Prevalence of Ticks Infected with Human Pathogens in the Lehigh Valley: Molecular Surveillance

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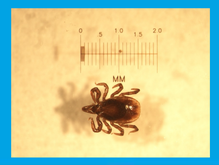

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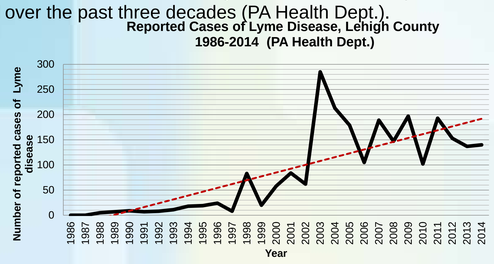
Background

Black-legged ticks (*Ixodes scapularis*) transmit *Borellia burgdorferi,* the spirochete that causes Lyme disease. The incidence of Lyme disease cases reported in the Lehigh Valley has increased over the past three decades (PA Health Dept.).



Other infectious diseases transmitted by *Ixodes scapularis* are of concern. These pathogens incude *Babesia microti* (causing human babesiosis), *Anaplasma phagocytophilum* (causing human granulocytic anaplasmosis), and *Borrelia miyamotoi* (causing tick-borne relapsing fever).

Reporting the prevalence of infection in an area can inform health care providers in making time-sensitive decisions involving prophylaxis and treatment in regards to tick-borne infectious disease.

Methods

Collect Nymphs → **Extract DNA** → **Real-Time PCR**

Over the summers of 2015 and 2016, at least 50 black-legged tick nymphs were tested from each of 11 sites within the Lehigh Valley. More than 1,150 ticks were tested for the presence/absence of human pathogens. The infection rate multiplied by tick abundance can be used to calculate the risk for acquiring a tick-borne pathogen, known as the **Entomological Risk Index (ERI)**.

After DNA extraction, samples were tested for the successful extraction of DNA and the four pathogens using Real-Time Polymerase Chain Reaction. Each pathogen was tested for individually using a specific primer and probe set and a negative and positive control.

An established threshold of 0.2 and 40 cycles were used to detect each pathogen. Negative samples were those with abnormal curves and those that did not cross threshold by 0.2.

Conventional PCR and sequencing of PCR products was then used to verify *B. microti*, *A. phagocytophilum*, and *B. miyamotoi*.

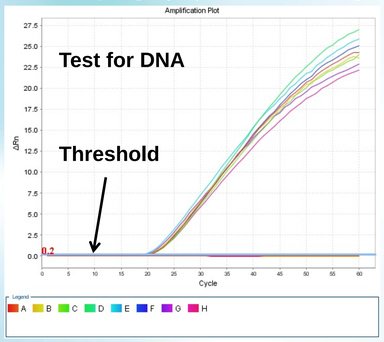


Figure 1:Real-Time PCR for ITS2 confirming the presence of tick DNA

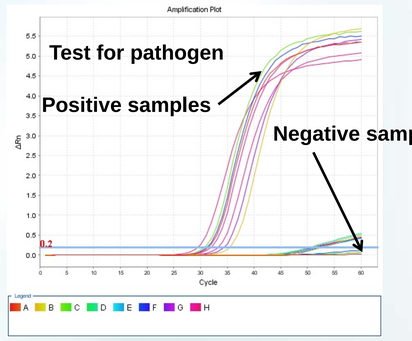


Figure 2:Real-Time PCR for 23s testing for *Borellia*

Results

Bayesian approach: Likelihood that the actual value is in an interval that surrounds sample value

Summer 2015
Infection Rates for Individual Pathogens

				Credible Interval	
Pathogen	**Positive**	**Sample**	**Rate**	**Lower**	**Upper**
B. burgdorferi	137	600	0.228	0.196	0.263
B. miyamotoi	0	600	0.000	0.000	0.003
B. microti*	9	600	0.015	0.007	0.026
A. phagocytophilum*	11	600	0.018	0.009	0.030
Unknown *Borrelia*	1	600	0.002	0.000	0.006

Table 1: Results from the 600 ticks that were collected within the Lehigh Valley and tested for pathogen DNA using qPCR. All credible intervals were calculated using the statistical package R. *Samples have been confirmed by Real-Time PCR and DNA sequencing.

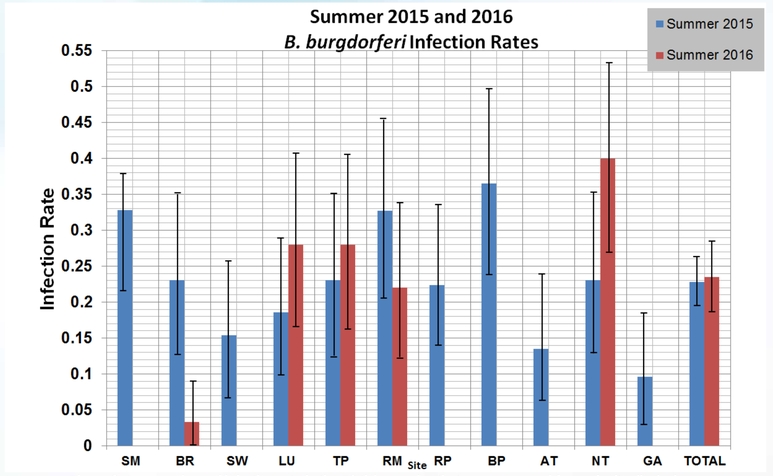


Figure 3: A comparison of infection rate in the 50 tick samples that were screened for each site displaying Bayesian Credible Intervals. >550 ticks were screened in 2015 and >300 ticks have been screened in 2016.

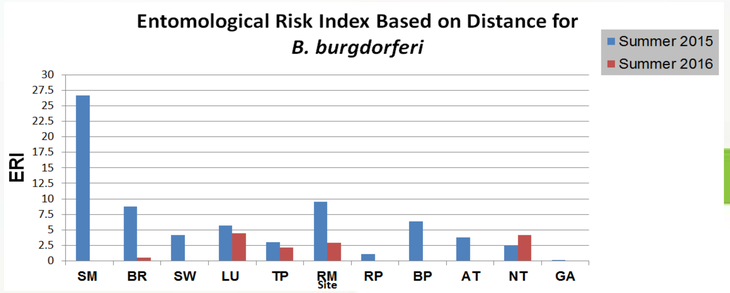


Figure 4: A comparison of ERI for 2016 sites tested to date

Discussion

B. burgdorferi infected black-legged ticks were abundant at all 11 study sites. This confirms previous research showing a widespread distribution of *B. burgdorferi* infected ticks in the Lehigh Valley region (Edwards *et al* . 2015).

No significant difference was observed between the **overall infection rate** of *B. burgdorferi* in blacklegged tick nymphs collected in 2015 and 2016 (P=0.8, Two- tailed T Test).

The **Entomological Risk Index** for *B. burgdorferi* are not statistically significant between 2015 and 2016 (P=0.3, Two-tailed T Test). Entomological risk incorporates both infection rate and tick abundance data.

Overall *B. burgdorferi* infection rates for summer 2015 and 2016 are not significantly different from a similar study performed in 2014 at different sites in the Lehigh Valley which were 18.3% (20/109 nymphs, 95% CI: 11.6-26.9%) positive for this pathogen (Edwards *et al.* 2015).

In summer 2015, *B. microti*, *A. phagocytophilum* were detected at low levels and *B. miyamotoi* was not detected. Ticks collected in Summer 2016 are currently being tested for infection with these pathogens..

The incidence of *B. burgdorferi* infected ticks in the Lehigh Valley may impact clinical decisions. Evidence-based guidelines used by health care providers describe how antimicrobial prophylaxis is recommended for **adult** patients with a recognized tick bite in areas with local infection rates of *B. burgdorferi* reported to be equal or over 20% (Wormser et al. 2006). In 2015 and 2016, the majority of our study sites within the Lehigh Valley exceeded this level of infection.

Future Work

- Our immediate goal is to complete testing of tick samples that were collected in 2016 and to complete the statistical analysis of our full 2015 and 2016 data set.
- Continue vector surveillance of black-legged ticks to track rate of infection, ERI, and tick abundance to compare with cases of reported tick-borne illness.
- Analyze whether year to year variations in clinically reported cases of Lyme disease correlate with our ERI estimates.

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