Detection of Lyme disease and anaplasmosis pathogens via PCR in Pennsylvania deer ked

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ABSTRACT: *Borrelia burgdorferi* and *Anaplasma phagocytophilum* are obligate intracellular parasites that maintain their life cycles in enzoonotic vector-host cycles with *Ixodes scapularis* as a vector. In addition to ticks, the hosts are commonly infested with insects from the Hippobosciidae family. This study confirms the presence of *B. burgdorferi* and *A. phagocytophilum* in deer keds (*Lipoptena cervi*) removed from white-tailed deer using PCR. Detection of these pathogens in deer ked represents a potential novel susceptibility of wildlife and also suggests the risk of transmission of these pathogens to humans and animals alike through the bite of an infected ectoparasite. This study represents the first instance in the U.S. of detection of tick-borne pathogens in a member of the Hippobosciidae family. *Journal of Vector Ecology* 41 (2): 292-294. 2016.

Keyword Index: *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Lipoptena cervi*, PCR.

INTRODUCTION

*Borrelia burgdorferi*, the causative agent of Lyme disease, is a motile gram-negative spirochete. Lyme disease is a progressive and potentially debilitating disease that is the most commonly reported vector-borne illness in the U.S. Infection rates with *Borrelia burgdorferi* have increased in the northeast and upper Midwest in the past 40 years (Steere et al. 2004). In 2014, these geographical regions reported 96% of all Lyme disease cases. The number of confirmed cases in Pennsylvania during 2014 was 6,470, with an incidence rate of 50.6/100,000 people. Massachusetts had the second largest number with 3,646 confirmed cases and an incidence rate of 54.1 per 100,000 people (Centers for Disease Control and Prevention. 2013. Lyme Disease Data).

*Anaplasma phagocytophilum* is a gram-negative, obligate intracellular bacterium that causes anaplasmosis. This bacterium obtains nutrients in an early endosome and then grows into a morula within the leukocytes, platelets, and erythrocytes (Dumler et al. 2005). During 2010 in the U.S., there were an estimated 1,750 cases of anaplasmosis, a 52% increase from the prior year. However, in that same year, there were no reported cases of human granulocytic anaplasmosis, a naturally infected reservoir host of these bacteria, in Pennsylvania. The goal of our study was to investigate whether *B. burgdorferi* and *A. phagocytophilum*, the agents of Lyme disease and anaplasmosis of humans and animals, respectively, occurs in deer keds collected from *O. virginianus*, which are a naturally infected reservoir host of these bacteria, in Pennsylvania.

MATERIALS AND METHODS

Collection of deer ked

Forty-eight *Lipoptena cervi* were collected in the summer of 2015 from Blair and Indiana counties on Pennsylvania State Game Lands 184 (40.33N, 78.28W) (nine keds) and 276 (40.65N, 79.09W) (39 keds), and were stored in 100% ethanol until DNA extraction was performed. The deer keds were removed from freshly harvested *O. virginianus*. All *L. cervi* collected were wingless adults. Samples were then prepared for DNA extraction by freezing for a minimum time of 15 min.

DNA extraction from *L. cervi* was conducted using the Qiagen DNeasy protocol according to the manufacturer's
presents small mammals and deer. These pathogens are tick-borne obligate intracellular parasites whose normal DNA was stored at -20°C until PCR analysis so quantification could be performed (Brown et al. 2015).

**DNA analysis**

The DNA was amplified by PCR. Briefly, the initial denaturing was at 95°C for a period of 2 min. 40 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C. On positive samples, a nested PCR assay targeting a 546-bp amplicon of the 16S rRNA gene was utilized for *A. phagocytophylum* (Brown et al. 2015). To identify specimens infected with *B. burgdorferi*, nested PCR amplifying a 390-bp amplicon from the fla gene was performed. PCRs for both organisms were done with Promega GoTaq Master Mix. An Eppendorf thermocycler was performed. PCRs for both organisms were done with Promega GoTaq Master Mix. An Eppendorf thermocycler was performed. PCRs for both organisms were done with Promega GoTaq Master Mix. An Eppendorf thermocycler was performed. PCRs for both organisms were done with Promega GoTaq Master Mix.

**RESULTS**

Forty-eight *Lipoptena cervi* were collected from southwestern Pennsylvania. There were 19 (39.58%) deer keds positive for *Borrelia burgdorferi* and 14 (29.12%) deer keds positive for *Anaplasma phagocytophilum*. Out of the 48 deer ked, three (6.25%) were co-infected with the etiologic agents of Lyme disease and anaplasmosis (Table 1).

**DISCUSSION**

*Borrelia burgdorferi* and *Anaplasma phagocytophilum* are tick-borne obligate intracellular parasites whose normal reservoirs are small mammals and deer. These pathogens circulate in the enzootic cycle, with *Ixodes scapularis* being the principal vector of these organisms in the northeastern and upper midwestern United States (Walker et al. 1996). Although *Ixodes scapularis* is primarily known for its role in transmitting these organisms, other blood-sucking arthropods infest the same hosts as *I. scapularis* and could acquire the pathogen upon taking a blood meal. One such ectoparasite of cervids, *Lipoptena cervi*, has been shown to harbor the DNA from these pathogens in Europe (Víchová et al. 2011, de Bruin et al. 2015). Also, *L. cervi* is able to transmit *A. phagocytophilum* from the reservoir hosts to susceptible sterile hosts via their bite (De La Fuente et al. 2005).

Humans are accidental hosts for *I. scapularis* and *L. cervi* (Berger 1989, Madslien et al. 2012). Deer ked often encounter humans and will temporarily parasitize them due to the presence of hair on the human body (Paakkonen et al. 2010). Hunters may be exposed to ectoparasites while harvesting white-tailed deer, increasing the risk of exposure to both *I. scapularis* and *L. cervi*.

In our study, deer keds were examined for the presence of *B. burgdorferi* and *A. phagocytophilum* using PCR. We found that both pathogens were present in the deer keds. Of the 48 deer keds examined, 19 were positive for *B. burgdorferi* DNA, seven from Blair county and 12 from Indiana county, and 14 were positive for *A. phagocytophilum*, one from Blair county and 13 from Indiana county (co-infection of three ked). Using a Fisher’s exact test, we found that the prevalence of *B. burgdorferi* is a statistically significant p=0.0195 between counties. However, the prevalence of *A. phagocytophilum* is non-significant (p=0.25). The overall prevalence of infection reached 62.5% for at least one pathogen across counties (30/48).

A study of the prevalence of these pathogens in *I. scapularis* from our laboratory showed similar prevalence rates of *B. burgdorferi* and *A. phagocytophilum* in these counties (Brown et al. 2015). Recently, the Pennsylvania Department of Environmental Protection studying all 67 counties, stated that prevalence rates of *B. burgdorferi* in *I. scapularis* within the eastern and central regions of Pennsylvania are higher than those in the southern and western regions (Hutchinson et al. 2015). The increased prevalence of *B. burgdorferi* in the central portions of PA within the deer tick helps to support the increased prevalence our study shows in Blair county, as Blair county is part of the central region of PA.

*Lipoptena cervi* typically seek reindeer, deer, moose, horses, and cattle as their hosts, but the concern is that they may begin to target other mammals (Kaunisto et al. 2009). Transmission of *B. burgdorferi* and *A. phagocytophilum* could migrate to other mammals and eventually reach humans. There is no evidence thus far that suggests that deer ked bite or transmit *B. burgdorferi* or *A. phagocytophilum* to humans. However, humans are accidental hosts for *L. cervi* and could be exposed to these pathogens via exposure to the keds, as it takes 15-20 mins for an adult deer ked to take a blood meal on a human (Haarlov 1964, Hackman et al. 1983, Rantanen et al. 1982). This is not to say that deer keds cannot spread the pathogens to a larger area or other mammalian hosts. Transmission of pathogens between wildlife, humans, and domestic animals is common when there is mutual contact between the groups.

While we do not have the infection data on the white-tailed deer from which these ked were harvested, we presume that the deer ked acquired the infection from a blood meal on an infected deer. As suggested by Víchová et al. (2011) in the study from Europe, deer ked may be mechanical vectors for the transmission of *B. burgdorferi* and *A. phagocytophilum*.

<table>
<thead>
<tr>
<th>Collection site</th>
<th>n</th>
<th>No. (%) PCR positive for <em>B. burgdorferi</em></th>
<th>No. (%) PCR positive for <em>A. phagocytophilum</em></th>
<th>No. (%) of co-infected samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blair</td>
<td>9</td>
<td>7 (77.7%)</td>
<td>1 (11.1%)</td>
<td>1 (11.1%)</td>
</tr>
<tr>
<td>Indiana</td>
<td>39</td>
<td>12 (30.8%)</td>
<td>13 (33.3%)</td>
<td>2 (5.13%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>48</td>
<td>19 (39.58%)</td>
<td>14 (29.12%)</td>
<td>3 (6.25%)</td>
</tr>
</tbody>
</table>

Table 1. Spatial distributions of *B. burgdorferi* and *A. phagocytophilum* in *L. cervi* collected in southwestern Pennsylvania.
This study provides the first incidence of \textit{B. burgdorferi} and \textit{A. phagocytophilum} in deer ked harvested from wild cervids in the United States. This also suggests that the risk for transmission of these pathogens via the bite of \textit{L. cervi} is present. Further research could explore the competency of the vector and whether it is biologic or mechanical.

\textbf{Acknowledgments}

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\textbf{REFERENCES CITED}

Bequaert, J. 1937. Notes on Hippoboscidae. 5. The Strain B31 (Clone 5A1), NR-13251. were obtained through BEI Resources, NIAID, NIH: at Johnstown for supporting this study. The following reagents

\begin{itemize}
\item Bequaert, J. 1937. Notes on Hippoboscidae. 5. The Strain B31 (Clone 5A1), NR-13251.
\item Bequaert, J. 1942. A monograph of the Melophaginae, or ked-flies, of sheep, goats, deer and antelopes (Diptera, Hippoboscidae). Entomol. Amer. 22 (new series): 1-220.
\item Bequaert, J. 1953. The Hippoboscidae or louse-flies (Diptera) of mammals and birds. Part I. Structure, physiology and natural history. Entomol. Amer. 36 (new series): 211-442.
\item Bequaert, J. 1957. The Hippoboscidae or louse-flies (Diptera) of mammals and birds. Part II. Taxonomy, evolution and revision of American genera and species. Entomol. Amer. 36 (new series): 417-611.
\item Haarlo N. 1964. Life cycle and distribution pattern of \textit{Lipoptena cervi} (L.) (Dipt., Hippobosc.) on Danish deer. Oikos 15: 93-129
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