.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number testing positive (percent testing positive)</th>
<th>Male (n = 305)</th>
<th>Female (n = 245)</th>
<th>Adults (n = 550)</th>
<th>Nymphs (n = 1040)</th>
<th>Overall (n = 1590)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Rickettsia amblyommii&quot;</td>
<td>176 (57.5)</td>
<td></td>
<td>155 (63.3)</td>
<td>331 (60.2)</td>
<td>540 (51.9)</td>
<td>871 (54.8)</td>
</tr>
<tr>
<td><em>Rickettsia</em> spp.</td>
<td>39 (12.8)</td>
<td></td>
<td>33 (13.5)</td>
<td>72 (13.1)</td>
<td>133 (12.3)</td>
<td>205 (12.9)</td>
</tr>
<tr>
<td>All <em>Rickettsia</em></td>
<td>215 (70.5)</td>
<td></td>
<td>188 (76.7)</td>
<td>403 (73.3)</td>
<td>673 (64.7)</td>
<td>1076 (67.7)</td>
</tr>
<tr>
<td><em>Ehrlichia chaffeensis</em></td>
<td>7 (2.3)</td>
<td></td>
<td>8 (3.3)</td>
<td>15 (2.7)</td>
<td>10 (0.96)</td>
<td>25 (1.6)</td>
</tr>
<tr>
<td>&quot;Borrelia lonestari&quot;</td>
<td>2 (0.7%)</td>
<td></td>
<td>0 (0.0%)</td>
<td>2 (0.4)</td>
<td>4 (0.4)</td>
<td>6 (0.4)</td>
</tr>
</tbody>
</table>

Competitive Exclusion of *Rickettsia*?

FIG. 3. Regression of percentage of *A. americanum* ticks infected with *Rickettsia amblyommii* and unidentified *Rickettsia* at 26 collection sites in Chatham County, North Carolina.

Table 6. Results of qPCR analyses for bacterial pathogens in ticks collected in Chatham County, NC in 2006.

<table>
<thead>
<tr>
<th>Bacterial agent</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Ixodes scapularis</em></td>
<td></td>
<td><em>Dermacentor variabilis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>nymphs</td>
<td>adults</td>
<td>males</td>
<td>females</td>
<td>total</td>
</tr>
<tr>
<td><em>Rickettsia</em> spp.</td>
<td>5/15 (33.3%)</td>
<td>1/4 (25.0%)</td>
<td>4/12 (33.3%)</td>
<td>12/24 (50.0%)</td>
<td>16/36 (44.4%)</td>
</tr>
<tr>
<td>“<em>Rickettsia amblyommii</em>”</td>
<td>None Tested</td>
<td>NT</td>
<td>1/12 (8.3%)</td>
<td>3/24 (12.5%)</td>
<td>4/36 (11.1%)</td>
</tr>
<tr>
<td><em>Rickettsia rickettsii</em></td>
<td>NT</td>
<td>NT</td>
<td>0/12 (0.0%)</td>
<td>0/24 (0.0%)</td>
<td>0/36 (0.0%)</td>
</tr>
<tr>
<td><em>Rickettsia montanensis</em></td>
<td>NT</td>
<td>NT</td>
<td>2/12 (16.7%)</td>
<td>5/24 (20.8%)</td>
<td>7/36 (19.4%)</td>
</tr>
<tr>
<td><em>Borrelia burgdorferi s. l.</em></td>
<td>6/15 (40.0%)</td>
<td>0/4 (0.0%)</td>
<td>NT*</td>
<td>NT*</td>
<td>NT*</td>
</tr>
</tbody>
</table>

Prospective Surveillance for tick-borne illness

• 79 sera submitted but only 51 met case criteria
• Sera from 51 patients screened at NC State Lab
• Paired sera available for 28 patients
  • 17 negative
  • 6 patients classified as probable RMSF
  • 5 confirmed HME

<table>
<thead>
<tr>
<th>Sample submitted</th>
<th>Number of probable cases</th>
<th>Number of confirmed cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative test</td>
<td>RMSF</td>
</tr>
<tr>
<td>Single serum (n = 22)</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Paired sera (n = 28)</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>All sera (n = 50)</td>
<td>28</td>
<td>16</td>
</tr>
</tbody>
</table>
Incongruent results

- Highest numbers of RMSF cases reported to CDC.
- But can’t confirm a single presumptive case of RMSF. Paired serologies demonstrate <4X change in IgG titers.
- Case patients presumptively diagnosed with RMSF exhibit mild to moderate symptoms.
- Low abundance of American dog ticks and high abundance of lone star ticks.
- High prevalence of another SFGR, “R. amblyommii”, in lone star ticks.
Serologic results from CDC: In general, patients sera gave higher IFA titers to “*R. amblyommi*” antigens.

<table>
<thead>
<tr>
<th>Patient</th>
<th>T1 (days)</th>
<th>T2 (days)</th>
<th><strong>R. rickettsia</strong> antigens</th>
<th><strong>“R. amblyommi”</strong> antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgM (mu)</td>
<td>IgG (gamma)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>acute</td>
<td>conv.</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>24</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>59</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>51</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>14</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>46</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

**T1** = date of onset to acute serum sample.

**T2** = date of acute to convalescent serum sample.
Summary

- Clinical and serological evidence suggested “R. amblyommii” infection in 5 patients
  - Acute febrile illness
  - Mild to moderate symptoms
  - No rash
  - Rapid improvement on doxycycline
- Survey studies revealed an overwhelming proportion of ticks in the environment were A. americanum, and many were infected with “R. amblyommii”.
- Ticks infected with Rickettsia spp. were collected from 7 (44%) of 16 home sites.
Limitations to our study?

- **Sampling issues**
  - Convenience sample, may not be generalizable
  - Small number of patients
  - Ticks were not collected from the residences of case patients

- **Heterologous serologic results**
  - High “*R. amblyommii*” titers may represent cross-reactions to other spotted-fever group rickettsiae.

- **No biopsy samples collected**
  - culture, PCR, immuno-histochemical staining
Questions

- Is “Rickettsia amblyommi” a human pathogen?
- If “R. amblyommi” is maintained in a zoonotic cycle, what is the wildlife reservoir host?
- What other Rickettsia spp. are harbored by black-legged, American and lone star ticks.
- What are spirochete-infected Ixodes scapularis nymphs feeding on?
Collaborating Agencies

- Dept. of Entomology, NC State University (Apperson and Watson)
- Public Health Pest Mgt. Branch, NC Dept. of Environment & Natural Resources (Engber)
- NC Division of Epidemiology, NC Dept. Health & Human Services (Engel and Dail)
- NC Laboratory of Public Health, NC Dept. Health & Human Services (Johnson)
- Rickettsial Zoonoses Branch, CDC (Nicholson)
- SE Cooperative Wildlife Disease Study Group, Univ. of Georgia (Mead and Yabsley)
Thanks for your attention.