CITIZEN SCIENTISTS DETECT PATHOGENS ASSOCIATED WITH TICK-BORNE ILLNESSES IN *Ixodes scapularis*

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Abstract

The *Ixodes scapularis* tick has been expanding its range across the upper Midwest and New England, which is likely to be increasing the risk to individuals of developing tick-borne illnesses. Ticks acquire pathogens from primary reservoir hosts like the white-footed mouse, *Peromyscus maniculatus,* and depending on the prevalence of the primary host in the environment there can be variability in the percentage of ticks carrying the pathogens associated with Lyme disease and Anaplasmosis. Public health data is limited by the number of samples that can be collected; however, with the advent of mobile technologies there are growing opportunities for the public to engage in citizen science. As part of a public education campaign to promote the adoption of behaviors that protect against Lyme disease, we have partnered with local middle school students in the extraction of tick DNA using Biomeme field sample preparation kits combined with portable real-time PCR analysis to test for the presence of pathogens. We have found that the students are able to successfully extract DNA that can be used in RT-PCR analyze and have found that 55% of ticks tested in Central Wisconsin carry *Borrelia burgdorferi* and 10% carry *Anaplasma phagocytophilium*. Importantly, we found a higher than random coincidence of the bacteria, which may impact disease transmission. Future work will involve pathogen strain analysis and a more in depth understanding of the benefits and challenges of engaging middle students in science.

Introduction

Figure 1. 63 countries are endemic for Lyme disease

https://www.gideononline.com/2008/05/07/lyme-disease-revisited/

Figure 2. Risk of Anaplasmosis overlaps with Lyme disease

What is risk of coinfection in the Upper Midwest?

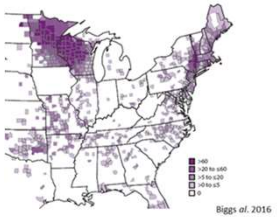
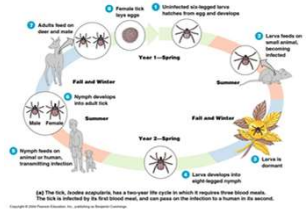


Figure 3. The life cycle of the deer tick (Ixodes scapularis)

The tick takes three blood meals over a two year life cycle. If the tick picks up *B. burgdorferi*, *A. phagocytophilium*, or *B. microti* from reservoir hosts, it can pass the pathogens to humans during subsequent blood meals.



Tick Collection & Community Engagement

Figure 4. Questing Ixodes scapularis and Dermacentor variabilis were collected by undergraduates or donated by community members.

Undergraduates collected ticks using drag clothes. Alternatively, ticks are donated by community members generating a geographically and temporally diverse tick bank.

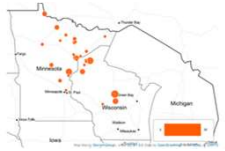


Figure 5. Ixodes scapularis and Dermacentor variabilis are documented. During outreach presentations co-delivered by undergraduates, middle and high school students are provided with relevant public health information about preventing Lyme disease (Seifert, et al. 2016). The students then took pictures of the ticks and differentiated the ticks by species, life stage, and sex (A). Both *Ixodes scapularis* (B) and *Dermacentor variabilis* (C) are commonly found in the upper midwest but only the *Ixodes scapularis* nymphs and adults can transmit *B. burgdorferi, A. phagocytophilium, and B. microti.*

A

B

C

DNA Isolation & Real-Time PCR Testing

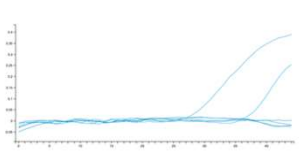
Figure 7. DNA is harvested from ticks by the students.

DNA extraction of ticks using the Biomeme 1-minute field prep extraction is more efficient and generates similar yield to laboratory based DNA extraction kits.

Figure 8. Mobile Real Time-PCR to detect pathogens and confirm species.

Sample was added to 3 wells containing lypholized mix and primers specific for the three pathogens and three tick species. Red and green fluorophores indicating amplification were detected using the Biomeme real-time PCR machine. The threshold cycle (Ct) is inverse to the amount of nucleic acid present in your sample.

fluorophore	Well 1- Species (Ct)	Well 2- Species (Ct)	Well 3- Species (Ct)
green	*Borrelia burgdorferi* (40.029)	*Dermacentor variabilis* (0)	*Amblyomma americanum* (0)
red	*Ixodes scapularis* (30.558)	*Anaplasma phagocytophilum* (0)	*Babesia microti* (0)



Results were subsequently confirmed testing each sample in triplicate using the Roche Lightcycler 480.

Prevalence of Pathogens in Ixodes scapularis

Figure 9. Samples generated by students give an idea of the co-infection rate.

We have tested 37 *Ixodes* scapularis samples, 8 samples extracted by students did not work, this chart includes data from 29 samples that worked in the RT-PCR (78%). From the 29 samples, 16 samples were positive for *Borrelia burgdorferi* (55.17%) and only 3 (10.34%) samples showed positive results for Anaplasma, and another 3 (10.34%) samples were positive for *Babesia microti*. We found that all samples that were positive for *Babesia microti* were positive for *Borrelia burgdorferi* too. None of the samples were positive for the three pathogens together.

ID	Sex- Species	Nanodrop	Ct Ixodes	Origin	Ct Borrelia	Ct Anaplasma	Ct Babesia
31	M-Ixodes	70.4	23.06	W16-13	34.8	0	0
48	M-Ixodes	91.05	35.503	W16-14	0	0	0
32	M-Ixodes	91.1	24.11	W16-11	34.76	0	34.5
44	F-Ixodes	93.8	21.977	W16-14	0	0	0
A	F-Ixodes	99.57	24.76	Linda-in bag winter	38.306	0	0
E	M-Ixodes	115.35	27.11	Linda4/17/17	0	20.37	0
D	M-Ixodes	115.7	26.44	Linda4/17/17	34.38	0	37.1
23	M-Ixodes	115.9	37.933	I45	0	0	0
20	F-Ixodes	123.7	32.07	I45	27	0	0
25	M-Ixodes	125.87	38.841	I45	0	0	0
37	M-Ixodes	126.6	34.793	W16-9	0	0	0
H	M-Ixodes	127.6	21.533	Linda4/18/17	33.938	0	0
M	F-Ixodes	139.6	20.78	Linda4/16/17	32.93	32.02	0
13	F-Ixodes	144.05	22.1	backyard	0	0	0
30	M-Ixodes	157	26.317	W16-13	35.18	0	0
36	M-Ixodes	158.8	32.832	W16-9	38.871	0	36.73
C	F-Ixodes	163.8	25.575	Linda in bag winter	0	0	0
24	M-Ixodes	166.3	30.649	I45	0	0	0
29	F-Ixodes	181.4	26.473	W16-13	0	0	0
33	M-Ixodes	183.9	31.02	W16-11	0	0	0
N	F-Ixodes	187.1	15.74	Linda4/16/17	30.26	0	0
K	M-Ixodes	191.3	25.146	Linda4/17/17	34.881	33.707	0
B	F-Ixodes	194.15	22.66	Linda in bag winter	33.27	0	0
28	F-Ixodes	197.5	30.56	I52	40.029	0	0
F	M-Ixodes	204.5	23.81	Linda4/17/17	32.473	0	0
G	M-Ixodes	226.45	24.52	Linda4/18/17	27	0	0
11	F-Ixodes	244.85	20.7	backyard	0	0	0
J	F-Ixodes	270.1	28.997	Linda4/17/17	40.484	0	0
35	M-Ixodes	423.3	32.179	W16-9	0	0	0

Acknowledgements

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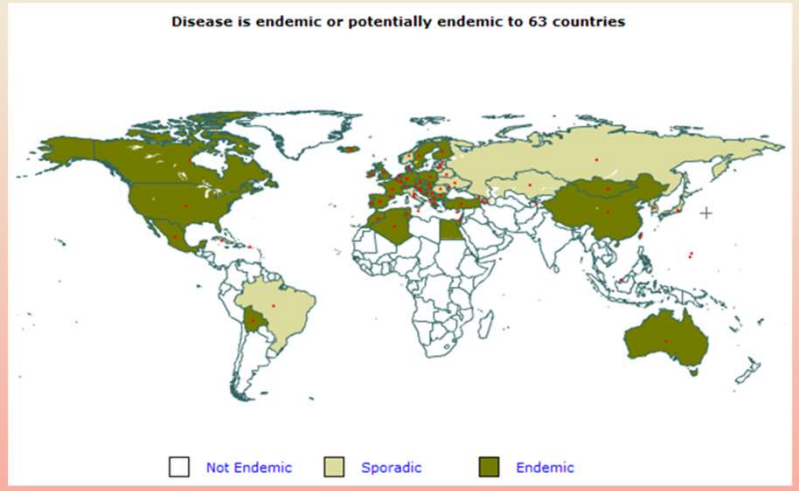
http://defiancecohealth.org/ticks/



https://www.gideononline.com/2008/05/07/lyme-disease-revisited/

http://www.deertickguard.com/html/tick.html