Establishing a Protocol for the Long-term Sampling and Analysis of Tick-borne Pathogens in the Lehigh Valley

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**Establishing a protocol for the long-term sampling and analysis of tick-borne pathogens in the Lehigh Valley**

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Lehigh Valley Health Network, Allentown, Pennsylvania

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**Ticks and Their Pathogens**

Deer ticks (Ixodes scapularis) are known vectors of several human pathogens including Borrelia burgdorferi, Anaplasma phagocytophilum (ha silaris), Babesia microti, and Borrelia miyamotoi. Dog ticks (Dermacentor variabilis) also transmit bacteria capable of causing human illnesses, such as Rickettsia rickettsii. Finally, Lone Star ticks (Amblyomma americanum) may induce mast cell diseases by transferring an alpha-gal sugar into the blood stream of the host.

**Lyme disease** is the most widespread vector-borne illness in the United States and is the most common tick-borne illness, including Lyme disease, which is endemic in this region. In 2013 Pennsylvania accounted for approximately 18% of the confirmed national cases of Lyme disease (CDC, 2016). Human Granulocytic Anaplasmosis (HGA) is caused by the intracellular bacterium, Anaplasma phagocytophilum that affects neutrophils. Babesia microti, a protozoan parasite of red blood cells, causes babesiosis. Babesiosis is emerging in the Lehigh Valley where three cases were reported in 2013. B. miyamotoi is a causative agent of tick-borne relapsing fever. One human case of infection was reported in New Jersey in 2012. Rickettsia rickettsii is the causative agent of Rocky Mountain Spotted Fever and is transmitted by Dog ticks. This infection is currently rare in Pennsylvania. In 2010, 0.2-1 ticks per 1 million persons were reported in the state according to the CDC.

**Collection, Extraction, and Analysis**

**Collection**

Ticks were collected by dragging a 1 m² white corduroy cloth in forested areas throughout Lehigh, Berks, and Northampton Counties. Ticks were collected into vials containing 70% ethanol and stored in a -20°C freezer until DNA extraction.

We recorded the temperature, humidity, dew point, weather conditions and vegetation characteristics to determine the environmental conditions of each collection site. Each tick was classified according to species and life stage, and adults were identified as male or female.

**Extraction and Analysis of Tick DNA**

Tick DNA is extracted using a QIAamp Mini Kit (Qiagen, Valencia, CA). A no-template control is also prepared to ensure that the reagents used in the extraction process are not cross contaminated with tick DNA. The extracted DNA will be tested for the presence of various tick-borne pathogens. All procedures for extraction and analysis are conducted according to the methods of Edwards et al. (2001).

**Choosing Collection Sites**

We used satellite images on Google Maps to locate closed-canopy forested areas in the Lehigh Valley. Each location was then placed on a map to ensure that we were representing a broad range of the Lehigh Valley region.

**Digital Documentation of Collection Sites**

- The iPhone TRAILS Application (Isoforsch GmbH) was used to map our paths for all “transects.” Topographic maps were available through this application from the work of the OpenStreetMap Community.
- TRAILS provided JPEG images of our locations on a topographical map of the area that we traveled that could then be layered on various different maps, including satellite images.
- By recording the precise locations of where ticks were collected, it will be possible to repeat the collections at the same sites in future years.
- Collections were timed for 30 minutes per “transect” in order to estimate the density of ticks in terms of time collecting and distance collected. These data will allow for risk assessments when combined with our molecular studies of infection rates.

**Criteria for selecting collection sites**

1. Can a sufficient number of ticks be collected to provide a minimally representative sample?
2. Is the understory possible to navigate?
3. Where is the site located in relation to other sites in the Lehigh Valley region?
4. Can we obtain permission to use the site?
5. Will this environment remain relatively unchanged for the next 20 years?

**Total Tick Counts by Site**

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Deer Tick Adults</th>
<th>Deer Tick Nymphs</th>
<th>Dog Tick Adults</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tretler Preserve</td>
<td>4</td>
<td>131</td>
<td>59</td>
<td>194</td>
</tr>
<tr>
<td>Raker Wildlife Preserve</td>
<td>0</td>
<td>95</td>
<td>5</td>
<td>100</td>
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<tr>
<td>Trees in Topon</td>
<td>3</td>
<td>136</td>
<td>3</td>
<td>142</td>
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<tr>
<td>Alberts Mt. Rd. Track</td>
<td>1</td>
<td>116</td>
<td>1</td>
<td>118</td>
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<tr>
<td>Reimert Memorial</td>
<td>1</td>
<td>168</td>
<td>1</td>
<td>170</td>
</tr>
<tr>
<td>Lehigh Uplands Preserve</td>
<td>15</td>
<td>209</td>
<td>0</td>
<td>224</td>
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<tr>
<td>S. Mountain Preserve</td>
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<td>276</td>
<td>0</td>
<td>277</td>
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<tr>
<td>Scholl Woodlands</td>
<td>0</td>
<td>113</td>
<td>0</td>
<td>113</td>
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<td>Robert Rodale Reserve</td>
<td>2</td>
<td>119</td>
<td>0</td>
<td>121</td>
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<tr>
<td>Burkhart Preserve</td>
<td>1</td>
<td>65</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>Graver Arboretum</td>
<td>2</td>
<td>68</td>
<td>1</td>
<td>71</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>30</strong></td>
<td><strong>1486</strong></td>
<td><strong>70</strong></td>
<td><strong>1596</strong></td>
</tr>
</tbody>
</table>

**Deer Tick Abundance by Site**

![Deer Tick Abundance by Site](https://example.com/deer-tick-abundance.png)

**References and Acknowledgements**

**REFERENCES**


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