NOVEL METHODS FOR SURVEYING RESERVOIR HOSTS AND VECTORS OF *BORRELIA BURGDORFERI* IN NORTHERN MINNESOTA

A Thesis
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCES

Dr. Benjamin L. Clarke & Dr. Lynne T. Bemis

October 2015

Acknowledgements

First and foremost, I would thank my advisory committee: Dr. Ron Moen, Dr. John Dahl and Dr. Teresa Rose-Hellekant. My thanks also to the faculty and staff of the Integrated Biological Sciences program, the faculty and staff of the Biology department, the Biomedical Sciences department faculty and staff, the Medical school faculty and staff, the Center of American Indian and Minority Health and the Native Americans into Medicine program, the Pathways program, the National Science Foundation and the faculty and staff for the GK-12 Fellowship program.

I am happy to acknowledge Dr. Amy Prunuske and undergraduate research assistants Samantha Toivenen and Shane Wilson for their work in partnership with my projects and ideas in studying Lyme disease and performing outreach, as well as the independent continuation of research on *Ixodes* ticks, *Borrelia burgdorferi*, and outreach to the community about Lyme disease. My thanks to Katie Nemeth who gave me resources to consider surveying students and publishing the outreach I performed. A special thanks to the high school teachers and principals and students who allowed me into their classrooms, and the volunteers from the Biology department, the Biomedical department, the Pharmacy department and the microscopy lab, especially Brian Blandi, the Geology Club, and the Planetarium at the University of MN-Duluth who entertained high school student visitors. I am also grateful to the Wild Ones Duluth community chapter and the Thistledew Correctional facility for allowing me to present on Lyme disease. Thank you to the Cloquet Forestry Center, the Thistledew Wildlife Refuge and the DNR, as well as the 1854 Treaty Authority, and James L. David Smith with the Department of Fisheries, Wildlife and Conservation Biology at the University of Minnesota-Twin Cities for allowing me to collaborate with them in the field. And thank you to the professors who wrote me letters of recommendation in applying to graduate school, especially Dr. Elizabeth Minor and the Large Lakes Observatory who provided my very first science lab experiences; Darrin Johnson with the Department of Physics; and Milan Kivacovic who introduced me to Dr. Arthur Johnson, who in turn introduced me, with enthusiasm, to Dr. Benjamin Clarke who coached me in applying to the Integrated Biological Sciences program and who has facilitated many opportunities for my research. A special thanks to Robert Miller who told me to go to college, and then made sure I had the resources necessary to get through an undergraduate degree at University of Minnesota-Duluth; to Shannon RedBrook, Sally Mayasich, and Jeannette Lang who helped me maneuver through the more difficult times, and especially to Dr. Lynne T. Bemis who encouraged my growth academically and personally, kept me focused, and cheered me to the finish line (and made it look easy.) To my colleagues, my mentors, and my friends at the University of Minnesota, the Duluth community and surrounding areas, for their unrelenting guidance, support and encouragement. To list you all by name would rival the page count of this thesis.

A special note of thanks to my husband, for his support while we spent months apart so I could finish this degree, for believing in me, and for wanting me to be successful.

Dedication

This thesis is dedicated to my grandfather, Vernon J. Aird, who viewed much of the world with appreciation, curiosity and wonder and who I think of when I look at the stars, and study cells under a microscope; and to my grandmother, Colleen M. LaJoie, who has always supported my "free-spirited" ways, who is a great source of comfort for me, and who I admire for her generosity and her ability to laugh.

Abstract

Lyme disease is the most prevalent tick-borne disease in North America and presents challenges to clinicians, researchers and the public in diagnosis, treatment and prevention. Lyme disease is caused by the spirochete, *Borrelia burgdorferi*, which is a zoonotic pathogen obligate upon hematophagous arthropod vectors and propagates in small mammal reservoir hosts.

Identifying factors governing zoonotic diseases within regions of high-risk provides local health and agricultural agencies with necessary information to formulate public policy and implement treatment protocols to abate the rise and expansion of infectious disease outbreaks. In the United States, the documented primary reservoir host of Lyme disease is the white-footed mouse, *Peromyscus leucopus*, and the arthropod vector is the deer tick, *Ixodes scapularis*.

Reducing the impact of Lyme disease will need novel methods for identifying both the reservoir host and the tick vector. The reservoir host, *Peromyscus leucopus* is difficult to distinguish from the virtually identical *Peromyscus maniculatu*s that also is present in Northern Minnesota, a region where Lyme disease is endemic. Collection of the *Ixodes* tick, the Lyme disease vector, is difficult as this is season dependent and differs from year to year.

This study develops new strategies to assess the extent of *Borrelia burgdorferi* in the local environment of Northern Minnesota. A selective and precise method to identify *Peromyscus* species was developed. This assay provides a reliable and definitive method to identify the reservoir host, *Peromyscus leucopus* from a physically identical and sympatric *Peromyscus* species, *Peromyscus maniculatus*. A new strategy to collect ticks for measuring the disbursement of *Borrelia* was employed. Students from local high schools were recruited to collect ticks. This strategy increased the available manpower to cover greater terrain, provided students with valuable experience in research methodology, and highlighted the prospect of increasing community engagement in university-based research projects.

Hypothesis

Two hypotheses drive this thesis. 1) If HRM is appropriate to discern between two closely related species of *Peromyscus*. 2) If a citizen science approach can bolster sample collection of *Ixodes* ticks.

First, we developed a species-specific genomic assay to identify *Peromyscus leucopus*, the white-footed mouse, the primary reservoir host for *Borrelia burgdorferi*, the causative agent of Lyme disease. Second, we integrated widespread recruitment of field assistance in collecting *Ixodes* ticks, the vectors responsible for spreading Lyme disease and other tick-borne illnesses to humans, with the development of a citizen science outreach curriculum. The curriculum exposed students to the scientific method and health-related precautions involving tick collection, the process of body tick surveillance and Lyme prevention. The result of these two projects included the integration of sampling methods for the mammalian reservoir host and the tick vector of Lyme disease while providing valuable public health outreach to communities.

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Introduction

The Integrated Biosciences program is designed to prepare graduate students for the complex challenges that face scientists and society today. The following manuscript describes Lyme disease as an important public health concern, the complexity of which involves the biology of the infectious bacteria, reservoir host, pathogenic host and vector, as well as the ecology and impact of climate factors, the evolution and epidemiology of tick-borne diseases, and a curriculum for communicating to the public about the biomedical research on Lyme disease and ways to prevent tick encounters.

Lyme disease is a tick-borne disease caused by the spirochete *Borrelia burgdorferi*, which can adversely affect musculoskeletal, cardiovascular, and neurological tissues in humans, and may lead to chronic, debilitating complications (Barthold et al. 1993; Steere and Glickstein 2004; Kannian et al. 2007). Lyme disease is the most prevalent vector-borne illness in the United States and Europe (Johns et al. 2001; Brisson et al. 2008; Radolf and Samuels 2010) and is becoming increasingly prevalent in the north central United States (Reed et al. 2003). As areas of Minnesota become increasingly susceptible to rodent and tick populations due to climatic trends, people are at heightened risk for exposure to Lyme disease, as well as to other emerging pathogens.

Understanding the ecology of Lyme disease is essential to managing it. A zoonosis such as *B. burgdorferi* transfers from non-human to human hosts via a primary vector, in this case by *Ixodes* ticks. Other well-known tick-transmitted pathogenic microorganisms are *Anaplasma marginale*, *Ehrlichia* spp., *Rickettsia* spp., *Haemobartonella* spp.

(Schabereiter-Gurtner et al. 2003) and tick-borne encephalitis virus, as well as other newly discovered viral diseases (Dong et al. 2008).

Exposure to Lyme disease is greatest in summer months when nymphal ticks are most active (Diuk-Wasser et al. 2012). The tick has a two-year life cycle from egg, to larvae, to nymph, and finally to adult. From May through September, eggs hatch into larvae, which usually feed on white-footed mice or other small mammals. If the mouse is infected with disease-causing organisms, the larval-stage will become infected and thus be able to transmit these organisms during its second or third feeding. After feeding, the larvae molt into nymphs and become dormant until next spring when it takes its second feeding. If the tick is carrying disease agents from its first feeding in the larval stage, it can transmit them during this second feeding. If the nymph was not already infected, it can become infected if the second meal host is carrying disease agents. In the fall of the second year, nymphs molt into adult ticks. The adult female ticks feed and mate on large animals in the fall or the following early spring. If the ticks are unsuccessful in getting a blood meal in the fall, they go dormant over winter and seek a meal in the spring. After feeding and mating, the female lays eggs and then dies. Adult male ticks attach but do not feed or become engorged. Because the adult males do not take a blood meal, they do not transmit Lyme disease. The greatest risk of Lyme disease for humans is from nymphal ticks (Swanson et al. 2006).

B. burgdorferi are transmitted from the mid-gut of the deer tick into the mammalian host during a blood meal. The mechanisms of this transmission are highly specific and regulated by bacteria-specific proteins. Two surface proteins in particular play a vital role

in controlling tick residency in the tick gut and are coordinately controlled by the feeding state of the tick. In the gut of the tick, *B. burgdorferi* is tethered through expression of an outer surface protein, OspA (Schwan and Piesman 2002; Piesman and Schwan 2010). The changes of temperature and pH from blood entering the gut causes a change in the expression of outer surface proteins from OspA to OspC (Schwan and Piesman 2002; Lybecker and Samuels 2007; Piesman and Schwan 2010; Samuels 2011). *B. burgdorferi* is then released from the gut and transferred into the mammalian host via tick saliva while the tick still feeds (Pal and Fikrig 2010). Eventual humoral responses by the receiving host are to OspA and OspC from *B. burgdorferi* and to Salp15, a saliva protein from *Ixodes scapularis* (Rosa 2005). Human immunological defenses are stunted by salivary proteins from the tick, which act to *B. burgdorferi's* benefit in a couple of ways: anesthetizing surrounding tissue and coating the spirochete bacteria by binding to OspC thus delaying detection (Anguita et al. 2002, 2003; Rosa 2005). Motility also plays a role in the spread of *B. burgdorferi* from the initial site of infection by then diffusing to other tissues within the host (Norris et al. 2010). However, the absence of OspC affects transfer of *B. burgdorferi* and the successful evasion of a host's immune system (Pal 2004; Grimm 2004; Tilly 2006, 2007), The reciprocal expression of these outer surface proteins, OspA and OspC, is regulated by a complex pathway that includes the small, non-coding RNA called DsrA. DsrA post-transcriptionally regulates the alternative sigma factor, RpoS, by binding to the upstream region of the RpoS mRNA. RpoS controls a number of virulence genes in more than one bacteria (Kazmierczak et al. 2005; Samuels 2011), including OspA and OspC in *B. burgdorferi* and hence is required for successful host infectivity by *B.*

burgdorferi (Samuels 2011.) RpoS is evolutionarily distinct in *Borrelia* from its function in other bacteria (Samuels 2011), and the entire mechanism of the DsrA small RNA has yet to be determined, although one of its functions is to regulate outer surface protein expression.

The vector-host relationship between *B. burgdorferi* with the generalist vector in *Ixodes* ticks has been co-evolving. The maturation of the tick plays a primary role in not only the spread of Lyme disease but also the evolutionary adaptations of *B. burgdorferi*. Outer surface proteins of *B. burgdorferi*, such as OspA, are highly specific to *Ixodes* and necessary for *B. burgdorferi* to survive the vector stage of transmission (Pal and Fikrig 2010). One approach to manage Lyme disease would be to reduce encounter rates by humans through ecological management of the tick population. Another route would be to prevent human-tick contact through education of the public on tick ecology and how to limit encounters with ticks and the pathogens they carry.

In the case of Lyme disease, creating awareness about the vector transmission of the disease directly impacts one of the goals of scientific research: to decrease the amount of people negatively affected by Lyme disease through a message of prevention and management. A citizen science approach arms the public with knowledge and tools that can help prevent tick encounters and allows for extensive sample collection when travel costs, personnel, and logistical demands constrain traditional research methods for collecting ticks (Dickinson et al. 2012). Citizen scientists can provide observations over an expansive area, and are most useful when involved in field-based activities over extensive spatial scales (Lepage and Francis 2002; Dickinson et al. 2012), as is the case with *Ixodes*

population patterns and tick-borne pathogens, especially as these dynamics are altered by climate change (Robinson et al. 2014). Examples of data collected by citizen science programs include water quality monitoring programs by the Minnesota Pollution Control Agency, The Cornell Lab of Ornithology, and Foldit, a collaborative online game that allowed participants to design virtual molecules, ultimately leading to the determination of the structure for a retroviral protease that promotes the spread of HIV and was previously unknown (Khatib et al. 2011). Data collected from citizen scientist field observations are relevant and often interesting especially when well-designed protocols, training materials, and professional assistance ensure the reliability of the data (Haag 2005; Cohn 2008).

The white-footed mouse, *Peromyscus leucopus*, has been identified as the most important reservoir for the vector-borne zoonotic disease Lyme Borreliosis (Schwanz et al. 2011; Brisson et al. 2008; LoGiudace et al. 2003; Donahue et al. 1987; Levine et al. 1985.) Reservoirs for zoonotic disease are determined by an animal's encounter rates with the disease, as well as an ability to maintain infection and pass it on to vector species, in this case the black-legged tick, *Ixodes scapularis*. A tick's first contact with *B. burgdorferi* seems to be through initial feeding on the white-footed mouse, a common host for immature ticks (Pal and Fikrig 2010). Studies conducted by Tupin et al. (2008) suggest that the white-footed mouse host experiences minimal ill effects from the bacterial infection, while deer, wolves and humans do present with inflammatory-related symptoms. With further studies into this intriguing cooperation and possible coevolution between rodent reservoir and *B. burgdorferi* spirochete, mechanisms of non-pathogenicity might be found and applied to clinical methods for treatment and prevention in humans. The risk of human

exposure to the pathogen hinges on the population dynamics of the primary reservoir and the eventual interaction with vector ticks.

This study expounds upon key ecological aspects of Lyme disease by providing novel methods for 1) differentiation of *Peromyscus* species of mice, the reservoir hosts to zoonotic disease, and 2) catching *Ixodes* ticks through public recruitment and a citizen science project.

Chapter 1:

A Novel method to distinguish the morphologically similar species *Peromyscus leucopus* from *Peromyscus maniculatus* using High-Resolution Melt (HRM) analysis.

Summary

A method applying high-resolution melt (HRM) analysis to PCR products copied and amplified from extracellular RNA (exRNA) has been developed to distinguish two morphologically similar *Peromyscus* species: *P. leucopus* and *P. maniculatus*. *P. leucopus* is considered the primary reservoir host of *Borrelia burgdorferi*, the causative agent for Lyme disease in North America. In northern Minnesota the habitat ranges of *P. leucopus* overlaps with that of *P. maniculatus*. Serum samples from live mice of both species were collected from cheek bleeds, extracellular RNA (exRNA) was extracted, and ribosomal RNA (rRNA) was amplified using reverse transcription and PCR followed by HRM analysis. The rRNA amplicon differed at seven nucleotides between the two species and the resulting HRM analysis allowed rapid species confirmation. The geographic distribution of these species is expected to vary with climate change and urban expansion. This novel method may be applied to identify changes in species distribution of a reservoir host impacting human health.

Introduction

Peromyscus leucopus (*P. leucopus*), the white-footed mouse, and *Peromyscus maniculatus* (*P. maniculatus*), commonly referred to as the deer mouse, are important environmental reservoirs for infectious microbes and viruses. Environmental disturbances such as climate change are likely transforming the distribution of competing *Peromyscus* species, and thus the regions associated with certain pathogens (Simon et al. 2014). Identifying factors governing the presence of zoonotic disease within a region provides local health and agricultural agencies with necessary information to formulate public policy and implement treatment protocols to abate the rise and expansion of disease outbreaks.

In Northern Minnesota, the white-footed mouse, *P. leucopus*, is identified as the predominant reservoir for the vector-borne zoonotic disease Lyme Borreliosis (Levine et al. 1985; Donahue et al. 1987; LoGiudice et al. 2003; Brisson et al. 2008; Mannelli et al. 2011; Schwanz et al. 2011.) The morphologically similar and sympatric species *P. maniculatus* is more often documented as a reservoir of Sin Nombre virus (Childs et al. 1994; Hjelle et al. 1995; Jonsson et al. 2010), although this pathogen is not observed in Northern Minnesota. *P. leucopus* and *P. maniculatus* are indiscernible in the field, yet are capable of being reservoirs for the same diseases. *P. leucopus* was reported to host hantavirus in New York (Hjelle et al. 1995), and *P. maniculatus* can serve as a competent reservoir host of *Borrelia* (Wright and Nielsen 1990; Moody et al. 1994; Brown and Lane 1994; Baum et al. 2012), yet studies rarely identify *P. maniculatus* as a factor considered in the maintenance of Lyme disease in regions where it is endemic (Rand et al. 1993). It may be that those conducting the sampling of Lyme disease infection rates in *Peromyscus*

do not confirm the species they are working with, and they rely on geographical regions or morphometric data alone, which may mislead epidemiological studies, and certainly leaves the species in question.

Methods to monitor reservoir host species accurately and quickly are necessary to develop a better understanding of the spread and risk of zoonotic diseases, such as Lyme disease. Previous studies that discriminate between *P. leucopus* and *P. maniculatus* have used a variety of morphological methods, serological methods, and DNA based assays. Morphological methods have the advantage of being useful in the field and less expensive to conduct than genetic tests but are less accurate (Tessier et al. 2004; Stephens et al. 2013). Confirmation of species for *P. leucopus* and *P. maniculatus* is not possible by external measurements alone, thus a molecular analysis is necessary (Lindquist et al. 2003).

We live trapped small mammals in Northern Minnesota. We measured external features of *Peromyscus* caught across a broad geographic area and found that anatomical measurements did not allow the discrimination of the two species. Thus, a molecular assay to accurately distinguish *Peromyscus* species was needed. In this study we developed a high-resolution melt (HRM) assay capable of distinguishing *P. leucopus* from *P. maniculatus*. The assay detects variation in a fragment of circulating 16S mitochondrial rRNA from a serum sample. HRM utilizes the distinctive melting of the PCR based amplicons to allow clear identification of *P. leucopus* and *P. maniculatus* when compared to known samples obtained from the *Peromyscus* Genomic Stock Center (PGSC). In order to test the new molecular HRM assay, we collected samples from an additional group of

animals and confirmed that the HRM assay allows the rapid genotyping of live caught animals.

Materials and Methods

Livetrapping methods

Twenty Sherman live traps were evenly spaced along two 100-meter transects. Peanut butter mixed with oats were rolled in wax paper and used to bait the traps and carrot slices were placed in the trap for moisture. Traps were set in the evening, before dark, between the hours of 5:00 p.m. to 10:00 p.m., and checked the following morning from 6:00 a.m. until 8:00 a.m. Mice were live-captured and handled in the field according to Institutional Animal Care and Use Committee (IACUC) #0806A37521. Mice were handled in the field to measure hind foot length and tail length with a flexible measuring tape.

Sample treatment: Cheek bleeds and serum separation

Blood was obtained from the facial vein (Sikes, Gannon, and The Animal Care Use Committee of the American Society of Mammalogists 2011). Twenty gauge needles were used to acquire 100 μL to 200 μL of blood by submandibular bleed. Blood samples were collected in non-treated Eppendorf tubes and allowed to clot. Anti-coagulants were avoided as these may interfere with reverse transcriptase or PCR amplification; heparin was specifically avoided because it inhibits PCR amplification (Khosravinia and Ramesha 2007). Samples were taken from the field and centrifuged at 4,000 G-force at 4°C for 10 minutes. Serum was taken from the top layer of fluid and at least 30 μL was used for RNA

extraction. The serum sample was treated with 700 μL of QIAzol (Qiagen) and all samples were immediately stored at -80°C.

RNA isolation and cDNA preparation

The quantity of RNA extracted from 30 μL of serum is sufficient for amplification by qRT-PCR methods. Total RNA was extracted using the Qiagen miRNeasy kit (cat. no. 217084) with QIAzol. Samples were processed as described by the suppliers and suspended in 30 μL of RNase free water. The maximum volume of RNA (12 μL) was converted to cDNA using the MiScript cDNA kit from Qiagen (cat. no. 218161). As a positive control, we analyzed the expression of a highly conserved mammalian microRNA, microRNA-21. All samples in this study met a threshold requirement in that microRNA-21 was detected in all samples, which confirmed successful RNA extraction from the limited serum samples available from each catch and release cheek bleed. Negative controls included a no template cDNA reaction with water, in addition to a water control. This was used throughout the PCR analysis in addition to the usual negative control of water alone.

Quantitative real time PCR conditions and Primer design

In order to determine the species, we developed an RT-PCR based molecular assay with the addition of a high-resolution melt (HRM). The internal control for RNA quality, microRNA-21 was amplified using the Type-it HRM kit for high-resolution melt (Qiagen, cat. no. 206542) with the forward primer microRNA-21 adjusted 5'- ccc TAG CTT ATC AGA CTG ATG TTG A -3', with 'ccc' being the adjusted aspect of the forward primer for microRNA-21, added to increase annealing temperature. The universal reverse primer was supplied with the MiScript SYBR Green PCR kit (Qiagen, cat. no. 218076).

The primers for the 16S mitochondrial rRNA of *Peromyscus* were designed to *P. leucopus* 16S rRNA sequence (GenBank accession number: JN181159.1) and includes the *P. leucopus* forward primer 5'- TTC ATA GGA GCT ATA GAG ATC AGT ACC G -3' and *P. leucopus* reverse primer 5'- GGT ACA AGG TTT AAT CTT TGC TTA TTT GTG CT -3'. The Type-it HRM PCR kit from Qiagen was used in conjunction with these primers for qRT-PCR. The success of HRM required the use of a saturating intercalating dye such as EVA Green rather than a nonsaturating intercalating SYBR Green product.

All PCR reactions were conducted on a Rotor-Gene Q PCR machine with HRM capability from Qiagen. PCR conditions included an initial denaturation at 95°C for 15 minutes, followed by 40 cycles of 95°C for 10 seconds and 62°C for 45 seconds acquiring at each cycle, high-resolution melt from 50°C to 90°C with a 0.1 degree increase per 2 seconds after a 90 second pre-melt conditioning first step. Gain was optimized on all tubes to give highest fluorescence at 95°C. The microRNA-21 amplification used the same conditions as the PCR for determining species, except the cycles were conducted at 60°C rather than 62°C. All PCR was accompanied with negative controls for PCR and RNA contamination using a no template control and a water control qRT-PCR.

T-vector cloning and direct sequencing

Confirmation of primer specificity for the targeted 16S rRNA sequence was conducted using the same *P. leucopus* primers and PCR conditions with the addition of GoGreen Taq polymerase (Promega). The amplified products were cloned into T-vector cloning vector pCR2.1 TOPO provided in the TOPO cloning kit (Life Technologies, product number K4560-01). Plasmids were sequenced at the University of Minnesota

Genomics Center (UMGC) via Sanger sequencing. Reported sequences were compared to established sequences for *Peromyscus* species using the Blast program. Sequences matching this region of the 16S mitochondrial rRNA region in *P. leucopus* are present in GenBank (accession number: JN181159.1). The complete nuclear genome for *P. maniculatus* is available at Baylor Human Genome Sequencing Center. However, the mitochondrial genome for this region of the 16S rRNA was unavailable for *P. maniculatus* at the time these primers were designed.

Results

Field Studies of *Peromyscus* species in Northern Minnesota

Biological metrics and samples were collected from mice live caught in regions of Northern Minnesota in order to identify the species that is the reservoir host for Lyme disease. Established metrics from Hazard (1982) provided a range of hind foot and tail length expected for each of the species present in this geographic region (Table 1.1). Field measurements for each mouse in our study included the length of the tail and hind foot (Fig. 1.1). Measurements of mice trapped in field studies conducted in Bagley Nature Area in Duluth, Minnesota, in the summer of 2012, in Thistledew Wildlife Refuge in Togo, Minnesota in the spring of 2013; an area in Beaver Bay, Minnesota in the summer 2013, and in Cloquet Forestry Center in Cloquet, Minnesota in the summer of 2013 are included in Figure 1.1.

These data demonstrate the difficulty in species confirmation using morphometric calculations and comparison to historic studies (Hazard, 1982). The measurements for

several mice fell within the expected size range for *P. leucopus*. Others were not within the expected measurement for either *P. leucopus* or *P. maniculatus*, and another group overlapped with two species (Fig. 1.1).

Developing a Molecular Identification of *Peromyscus* Species

In order to develop a rapid assay for distinguishing these two species we obtained serum samples from stock colonies of these two species, collected in accordance with our protocol (Materials and Methods). We included five *P. leucopus* and five *P. maniculatus* supplied by the *Peromyscus* Genetic Stock Center (PGSC) (University of South Carolina) to validate the genetic identity for each species. We also trapped ten mice from Bagley Nature Area in Duluth, Minnesota in the year 2014, five in the spring and five in the fall (Fig. 1.2) to demonstrate the utility for testing field samples using a molecular assay.

The mice from the PGSC clearly grouped together by measurement of hind foot and tail length demonstrating the importance of morphometric measurements coming from homogeneous stock. However, their measurements did not fit within the expected sizes for *Peromyscus* mice in Minnesota based upon the measurements as reported by Hazard (1982) (boxes in Fig. 1.2). The wild-caught mice again showed variation that prevented unambiguous identification of species using these morphometrics. The wild mice did not coincide with a definable species group when compared with known samples (Fig. 1.2).

Methods development of RNA Extraction and Amplification

Whole blood collected from cheek bleeds in this catch and release study ranged from 100 μL to 200 μL in volume. In this pilot study we extracted RNA from 30 μL aliquots of serum from each animal. Total RNA isolated from serum is difficult to quantify

due to the low levels of cell-free circulating RNA. Thus, the maximum volume of the extracted RNA was used for reverse transcription into cDNA. In order to confirm that RNA of amplifiable quality was present in each sample of cDNA, microRNA-21 was amplified in a quantitative RT-PCR (qRT-PCR) reaction using a primer specific for microRNA-21 and adjusted to allow a higher melt temperature. Amplification of microRNA-21 was used due to its high conservation across species, that it is identical in the lab mouse and in humans, and microRNA-21 is routinely found in serum and plasma (Arroyo et al. 2011). We therefore expected microRNA-21 to be an internal control in the serum from both *P. leucopus* and *P. maniculatus* (Fig. 1.3). The melt curves shown in Figure 1.3 overlap, confirming that microRNA-21 is conserved across the two species and that amplifiable cDNA was obtained from the RNA in serum.

Identifying a region of 16s rRNA for genotyping

In order to develop a high-resolution melt (HRM) assay that would be able to rapidly distinguish the two species we designed primers based on a segment of a circulating rRNA that had been previously identified. Primers were designed to amplify a segment of 16S rRNA from *P. leucopus* and *P. maniculatus* based on a region of homology shared between the lab species *Mus musculus (M. musculus*), and the known sequence for this region in *P. leucopus* (GenBank accession number: JN181159.1; Table 1.2). Although the entire genomic sequence is available in GenBank for *P. maniculatus*, this region of the mitochondrial genome was not known at the time of these experiments. The concept guiding this experiment is to utilize highly conserved regions in the two known species, *M. musculus* and *P. leucopus*, that surround a region of variability and test whether that

amplicon allows species identification between *P. leucopus* and *P. maniculatus* using HRM technology. The HRM assay requires enough variability within the amplicon to allow changes in melting of the PCR amplicon. HRM allows distinguishing the species directly from a qRT-PCR assay once the method is established using samples from the mice obtained from known colonies. Each time the assay is conducted, it will require the use of known samples for comparison.

High-Resolution Melt assay to distinguish *P. leucopus* from *P. maniculatus*

The *Peromyscus* specific primers bound to the cDNA and amplified a product in both *P. leucopus* and *P. maniculatus* samples obtained from the PGSC (Table 1.3). As shown in Table 1.3, the small amplicon contained a significant number of different bases, and this allowed a robust HRM analysis (Fig. 1.4). The heterogeneity of the sequence between *P. leucopus* and *P. maniculatus* promotes greater diversity in annealing between amplicons thus enhancing identification of differences between the two species when the PCR product is melted (Fig. 1.4). The genotype of each species was evident in HRM analysis and was confirmed by direct sequencing (Table 1.3).

The HRM analysis was first conducted on five known *P. leucopus* samples and on five known *P. maniculatus* samples obtained from the PGSC. In contrast to the HRM melt curves for microRNA-21 (Fig. 1.3), for which melt curves between species is identical, samples amplified with the *Peromyscus* specific primers clearly distinguish the two species. Each cDNA amplicon of *Peromyscus* specific 16S mitochondrial rRNA sequence was analyzed in two ways using the HRM program (Qiagen). The graph on the left in Figure 1.4 represents normalized melt curves and distinguishes *P. leucopus* melt curves

from *P. maniculatus* melt curves. The graph to the right of the normalized melt curves in Figure 1.4 is a difference curve for which one sample is chosen as the reference sample (a sample confirmed as *P. leucopus*) and against which all other samples are compared. These results demonstrate differences between known *P. leucopus* and *P. maniculatus* serum samples. Figure 1.4 shows the samples collected in our catch and release study in the spring and the fall, respectively, clearly segregate with the *P. leucopus* group by normalized melt curve and by difference curve. Peaks for the wild-caught samples in the difference curve graphs are either above or below the *P. leucopus* control sample, but they are distinct from *P. maniculatus* samples (arrows point to Bagley Nature Area samples). Each of the cDNA reactions from the catch and release program was also confirmed by direct sequence analysis, as described above, and all samples collected from the Bagley Nature Area were confirmed to be of the same sequence as the *P. leucopus* species sample from the *Peromyscus* Genetic Stock Center (Table 1.3).

Discussion

Identifying the *Peromyscus* species is a crucial step before mapping ecological niches and assigning roles as reservoirs in enzootic cycles of disease. Ultimately defining the reservoir host allows the development of interventions against the spread of disease. The reservoir population is central to sustaining the pathogen and propagating the vector-borne transmission of zoonotic disease, which adversely impacts susceptible inhabitants of a region (Oliver Jr. et al. 2003; Golovchenko et al. 2014). Catch and release programs to monitor species are critical to understanding biodiversity and the spread of infectious

disease, especially as changes in the ecosystem due to events such as climate change will impact these relationships. Climate change increasingly influences *Peromyscus* distribution (Myers et al. 2009; Rogic et al. 2013; Roy-Dufresne et al. 2013; Simon et al. 2014). In our study, we expected to find *P. leucopus* amidst our samples with relation to incidence of Lyme disease increasing in northern counties of Minnesota, but we also expected to find *P. maniculatus* in accordance with reported habitat ranges (Hazard 1982). A recent study shows *Peromyscus leucopus* distribution in Minnesota with a northern limit in central Minnesota (Bedford and Hoekstra, 2015). But changing climate has provided an opportunity for the white-footed mouse to move beyond estimated northern borders (Roy-Dufresne et al. 2013). Our study confirms that *P. leucopus* is as far north as Duluth, Minnesota, which is farther than reported by Bedford and Hoekstra (2015).

Our initial study involved capturing mice in the field and demonstrated a weakness in confidently assessing population changes in *Peromyscus* species by physical measurements. For this study, we used certain physical measurements of mice which are thought to be independent of age or gender following weaning (Rich et al. 1996). We used hind foot and tail measurements because juvenile *Peromyscus* have been reported to reach adult-sized hind foot and tail length well before they adopt adult weight and pelage color (Kamler et al. 1998). While field measurements have the advantage of omitting a need for collection and preservation of a biological sample, our findings confirm that identifying mouse species by morphological differences is limited and inconsistent. The advent of more accurate and reasonably priced methods to monitor and identify species would lower the barriers to performing monitoring studies.

Our goal was to develop a method to identify *Peromyscus* species by a molecular analysis of small serum quantities, thus providing a reliable and sensitive method without permanently injuring the animal. Our ultimate goal was to test for serological evidence of tick-borne diseases in murine populations in the wild, thus we focused on developing a serological test for discerning physically indistinguishable murine species that are reservoir hosts of zoonotic disease. A serological test is also used to confirm that the mice are infected with *B. burgdorferi* and this method also requires the use of serum. We demonstrated herein that a rapid test for extracellular RNA (exRNA) from serum clearly distinguishes physically similar *Peromyscus* species. The advantage of using exRNA for these studies is its presence and stability in serum (Arroyo et al. 2011; Witwer et al. 2013). Furthermore, RNA has the potential for broader applications in that multiple analytes may be detected from a single 30 μL serum sample. Studies have shown exRNA can be used as a biomarker for disease (Chen et al. 2008; Reid et al. 2011; Wang et al. 2012).

The development and application of a novel method requires the appropriate controls including positive controls for species as well as positive controls for RNA quality and amplifiable cDNA obtained from serum. The highly conserved microRNA-21 provides an internal standard to confirm sample quality because it is found in all mammalian serum samples tested. Samples with negligible microRNA-21 would be omitted from further analysis due to a consideration of poor sample quality that could be a result of storage or collection method. We identified adequate amplification of microRNA-21 in all our samples and included those samples for further study (Fig. 1.3). Thus, we demonstrated that microRNA-21 can be used as an internal calibrator to assess RNA

quality of signal detection and is an important control. Samples with non-detectable microRNA-21 may be considered inadequate and eliminated from study.

Using mitochondrial ribosomal RNA is a well-established approach to species identification (Munshi-South and Nagy 2014). The 16S mitochondrial rRNA gene has previously been used to classify species based upon unique species-specific sequences (Sarri et al. 2013). We chose to use a mitochondrial ribosomal RNA sequence that we detected in the serum. The 16S rRNA gene is a mitochondrial transcript, characterized in human samples to also produce four different RNA transcripts (Maximov et al. 2002) and a long noncoding RNA molecule that results in the expression of a small peptide (Tajima et al. 2002). Studies have shown that rRNA is frequently found in patient serum (Huang et al. 2013). Another study demonstrates a gene homologous to the 16S mitochondrial rRNA encodes a neuropeptide (Guo et al. 2003; Lee et al. 2013), otherwise the role of 16S mitochondrial rRNA outside of the mitochondria is not understood. Remarkably, this approach provides a usable biomarker of species (Fig. 1.4).

The method developed in this study illustrates that HRM can reliably demonstrate differences between two closely related species. Sequencing of the amplicon from these two species would not be required, however, it is recommended that a positive control for each species be obtained from the PGSC and be included in each analysis.

We believe this assay has broader applicability for wildlife studies that require reliable identification of closely related species and the ability to discriminate between subspecies. The HRM method developed in this study allows the amplification of a region of the 16S mitochondrial rRNA, which differs at seven nucleotides between *P. leucopus*

and *P. maniculatus.* The novel method developed here clearly distinguishes the two closely related *Peromyscus* species; however, because our study was small we did not find evidence of the presence of *P. maniculatus* in the areas where traps were set. Future studies could include additional *Peromyscus* species if positive controls are available, in addition to subspecies of *P. maniculatus*, i.e. *Peromyscus maniculatus gracilis* (*P. m. gracilis*). A stock colony for *P. m. gracilis* has yet to be developed in the United States (Bedford and Hoekstra 2015) and confirmation of subspecies detection by a molecular assay would require a positive control. *P. m. gracilis*, also known as the wood mouse, is largely unrecognized in *Peromyscus* literature and there are scant references to its range in Northern Minnesota. *P. m. gracilis* is similar to *P. leucopus*, but no mention exists of genetic confirmation of the species in small mammal surveys in the wild.

Peromyscus mice are abundant in the wild and easy to capture and maintain in the laboratory (Dewey and Dawson 2001; Bradley et al. 2007; Shorter et al. 2012). With *Peromyscus* increasing in value as a model species with so many research applications in so many research fields, further studies in this regionally specific subspecies may be of value and is an area needing to be developed in *Peromyscus* studies.

Table 1.1. Range of measurements of *Peromyscus* species as recorded by Hazard, 1982*, from *Peromyscus* Genetic Stock Center (PGSC), and multiple field sties in northern Minnesota.

	Body Measurement:			
	Hind foot length		Tail length	
	Min (mm)	Max (mm)	Min (mm)	Max (mm)
**P. maniculatus gracilis* (Hazard, 1982)	20.0	22.0	80.0	104.0
**P. leucopus* (Hazard, 1982)	19.0	22.0	65.0	84.0
**P. maniculatus bairdi* (Hazard, 1982)	16.0	19.5	56.0	69.0
PGSC *P. leucopus* (Fall 2014)	19.0	20.0	60.0	65.0
PGSC *P. maniculatus* (Fall 2014)	17.0	20.0	50.0	54.0
Bagley Nature Area (Spring 2014)	19.0	20.0	66.0	85.0
Bagley Nature Area (Fall 2014)	13.0	20.0	61.0	82.0
Thistledew Wildlife Refuge (2013)	20.0	21.0	75.0	90.0
Cloquet Forestry Center (2103)	19.0	20.0	78.0	102.0
Beaver Bay (2013)	19.0	21.0	63.0	89.0
Bagley Nature Area (Summer 2012)	18.0	21.0	72.0	78.0

Table 1.2. Identification of a homologous regions of the mitochondrial 16S rRNA from *P. leucopus* and *M. musculus* that surrounds a region of variability between species. Primers were designed to bind to homologous regions (methods and materials). The regions of variability are shown. Expected differences between *P. leucopus* and *M. Musculus* are designated with spaces and bolded letters.

Source	Sample		Sequence	
GenBank JN181159.1	*P. leucopus*	198	5′-CAAGGGAAGAGTAAAACAC-AATCAATTAGCACAAATAAGCAAAGATTAAA-3′	247
GenBank JF286601	*M. musculus*	1216	5′-CAAGGGAA**AGA**T**G**AAA**G**AC**T**AAT**T**AA**A**-AG**T**A**AG**AA**C**AAGCAAAGATTAAA-3′	1265

Table 1.3. Direct sequencing of PCR products to confirm species differences. Primers surrounding the sequences shown were designed to amplify *P. leucopus* with the expectation that the primer binding sites will be conserved and the sequence in between (shown below) will have variability between *Peromyscus* species. Samples of *Peromyscus* serum obtained from the PGSC. Sequences generated by Sanger sequencing at the University of Minnesota are compared to GenBank reported sequence in the same 16S mitochondrial rRNA region. Differences in sequence are defined by dashes where bases are missing and by bolded lettering when a nucleotide differs from that in the GenBank sequence. One base in all *P. leucopus* samples differs from that reported in GenBank.

Source	**Sample**	**Sequence**
GenBank (JN181159.1)	*P. leucopus*	CAAGGGAAGAGTAAAACACAATCAATTA
PGSC	*P. leucopus*	CAAGGGAAGAG**C**AAAACACAATCAATTA
PGSC	*P. maniculatus*	CAAGGGAAGA**AT**AAA-**T**ACAA**G**CAA---

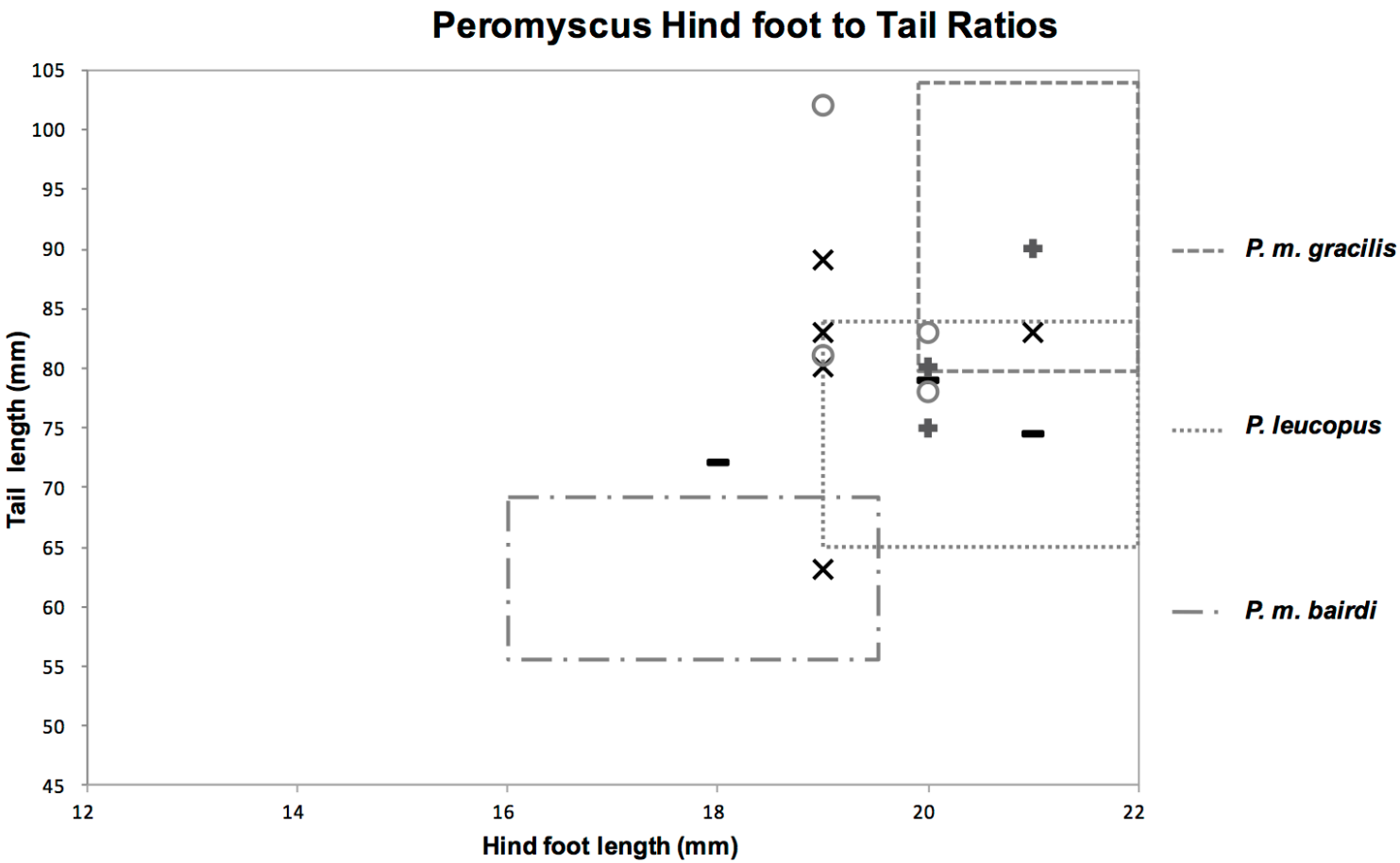

Figure 1.1. Measurements of hind foot length and tail length of *Peromyscus* mice caught at multiple sites in Northern Minnesota. Catch and release sites included the Bagley Nature Area (-), Thistledew Wildlife Refuge (+), Beaver Bay (x), and the Cloquet Forestry Center (o). The samples show a wide distribution across the expected sizes for *Peromyscus maniculatus* (lower and upper box) and *Peromyscus leucopus* (middle box) as determined by Hazard, 1982 (Table 1.1), with most samples falling within expected sizes for *Peromyscus leucopus.*

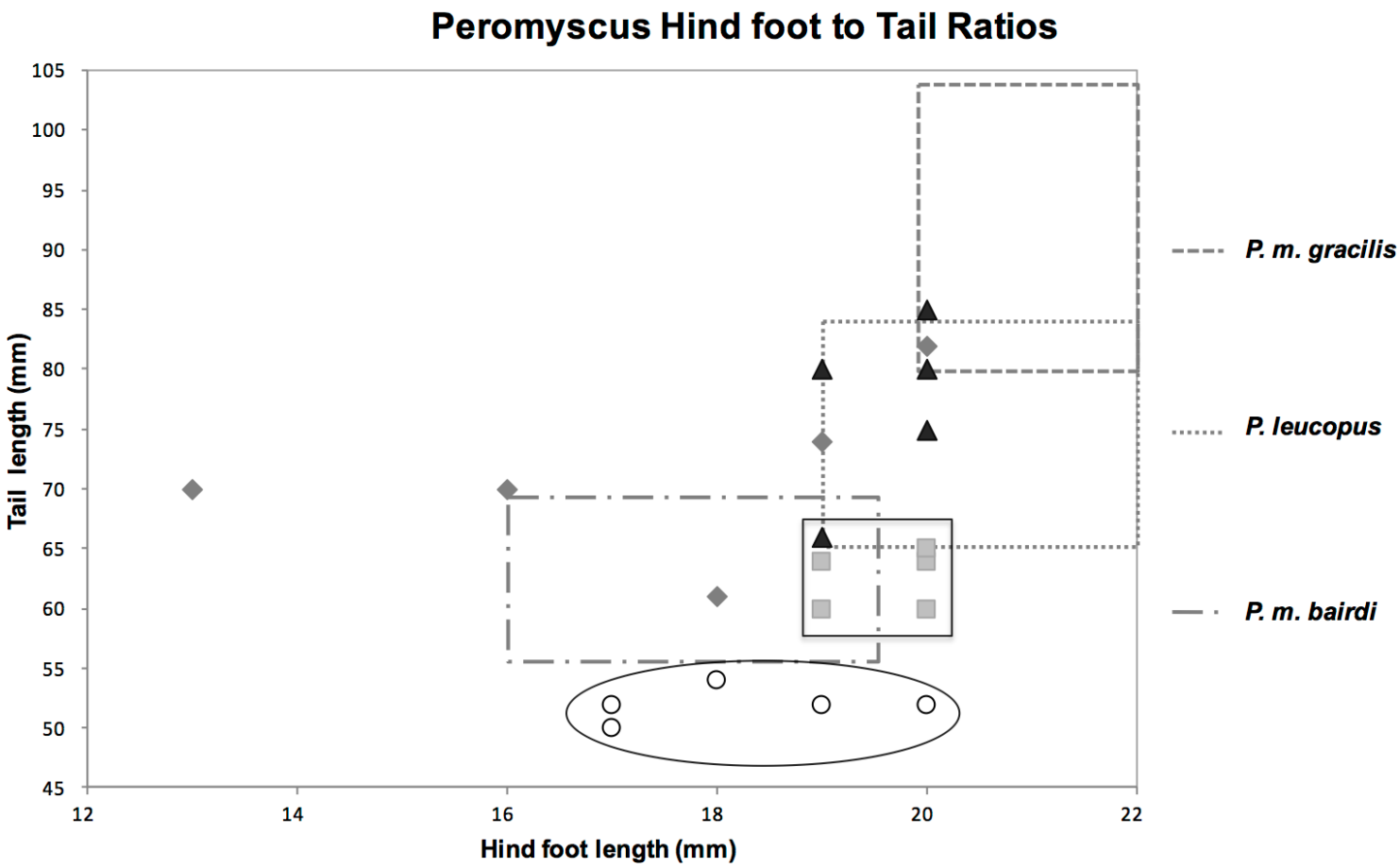

Figure 1.2. Measurements of hind foot, ear and tail length of *Peromyscus* species. Measurements from five *P. leucopus* (squares) and five *P. maniculatus* (circles) were obtained from live mice at the *Peromyscus* Genetic Stock Center (PGSC). Measurements were also collected from an additional ten mice from the Bagley Nature Area by trapping five mice in the spring (triangles) and five in the fall (diamonds). Measurements were obtained in the field for the Bagley Nature Area mice using the catch and release methods described previously.

HRM of microRNA-21 in samples from *Peromyscus* species

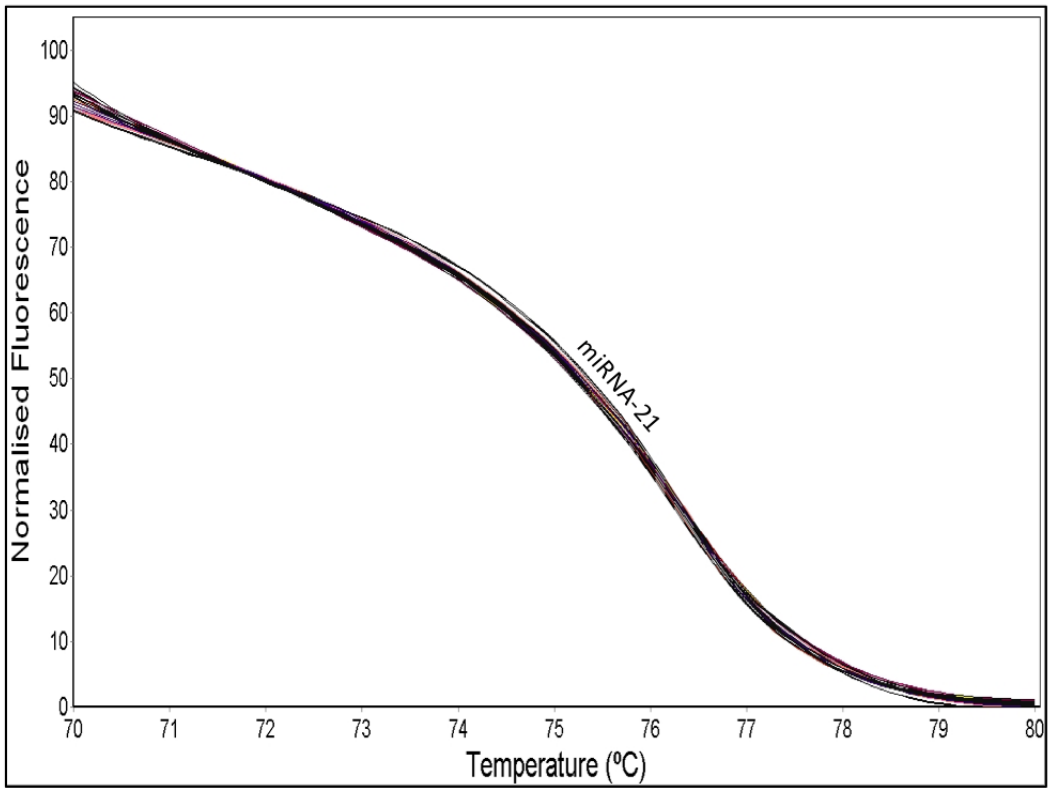

Figure 1.3. Normalized High Resolution Melt curves following the amplification of microRNA-21. The RNA extracted from serum is not accurately quantified on a spectrophotometer, requiring that a constitutively present RNA must be amplified to confirm RNA isolation and amplification. These melt curves illustrate that RNA isolated from all samples, *P. leucopus* and *P. maniculatus* as acquired by the *Peromyscus* Genetic Stock Center (PGSC) and wild samples collected from Bagley Nature Area, was amplifiable.

Normalized HRM and Difference Curves of 16S mitochondrial rRNA amplified from serum samples of *P. leu*, *P. mani* acquired from the PGSC in the spring of 2014.

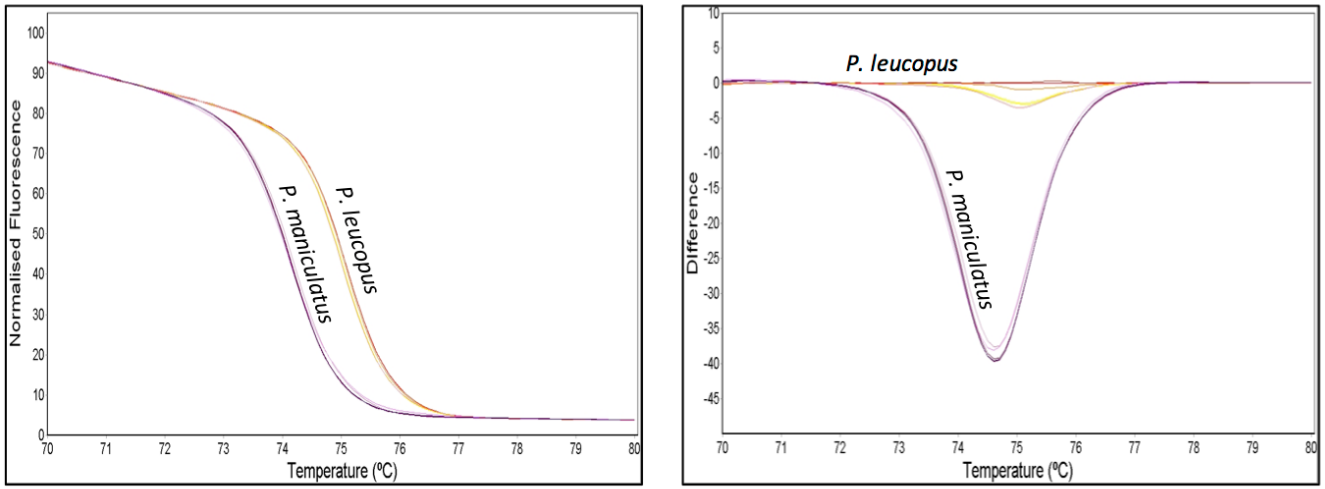

Figure 1.4: Development of a High Resolution Melt (HRM) assay to distinguish *P. leucopus* from *P. maniculatus* by amplification of 16S mitochondrial rRNA in samples from the serum acquired by the *Peromyscus* Genetic Stock Center (PGSC). Normalized HRM (left) for controls from the PGSC, five samples of *P. leucopus* mice, and five samples of *P. maniculatus*; difference curve (right) of the same samples with one *P. leucopus* sample selected as the genotype to which all other samples are compared. All samples from PGSC that were identified by the stock center as *P. leucopus* grouped together, while samples identified by the stock center as *P. maniculatus* grouped together and did not overlap with *P. leucopus*.

Normalized HRM and Difference Curves of 16S mitochondrial rRNA, amplified from serum samples of *P. leu*, *P. mani* acquired from the PGSC and unknown wild-caught samples from Bagley Nature Area in the spring of 2014.

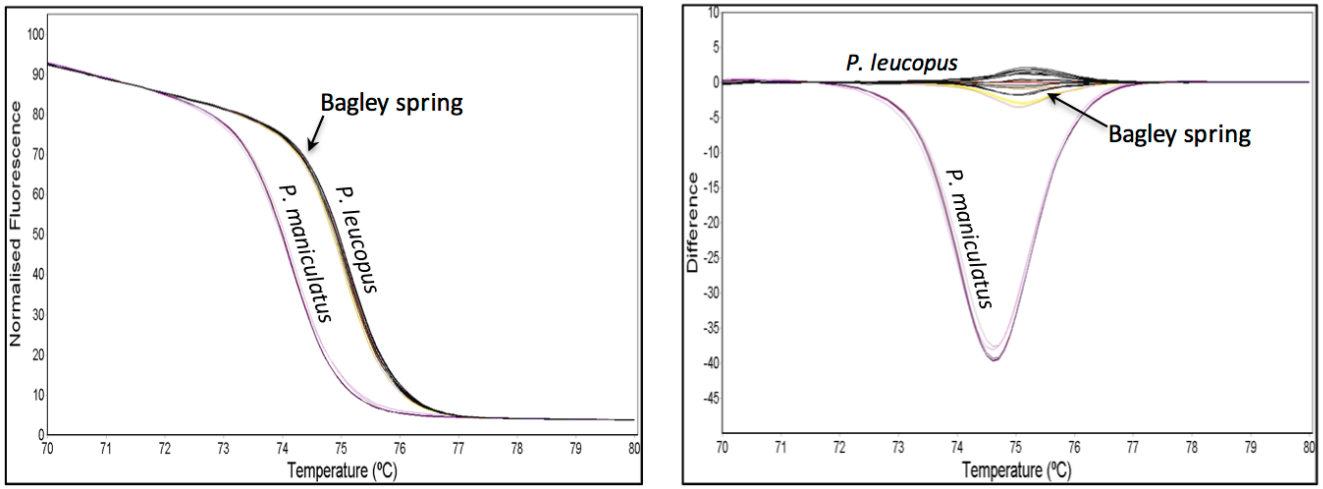

Figure 1.5: Normalized High Resolution Melt (HRM) curves of the samples from *Peromyscus* Genetic Stock Center (PGSC) are used as controls and then compared to samples wild-caught in Bagley Nature Area in the spring season of 2014. Normalized HRM (left) with control samples *P. leucopus* and *P. maniculatus* from PGSC, along with samples of unknown *Peromyscus* species obtained from Bagley Nature Area in the spring (Bagley spring); difference curve (right) where PGSC samples and spring Bagley Nature Area samples are compared against a selected PGSC *P. leucopus* sample. Arrows are used to designate the samples from Bagley Nature Area, and melt curves are shown in black. Note that the Normalized HRM curves of the Bagley spring samples overlap known *P. leucopus* samples from PGSC.

Normalized HRM and Difference Curves of 16S mitochondrial rRNA, amplified from serum samples of *P. leu*, *P. mani* acquired from the PGSC and unknown wild-caught samples from Bagley Nature Area in the fall of 2014.

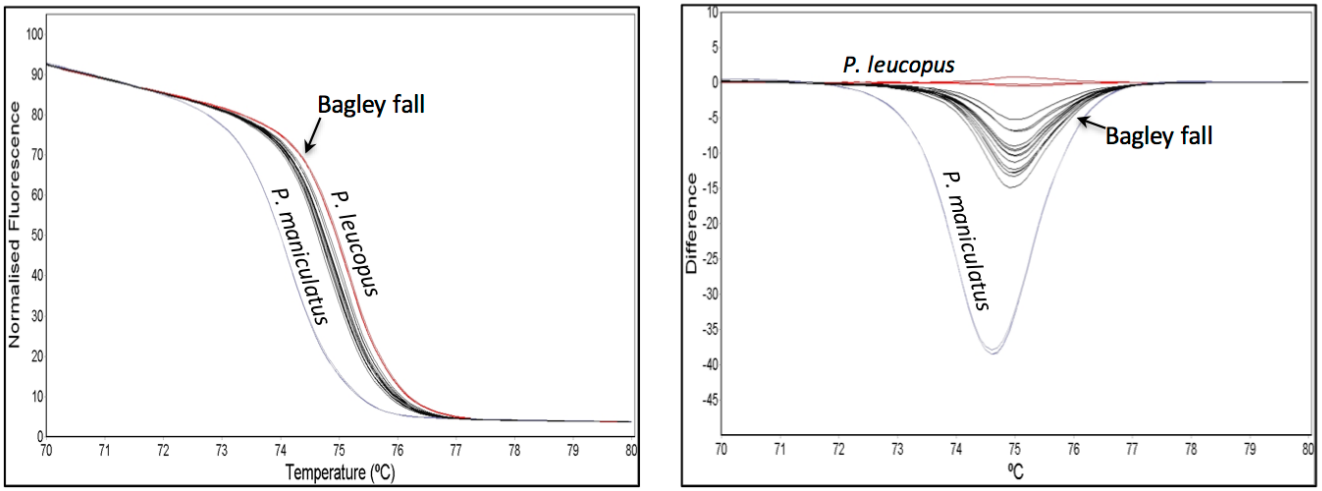

Figure 1.6: Normalized High Resolution Melt (HRM) curves of the samples from *Peromyscus* Genetic Stock Center (PGSC) are used as controls and then compared to samples wild-caught in Bagley Nature Area in the fall season of 2014. Normalized HRM (left) with control samples *P. leucopus* and *P. maniculatus*, along with samples of unknown *Peromyscus* species obtained from Bagley Nature Area in the fall (Bagley fall); difference curve (right) where stock center samples and fall Bagley Nature Area samples are compared against a selected PGSC *P. leucopus* sample. Arrows are used to designate the samples from Bagley Nature Area, and melt curves are shown in black. Note that the Normalized HRM curves of Bagley fall are with known *P. leucopus* samples from PGSC.

Chapter 2:

Community partnership designed to promote Lyme disease prevention and engagement in citizen science

Summary

This study outlines one project designed to promote disease prevention and to cultivate an interest in science through a citizen science project. This project was developed in cooperation with the University of Minnesota-Duluth and area high schools. Research aimed at monitoring Lyme disease in deer ticks in Northern Minnesota was brought to classrooms in rural high schools where students were introduced to the zoonotic aspects of transmission and to the health risks of Lyme disease and then invited to join in collecting field data on ticks. Authentic engagement in research is a noteworthy method for enhancing student understanding of Science, Technology, Engineering, and Math (STEM) topics. One of the primary goals of this lesson plan was to increase student interest and understanding of science through an authentic research experience with a topic that has implications on the health of the surrounding community. Learning about Lyme disease provided a platform that armed students with awareness of the health implications of the disease and methods of prevention against transmission of zoonotic diseases by the vectors, *Ixodes* ticks. To measure changes in student knowledge and attitude, students completed surveys before and after the Lesson on Lyme disease. Surveys were used to gauge how students felt about science and science careers as being important in their foreseeable future. One finding was that students predominantly agreed with the statement that science and careers in science are valuable to society, yet students answered other aspects of the survey to the effect that they did not see themselves fulfilling those roles. Other results from the surveys showed trends of an increased general interest in science after participating in the Lesson on Lyme disease.

Introduction

Lyme disease is a tick-borne infection caused by a spiral-shaped spirochete bacteria called *Borrelia burgdorferi*. Early signs of infection by the bacteria causing Lyme disease may include fever and flu-like symptoms, as well as possible erythema migrans, a characteristic bulls-eye rash around the tick bite. If detected early, Lyme disease is treated by antibiotics. However, if left untreated the bacteria may become systemic and may lead to more severe complications such as arthritis, Lyme carditis, and Lyme meningitis. The best way to prevent Lyme disease is to prevent being bitten by a tick.

As areas of Minnesota become increasingly prone to rodent and tick populations due to changes in climatic trends, people are at heightened risk for exposure to *B. burgdorferi*. Assessing the bacteria's ecological niche is dependent upon catching the animals it frequently resides in. One of those animals, the deer tick, is the primary vector. Of the types of ticks that exist in Minnesota, there are two that predominantly come in contact with humans: the deer tick (also known as the black-legged tick) *Ixodes scapularis,* and the wood tick, *Dermacentor variabilis*. Distinguishing between wood ticks and deer ticks can be difficult for the public; this is an important distinction, as only deer ticks are capable of transmitting *B. burgdorferi* bacteria to humans. To prevent tick-borne infection, it is also important to know to check for ticks frequently, where to check, and how to properly remove ticks.

Providing education about Lyme disease and *Ixodes* ticks through a citizen science outreach program would allow for extensive sample collection (Dickinson et al. 2012) of ticks, that would benefit research aims of understanding disease dynamics. It would also provide the public with a personal investment in research relevant to their own health and the health of their communities (Fig. 2.1). When people are involved in the scientific process, as they are in citizen science projects, there is a heightened appreciation for the value of science and scientific inquiry in society (Raelin 1997; Lombardi 2007; March et al. 2011).

Science and math are stumbling blocks for many individuals. In the U.S., student scores are significantly lower in these areas than in other countries (National Research Council 2013; Kelly 2013; U.S. Department of Education. 2013). However, these skills are in high demand considering the environmental, medical, and technological challenges that we face as a global community. An increasing need for scientific expertise require a greater number of undergraduates attaining degrees in and entering into STEM professions (National Science Board 2015). K-12 science education often emphasizes discrete facts rather than the problem-solving skills needed to address these challenges (National Research Council 2012). Yet studies have shown that students are more apt to have greater learning gains when the study material is personally relevant and involves authentic research (Bransford et al. 1999). Experimental aspects of research are not easily incorporated into secondary education models, so we propose a collaborative partnership between educational institutes of research and rural high schools in surrounding areas.

This study attempts to overcome barriers to understanding science and to engage students in higher order thinking by adopting multiple theories on education and learning that emphasizes modeling (Bandura 1977), structured knowledge (Bruner 1996), and authentic learning (Brown et al. 1989) through a citizen science project. Theories on learning suggest that engaging students in experiential and project-based learning activities will facilitate both learning and practice (OECD 2012; Nebeker 2014). Such methods are not a new practice in biology education, but authentic experimentation is not easily available in a high school science classroom. This is especially true in rural districts. This lesson proposes partnering with scientists on a valid research topic where work conducted in the classroom can be concluded in a research lab and disseminated back to the community.

Scientists are being encouraged and even expected to engage in community outreach, but particular segments of the population, especially in rural areas (<20,000 people) are much less likely to have interactions with scientists. Scientist visits to the classroom have the potential to dispel certain stereotypes students may have about scientists' careers that may limit their desire a pursue a STEM degree while also providing students with a first-hand account of scientific concepts, skills, and relevance (Laursen 2007; PCAST 2010; Fitzakerley et al. 2013).

Therefore, the goal of our study was to determine whether participation in the Lyme disease lesson and activity led to increased confidence in and ability to correctly identify deer ticks. We also measured if such activities have an impact on students' interest in science and pursuing a science degree in college.

Intended Audience

The activity was developed for use with high school students and was implemented in rural high schools, a tribal school, and a correctional facility. The activity could be adapted for application with younger students, undergraduates, or a public education program. The activity involves a one-hour interactive lecture followed by at least one hour of fieldwork. This lesson was implemented in an area of Minnesota known to have a high prevalence of Lyme disease. Even in areas that are not reported as high prevalence of Lyme disease, this activity could be modified to address other tick-borne illnesses in the U.S.

Prerequisite knowledge

Prerequisite knowledge was provided in a presentation at the onset of the lesson. Prior exposure to microbiology and ecology would be beneficial, but is not necessary.

Learning objectives

At the completion of this activity, students will be able to

1. Differentiate between a deer tick and wood tick.
2. Describe symptoms associated with Lyme disease.
3. Identify strategies to prevent Lyme disease.
4. Collect field data.
5. Develop hypotheses to solve a problem.
6. Consider pursuing a science degree.
7. Identify methods to use scientific data to educate community

Methods

Part 1. The Lecture

A power-point introductory presentation (Appendix 7) highlights aspects of Lyme disease transmission, bacterium and host ecologies, vector transmission, how to advocate prevention of tick attachment and disease, practices for capturing ticks, conducting a field survey, and recording data as well as general background on scientific method. Students are also trained on how to differentiate between blacklegged deer ticks, *Ixodes scapularis*, and dog ticks (or wood ticks), *Dermacentor variabilis*.

Instructors are encouraged to interact with students during the presentation by asking students if they know someone affected by Lyme disease, to give examples of symptoms of Lyme disease, why the research might be important, how they would go about hunting for ticks, and why we might want to capture information about terrain, time of day, season, etc. Students are required to reflect on the scientific method as well as develop their own hypotheses for capturing ticks and determining the risk of Lyme disease in their area. After witnessing the presentation on introductory knowledge on Lyme disease, students are invited to participate in fieldwork.

Part 2. Fieldwork

Students are divided into groups of three or four students and each student is assigned a job that contributes to the team effort of collecting ticks and field data. During the in-class presentations students have an opportunity to come up with ways of collecting ticks. Prior experimentation by Falco and Fish (1992) has shown that drag sampling is effective and at least one group should conduct sampling by this method. To make a drag

cloth, a square meter of cloth is attached at one end to a stick, and lead sinkers are secured to the other end to ensure the cloth drags on the ground. Securing string to the stick end makes it easier for students to drag the cloth without having to bend down as they walk along transects.

Students can conduct the survey in any grassy area around the school during regular school hours or a field trip to a wooded area may increase the number of ticks collected. One student is designated "the Dragger" throughout one field session. Students are instructed that this maintains consistency in height and speed of the collection method to limit experimental variability. The dragger walks along a 100 meter transect and drags the "drag cloth" on the ground, keeping the cloth low to the ground in the hopes of nabbing ticks from the grassy or wooded area. Students should check for ticks every 10 meters, along multiple 100-meter transects.

Another student can be assigned as "the Collector" who helps locate ticks on the cloth and picks them off. The Collector wears gloves and places the ticks in the vials or Eppendorf tubes with a tweezers and records the date, time, and place (a general location or GPS location) where each tick was found on the container. If more than one tick is found in an area of grouped transects, they may all be placed in one vial. The goal is to establish a general area where deer ticks are prevalent. Students should use the tick ID card to distinguish deer ticks from wood ticks. Wood ticks may be collected but are of less importance to the study. The Collector reports to "the Recorder" how many ticks are gathered at each stop, as well as what type of tick, what life stage, and the gender of each tick.

The Recorder records on the survey sheet the information gathered by the Dragger and the Collector and also notes information about the weather, temperature, and terrain including specifics on the flora, fauna and soil of their surroundings. Data sheets (Appendix 3) are included in a Tick Kit, which also contains tweezers, lab gloves, small Eppendorf tubes filled with isopropanol or ethanol hand sanitizer, markers, a GPS, and tick repellent such as diethyltoluamide (DEET) or Permethrin. Additional collecting can be done at the discretion of the instructor and per the interest of the student depending on the hypotheses generated during the in-class session. Students should wear long pants while collecting the ticks, apply a tick repellant, and check for ticks at the end of the activity. Participation was always completely voluntary.

Part 3. A Day of Science: Science Opportunities at the University.

A final component to this collaboration involves inviting classes to tour the lab facilities where the samples (ticks) are analyzed. Giving students an opportunity to tour the University facilities in the Biomedical Sciences department provides a basis of familiarity that may help students build confidence in considering science majors in post-secondary pursuits.

Students are invited to an Experience Science Day at the University of Minnesota-Duluth in exploration of various science education opportunities at the college level. This Experience Science Day includes a show at the Planetarium, a presentation and guided tour by the Geology Department Club, a lesson in human anatomy with plasticized limbs in the Medical School, a tour of the microscopy lab featuring scanning electron microscopes and confocal microscopes to view detailed

surface anatomy of the deer tick and fluorescent bacterial detection methods. The university visit is also an informal opportunity to meet and talk with graduate students, faculty and staff from all science departments and a chance to discuss experiences in pursuing professional degrees and careers.

Survey Assessment

A formal survey was given a day after the field experience to students from three different schools (Appendix 5). This survey evaluated student gains in knowledge in science appreciation or interest in post-secondary education. Students were also surveyed prior to participating in the lesson. Survey data was de-identified. Before and after survey data was matched up by student chosen code names combined with numbers from their student ID, or the first two letters of the students last name followed by a favorite food (example: NeTaco). Descriptive statistics were calculated in excel and significance between the before and after surveys was calculated with the statistical software JMP using a paired t-test. This study was deemed exempt from review by the University of Minnesota IRB 1309E42103.

Safety issues

Recruiting students to collect deer ticks does pose a risk that the students will be bitten by a tick and become infected with a tick-borne illness. Participation in the activity is voluntary and we recommend any minor's parents be notified in advance to students participating in the activity. Even if a student does not directly participate in the activity, they will benefit from hearing the interactive presentation and helping to generate hypotheses. If the activity takes place in an area around the school such as the high

school football field, students will often be exposed to this area outside of the fieldwork. The activity will help to educate students about how to safeguard against Lyme disease since students will be required to dress in long pants to prevent ticks from attaching to their skin, apply a tick repellant, and learn to do daily tick checks. Students learn proper tick removal technique by slowly pulling an attached tick using tweezers and never directly touching the ticks since gloves are worn. Individuals wearing Permethrin-treated socks and shoes resulted in 75% fewer tick bites than individuals wearing untreated items (Miller et al. 2011) and applying DEET to exposed skin minimizes the risk of infection.

Results

As part of a partnership between the University and local high schools, a master's student gave an interactive lecture on Lyme disease and worked directly with students to collect ticks for the master's student's research project designed to better characterize the upper Midwest region of risk for exposure to *B. burgdorferi*. Students first listened to a presentation about Lyme disease and then devised strategies to collect ticks. Students completed fieldwork in groups to collect ticks and recorded relevant field data (Fig. 2.2) such as ecological terrain, weather, time of day, tick species collected, and GPS coordinates.

One hundred and seventy ticks from approximately 40 locations have been submitted as part of the project. From these ticks, researchers have been able to culture a novel Minnesota strain of *B. burgdorferi* to use in their research.

Survey Findings

Over 2,000 students and approximately 50 community members at nine different educational facilities and two public forums were presented with "A Lesson on Lyme disease" and participated in the tick collecting. Of these, two hundred nineteen students from three different high schools completed a survey after participating in the activity. The general open response feedback from participants was extremely positive. Students enjoyed learning about ticks and wanted to know more about Lyme disease and ticks after the presentation. Students felt the activity was valuable because "…I felt that I was helping people accomplish something …" It also changed some of the students' attitudes toward the ticks. "I had a lot of fun… Looking for ticks and being able to see them without being scared." In addition, the students liked being part of the scientific process and being engaged in hands on learning. Even if students did not collect any deer ticks during the activity, they were excited to come up with possible strategies to increase tick yield in the future. These ideas included changing the dragging technique, altering the drag cloth shape or material, asking local residents where ticks have been spotted and where to drag, or identifying a chemical that would attract ticks (Table 2.2).

We used the survey to first establish how comfortable the students were with our learning objectives before the presentation and the activity (Table 2.1). We found that students from three different rural high schools had low confidence in their ability to differentiate between a deer tick and wood tick, had low awareness of how to prevent Lyme disease and its associated symptoms, and were interested in going to college and graduate school but not in a STEM field. We resurveyed a subset of these students to

report their confidence on the learning objectives after the activity (Fig. 2.3). Specifically, students reported increased awareness of the symptoms associated Lyme disease, of how to prevent Lyme disease, and of how to correctly identify a deer tick with p-values less than 0.0001 in a paired t-test. We also found that after participating in the activity students had an increased desire to pursue science in college and in graduate school with significance below 0.05 p-value cut-off.

It is possible that students could report being able to identify ticks but not be good at actually performing the task. During the presentation, students were provided a small vial of wood ticks and a certain number of deer ticks suspended in hand sanitizer and were asked to identify how many deer ticks were contained within the vial. On the post-activity survey, students were given pictures of a deer tick and a wood tick and asked to circle the deer tick. Eighty-three percent of the students that answered the question on the survey correctly circled the deer tick.

Student attitudes toward science contribute to retention and enrollment in science courses. After participating in the activity, students completed a modified Attitudes Toward Science Inventory (Gogolin and Swartz 1992) to assess student feelings toward science. The items students rated most highly indicated that students feel science is important for a country's development and for understanding the natural world. However, the data also show that students were less likely to agree that "knowing science is important in order to get a good job" and "I like the challenge of science assignments." Overall, student participation in authentic science research as citizen scientists met the objectives of increasing awareness about Lyme disease, allowing students opportunities

to engage in hypothesis generation, and encouraged students to consider pursuing science degrees.

Discussion

Authentic engagement in research is a noteworthy method for enhancing student understanding of Science, Technology, Engineering, and Math (STEM) topics. This study outlines one project designed to promote disease prevention and to cultivate an interest in science through a citizen science project developed in cooperation with the University of Minnesota-Duluth and area high schools. Research aimed at monitoring Lyme disease in deer ticks in Northern Minnesota is brought to classrooms in rural high schools where students are introduced to the zoonotic aspects and the health risks of Lyme disease and invited to join in collecting field data on ticks. The primary goal of this lesson plan is to increase student interest and understanding of science through an authentic research experience, with a topic that has implications on the health of the surrounding community. Learning about Lyme disease also provides a platform to arm students with awareness of the illnesses implications and methods of prevention against the vectors of the zoonotic disease, *Ixodes* ticks. To measure changes in students' knowledge and attitudes, students completed surveys before and after the lesson. Surveys were used to gauge how students felt about science and science careers as being important in their foreseeable future. One finding was that students predominantly agreed with the statement that science and careers in science are valuable to society, even when students answered other aspects of the survey to the effect that they did not see

themselves fulfilling those roles. Future STEM lessons and outreach may place greater emphasis on science as a career opportunity, while assessing why students feel employment within a science field is less feasible. Other results from the surveys showed trends of an increased interest in science after participating in the lesson on Lyme disease, generally. This outreach program attempted to inspire more STEM-capable students and encourage a STEM competent society, and exposure to lessons on science by practitioners of science may make this more achievable.

In this project, a graduate student research project investigating rates of Lyme disease in populations of *Ixodes* ticks in Northern Minnesota was brought to the classrooms of rural high schools. The high school students were introduced to the zoonosis and the health risks of Lyme disease and invited to join in collecting field data on ticks, thus delivering a lesson through scientific outreach that has an impact on the health of those communities receiving the outreach.

The results of our surveys suggest student participation in authentic science research positively impacts student interest and understanding of science. It is important to incorporate science lessons that involve student participation. When students observe science in action the process becomes easier to understand, easier to retain and student self-efficacy in the practice of science may increase (Lombardi 2007). This is important, especially in STEM education because many teaching approaches still utilize rote memorization of facts lends to a deficit in American student comprehension of STEM concepts. Students reported on surveys that the "hands on experiences are the most interesting." They also indicated a lack of information on the routes to professional

science careers and what those careers entail: "*How do you become a scientist …I know you go to college but what about after"*. These reflections reinforce the idea that students are not exposed to contextualized applications of science.

Students seemed more enthused about science when exposed to authentic scientific research. A few students asked how to continue participating in the research outside of the classroom. Teachers also appreciated the visits to the classroom by a student scientist and extended invitations to return to their classrooms and present to multiple classes. Teachers went on to adapt this lesson into their ongoing curriculum and community members were also excited to submit ticks to use in this research.

In developing the power point presentation, there were questions and comments that frequently came up, and the lesson was constantly evolving based on the feedback provided by students. Engaging students in developing their own proposals about why Lyme disease affects individuals in certain areas as opposed to others, about how to collect ticks, and about how to use information about climate, time of day, season, ecological factors are key to students practicing the scientific process. Students' confidence was enhanced in their ability to act as scientists with a sense of ownership in the direction of the research. Students demonstrated an understanding of scientific processes and creating a hypothesis and volunteered their own suggestions. Fieldwork is not always successful in collection of specimens, but this lends a lesson in the importance of persistence and the need to constantly self-evaluate methods to optimize experimental outcomes.

Technology, as with the ease of access to and the social interconnectedness of the Internet, allows for outreach on a broad scale. Students and community members could learn more about the Lyme disease research occurring at the University of Minnesota-Duluth through a website implemented by Samantha Toivenon, <http://d.umn.edu/lyme/>. This technological aspect to outreach was funded in part by an Undergraduate Research Opportunity Program award. The power point lecture and classroom activity for the Lesson on Lyme disease is available for download by teachers. Important resources are organized and also made available through this website, making credible resources available to the public that reinforces safe practices when doing activities that may expose their person to ticks and put them at risk for tick-borne disease. Information is available on how to safely remove ticks, and where to send ticks if people opted to send them in to the Biomedical laboratory at University of Minnesota-Duluth for further research.

Future Directions

Assessing the long-term impact of a science exercise on a student's eventual career choice is important for developing effective curriculum. Long-term surveys could follow a student who was exposed to citizen science research into their college career, and could be made available online as a valuable measure of overall success with this approach. Follow up is important to establish if health information related to Lyme disease is retained and if the intervention did alter behaviors by encouraging students to engage in tick checks. Maintaining a relationship with teachers and schools through science outreach by providing socially applicable health-related research topics will

produce more information on how to enhance student confidence and interest in science while reinforcing positive trends seen already.

To further facilitate familiarity with post-secondary STEM opportunities, some of the students attended a Day of Science and visited where the graduate research took place, witnessing how the samples and data would be used for authentic scientific research. Having a comparative study that surveyed groups of students involved in witnessing where samples end up and the methods used in the lab to examine those samples, versus students who do not, would add values of importance as to the inclusion of students visiting research labs and understanding the purpose of the samples.

Table 2.1: Student confidence with the learning objectives before participating in the activity (1 = strongly disagree; 5 = strongly agree)

	School 1 n=23	**School 2** n=75	**School 3** n=177
Correctly identify a deer tick from a wood tick	2.96±1.55	2.60±1.11	3.24±1.36
Aware of symptoms associated with Lyme disease	3.13+1.29	2.16±0.97	2.69±1.06
Aware of how to prevent Lyme disease	3.00±1.24	2.04±1.02	2.33±1.04
Interested in participating in scientific research	4.02±0.86	3.22±1.12	2.79±1.09
Interested in going to college	4.65±0.77	4.62+0.72	4.15±1.11
Interested in pursuing science in college	3.39±1.20	3.03+1.28	2.56+1.30
Interested in going to graduate school	4.5±0.84	4.00+1.24	3.52±1.35
Interested in pursuing science in graduate school	3.02±1.22	2.91±1.20	2.33±1.26

Table 2.2: Student generated hypotheses to increase tick yield.

1. *"I think that the ticks would be easier to collect if we didn't potentially knock them off the grass, so maybe putting a drag in front of ourselves???"*
2. *"I thought that maybe the sheets should be longer. Not wider, but longer. This is because when you first drag the cloth you may disturb the tick, but it might not latch on right away. By dragging a longer cloth, you might have a better chance of getting a tick."*
3. *"...coveralls rather than a drag."*
4. *"Maybe ask locals where they have had a lot of ticks. Thus helping your chances of getting deer ticks. Maybe do collecting in tall grass fields."*
5. *"Pursue the idea of the waders / some kind of attraction on your legs as you're walking because no matter what you'd get ticks on your legs, so why not be able to catch them easier."*
6. *"Having a complete outfit* [made] *of the fiber they stick to."*

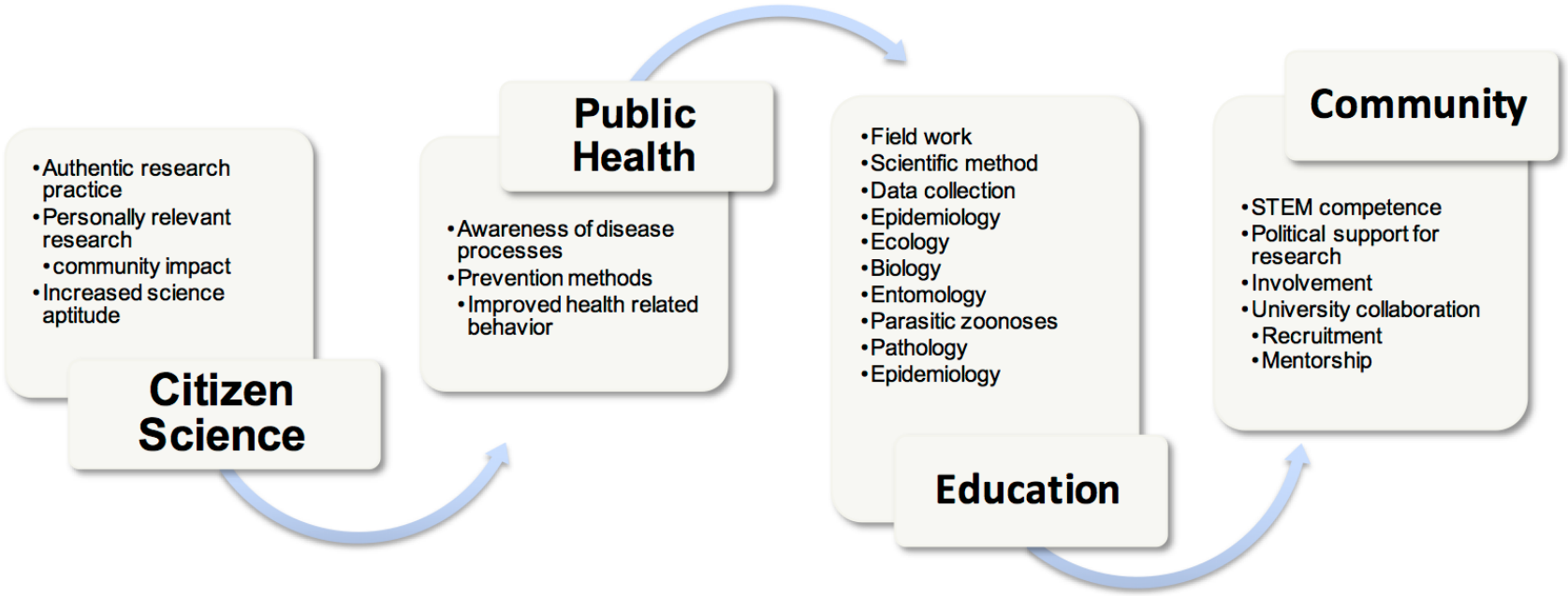

Figure 2.1: Students are educated through a multi-tiered approach, becoming involved in a scientific problem that affects the health and well being of themselves and their communities, and addressing the issue through participation in authentic scientific research. Ultimately, science becomes more accessible as and important tool in the lives of those students, as they grow in their roles as active community members and citizens of the United States.

A)

B)

Figure 2.2: Examples of students conducting the field work in woods near their high school. Drag cloth on the ground (A) and in use (B).

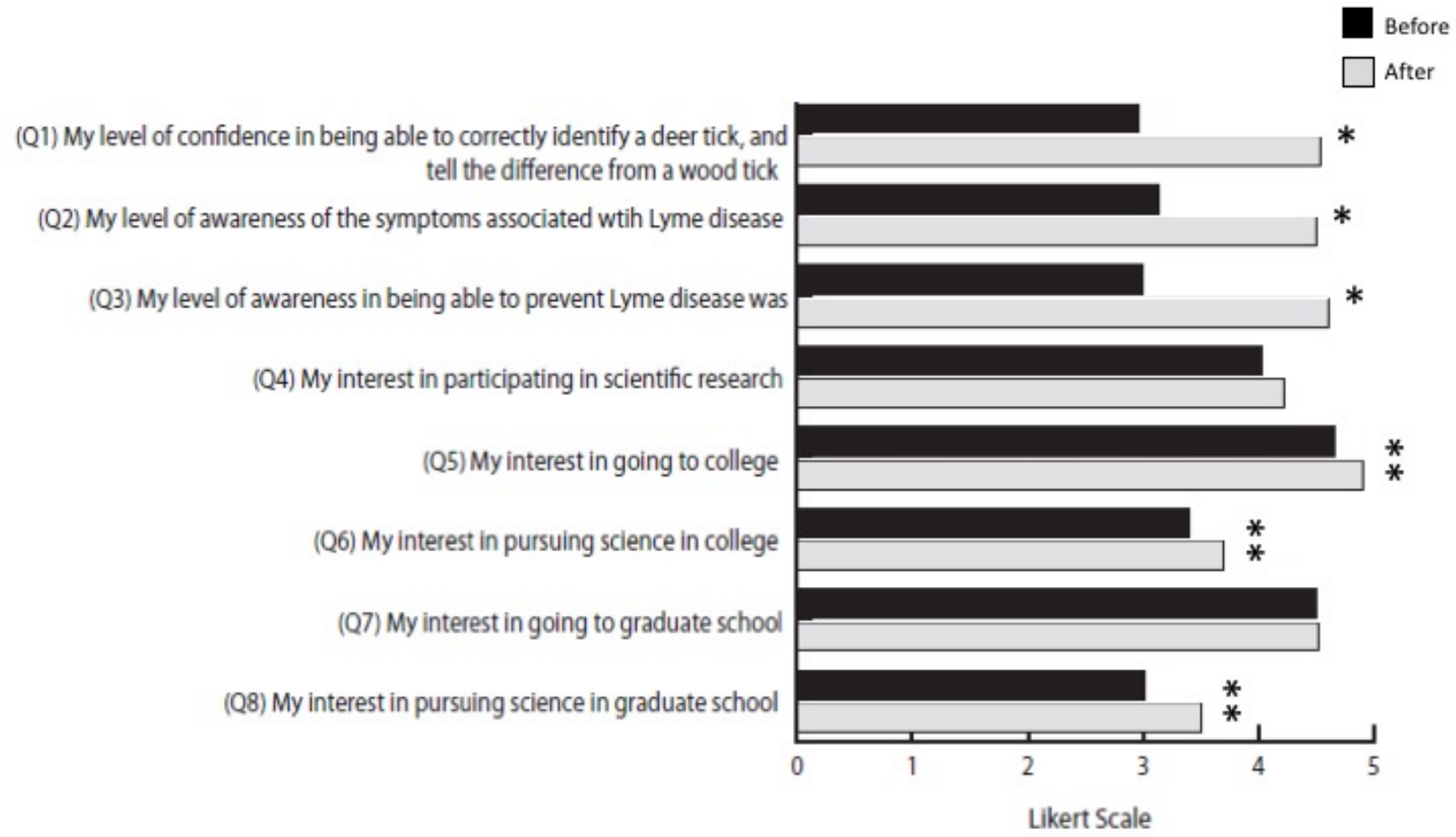

Figure 2.3. Student reported ability to achieve the learning objectives before and after participation in the activity. Students were asked to rate their agreement with each of the following statements (1 = strongly disagree, 5 = strongly agree). Questions 1, 2, 3 were statistically significant (*p-value <0.0001) as were questions 5, 6, 8 (**p-value <0.05).

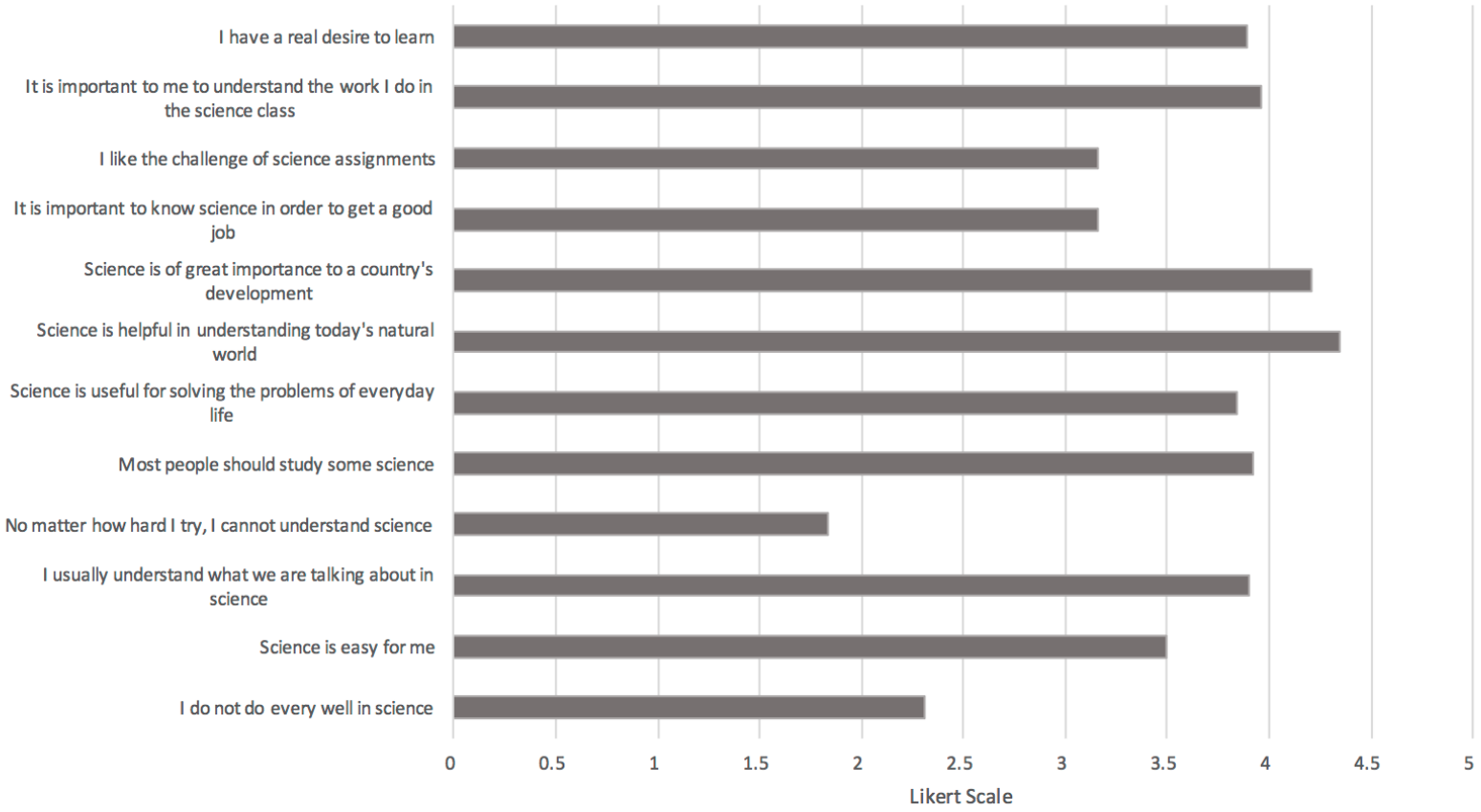

Figure 2.4: Science attitudes were collected from students after participation in the Lesson on Lyme disease activity using the Modified Attitudes Towards Science Inventory. (1 = strongly disagree, 5 = strongly agree).

Conclusion

There were two hypotheses tested in this thesis: *Peromyscus* species could be distinguished by a genomic assay, ultimately contributing to the study of Lyme disease by adding to the understanding of the reservoir host for the etiological agent of Lyme; and *Ixodes* tick yield would be increased through outreach to communities in the form of a citizen science project, while also extending public awareness on prevention of Lyme disease through tick management.

Peromyscus leucopus, a reservoir host for Lyme disease, is migrating further north in the North American continent (Roy-Dufresne et al. 2013; Simon et al. 2014). Ranges for *Ixodes* ticks, the vector for Lyme disease, are expanding as well (Robinson et al. 2014). Researching host and vector ecology is important to understand trends and risks associated with the spread and infection rate Lyme disease in humans, and to inform the public on emerging diseases. Sampling deer ticks using only localized or individual efforts greatly hinder attaining an adequate sample size for producing significant results. Our *Ixodes* studies provided outreach to communities that both provided a public health message about Lyme disease while also working with volunteers to achieve a high sample yield of *Ixodes*. Continuation of this outreach has been extended to forest workers, in part through the development of the website. Making the outreach educational material easily accessible through online resources is an important model as Lyme disease affects new geographic regions for the first time, as both *Ixodes scapularis* and *Peromyscus leucopus* broaden their ranges.

In this report we demonstrate a direct and reliable assay to discriminate between two very similar species of *Peromyscus* using primers that target the 16S mitochondrial rRNA isolated from serum samples from live-caught mice with qRT-PCR and HRM technology. A useful addition to this assay is to include a direct test for the Lyme disease pathogen. We are currently examining a method to simultaneously monitor exRNA from the pathogen *B. burgdorferi* with our HRM assay for *Peromyscus* species to identify active infection. The existence of exRNA from the pathogen is plausible based on our understanding of exRNA derived from pathogens in other mammals and in ticks (Yang et al. 2015; Ornstein and Barbour 2006). In future studies we expect that adapting the method described here to identify the mouse species and their pathogen load will be possible from one 30 μL serum sample.

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Appendix 1.

Primers

Primer	Target	Sequence
miR 21 forward	microRNA 21	ACC AGA CAG AAG GAC CAG AGT TTC TGA TTA
Universal reverse	microRNA 21	*Proprietary c/o Qiagen mat. no. 1046471
P. leu forward	*Peromyscus leucopus* 16s mitochondrial rRNA	TTT CAT AGG AGC TAT AGA GAT CAG TAC CG
P. leu reverse	*Peromyscus leucopus* 16s mitochondrial rRNA	GGT ACA AGG TTT AAT CTT TGC TTA TTT GT

Appendix 2.

Classroom Activity

Title: Lesson on Lyme disease: a citizen science project provides field research experience to secondary students.

Objectives:

- Gain hands on fieldwork and data collection experience while contributing to a current ecological assessment of disease.
- Collaborate with scientists.
- Appreciate the many controlled aspects of conducting a field survey and developing a hypothesis based on scientific method.
- Provide education on deer ticks and Lyme disease as well as tools for preventing infection.

Materials:

The Tick Kit -

- Tick drag cloth
- Tick data sheet
- Tick ID cards
- tweezers
- lab gloves
- vials
- labels
- markers
- GPS
- Tick repellent

Student Prior Knowledge:

Student prior knowledge about Lyme disease and deer ticks varied school to school. Students from the Duluth and Twin Cities areas had scant awareness of ticks and the Lyme disease process, and how to prevent it. Meanwhile, rural areas such as Esko and Eveleth had significantly greater understanding of ticks and disease threats associated.

Introduction: (15 min.)

Local scale: Introduce an issue of local health concern. The first question posed to the students is whether or not they know of anyone who has found a deer tick on them, or had Lyme disease.

Global scale: Epidemiology of Lyme disease is a National concern as the East coast and North Midwest states are teeming with deer ticks and a risk for Lyme disease, while Southern states like Texas and Pacific coast states such as California are presenting with confirmed cases of Lyme. The world over, there are approximately 13 species of Borrelia resulting in Lyme Borreliosis. As spirochetes are evolutionarily highly adaptable to host environments, and as climatic warming trends enhance habitats for reservoir hosts and vectors, anticipated incidence for zoonotic disease is increasing.

Students are provided with a power point introductory presentation that highlights aspects of Lyme disease transmission, bacterium and host ecologies, vector transmission, how to advocate prevention of tick attachment and disease, and practices for capturing ticks, conducting a field survey and recording data. Students are also trained on how to differentiate between deer ticks, *Ixodes scapularis*, and dog ticks (or wood ticks), *Dermacentor variabilis*. This is an important distinction, as only deer ticks are capable of transmitting *Borrelia* bacteria to humans.

*See power point (Appendix 8).

Lesson/Activities: (30 min – 3 hours)

Students are assigned to groups of three. Each group is equipped with all the contents of one tick kit, and each student has a specific job that contributes to the team in the effort of collecting ticks.

The Dragger: the same person remains the dragger throughout one field session. This maintains consistency in height and speed of the collection method, in keeping with scientific convention. The dragger walks along a 100 m transect and drags the drag cloth on the ground, keeping the cloth low to the ground in the hopes of nabbing ticks from the grassy or wooded area. Students should check for ticks every 10 meters along each 100 meter transect.

The Collector: helps locate ticks on the cloth and pick them off. The collector carries the vials that the ticks will be stored in, along with markers and labels to record the date, time and place (a general location or GPS location on vials) where each tick was found. If more than one tick is found in an area of grouped transects, they may all be placed in one vial. The goal is to establish a general area where deer ticks are prevalent. Wood ticks may be collected as well, but are of less significance to the study. The collector reports how many ticks are gathered at each stop, and what type, what life stage and what gender each tick is to the Recorder.

The Recorder: collects information from the Dragger and the Collector while also capturing information on a formal data sheet throughout the field survey. Students should write in detail about the weather, about the terrain where the survey is conducted, including specifics on the flora, fauna and soil of their surroundings in order to attempt to establish trends in tick heightened activity by place or time of day or type of weather.

Students can conduct this survey in any grassy area around the school or their neighborhoods during or after regular school hours.

A field trip can be and has been established around this activity resulting in more fruitful conclusions and is recommended.

Conclusion/Assessment: (15 min.)

A formal survey in evaluation of student knowledge gain and any enhancement in science appreciation or interest in post secondary education is given a day after the field experience. To most effectively assess any gain in knowledge, a pre-survey should be given. Also, a control group can be established with a survey for non-participants that is to be given to students in science classes at the same institution who do not participate in the field work and were not exposed to the presentation on deer ticks and Lyme disease.

*See surveys (Appendix 5, 6).

Day of Science Outreach
In working with area high schools, in exchange for students conducting field research, I created a field trip for them to visit University of Minnesota-Duluth Medical school, casually referred to as Experience Science Day at UMD. The visit consists of introductions to aspects of science at the University.

A sample field trip:

1 hr	Planetarium lesson =	Star gazing and black hole documentary. Led by Planetarium Director
30 min, 1 hr	Anatomy lesson =	Complete with plastonics. Led by Faculty/Staff of Medical School
30 min, 1 hr	Geology tour =	Holographic topography maps, and handling of rocks throughout ages. Led by Geology Student Group

30 min, Microscopy = Electron microscopy of deer ticks! Led by Geology Faculty/Staff

30 min, 1hr Lunch panel = Provide a lunch and invite Faculty and Staff from the experience day, as well as Graduate Students, Undergraduate Students, Professors, Mentors, and Technicians in various science fields and posts to visit with students in a casual fashion to discuss first hand experiences and opportunities in the sciences.

MINNESOTA ACADEMIC STANDARDS THIS LESSON MEETS:

9.1.1.1.2
Understand that scientists conduct investigations for a variety of reasons, including: to discover new aspects of the natural world, to explain observed phenomena, to test the conclusions of prior investigations, or to test the predictions of current theories.

9.1.1.1.4
Explain how societal and scientific ethics impact research practices.

9.1.1.1.6
Describe how changes in scientific knowledge generally occur in incremental steps that include and build on earlier knowledge.

9.1.1.1.7
Explain how scientific and technological innovations —as well as new evidence— can challenge portions of, or entire accepted theories and models including, but not limited to: cell theory, atomic theory, theory of evolution, plate tectonic theory, germ theory of disease, and the big bang theory.

9.1.1.2.1
Formulate a testable hypothesis, design and conduct an experiment to test the hypothesis, analyze the data, consider alternative explanations and draw conclusions supported by evidence from the investigation.

9.1.1.2.2
Evaluate the explanations proposed by others by examining and comparing evidence, identifying faulty reasoning, pointing out statements that go beyond the scientifically acceptable evidence, and suggesting alternative scientific explanations.

9.1.1.2.3
Identify the critical assumptions and logic used in a line of reasoning to judge the validity of a claim.

9.1.2.1.1
Understand that engineering designs and products are often continually checked and critiqued for alternatives, risks, costs and benefits, so that subsequent designs are refined and improved.

9.1.2.1.2
Recognize that risk analysis is used to determine the potential positive and negative consequences of using a new technology or design, including the evaluation of causes and effects of failures.

9.1.3.1.3
Describe how positive and/or negative feedback occur in systems.

9.1.3.2.1
Provide examples of how diverse cultures, including natives from all of the Americas, have contributed scientific and mathematical ideas and technological inventions.

9.1.3.2.2
Analyze possible careers in science and engineering in terms of education requirements, working practices and rewards.

9.1.3.4.2
Determine and use appropriate safety procedures, tools, computers and measurement instruments in science and engineering contexts.

9.1.3.4.4
Relate the reliability of data to consistency of results, identify sources of error, and suggest ways to improve data collection and analysis.

9.3.4.1.1
Analyze the benefits, costs, risks and tradeoffs associated with natural hazards, including the selection of land use and engineering mitigation.

9.3.4.1.2
Explain how human activity and natural processes are altering the hydrosphere, biosphere, lithosphere and atmosphere, including pollution, topography and climate.

9.4.1.1.1
Explain how cell processes are influenced by internal and external factors, such as pH and temperature, and how cells and organisms respond to changes in their environment to maintain homeostasis.

9.4.1.2.3
Describe how viruses, prokaryotic cells and eukaryotic cells differ in relative size, complexity and general structure.

9.4.4.1.1
Describe the social, economic and ecological risks and benefits of biotechnology in agriculture and medicine.

9.4.4.1.2
Describe the social, economic and ecological risks and benefits of changing a natural ecosystem as a result of human activity.

9.4.4.2.1
Describe how some diseases can sometimes be predicted by genetic testing and how this affects parental and community decisions.

9.4.4.2.2
Explain how the body produces antibodies to fight disease and how vaccines assist this process.

9.4.4.2.3
Describe how the immune system sometimes attacks some of the body’s own cells and how some allergic reactions are caused by the body's immune responses to usually harmless environmental substances.

9.4.4.2.4
Explain how environmental factors and personal decisions, such as water quality, air quality and smoking affect personal and community health.

Appendix 3.

Data Sheet: *Ixodes Scapularis* (Deer Tick) Collection

Name:	**Program:**	**Date:**
		Time of Day:

GPS	Latitude	Longitude

Place	Nearest City:	State:

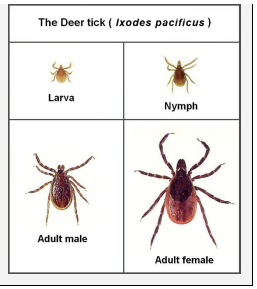
Weather Description:

Temp:	Humidity:

Soil Type	Sandy ☐ Sandy Loam ☐ Loam/Clay ☐ Clay ☐ Soil pH: Litter depth (mm):
	Soil Temp: Soil moisture:
Topology	

Describe Plants/Trees

Vial	**Time**	**Deer Ticks**		**Wood Ticks**	
#	**Of Collection**	**Nymphs**	**Adult**	**Nymph**	**Adult**
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					



Appendix 4.

GENERAL SCIENCE ATTITUDE INQUIRY
Please read the statements below and indicate the degree to which you agree with the statement as reflecting your attitude towards science.

			Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
1.	I do not do very well in science		**SA**	**A**	**N**	**D**	**SD**
2.	Science is easy for me		**SA**	**A**	**N**	**D**	**SD**
3.	I usually understand what we are talking about in science class		**SA**	**A**	**N**	**D**	**SD**
4.	No matter how hard I try, I cannot understand science		**SA**	**A**	**N**	**D**	**SD**
5.	Most people should study some science		**SA**	**A**	**N**	**D**	**SD**
6.	Science is useful for solving problems of everyday life		**SA**	**A**	**N**	**D**	**SD**
7.	Science is helpful in understanding our world		**SA**	**A**	**N**	**D**	**SD**
8.	Science is of great importance to a country's success and continued development		**SA**	**A**	**N**	**D**	**SD**
9.	It is important to know science in order to get a good job		**SA**	**A**	**N**	**D**	**SD**
10.	I like the challnege of science assignments		**SA**	**A**	**N**	**D**	**SD**
11.	I have a real desire to learn		**SA**	**A**	**N**	**D**	**SD**
12.	It is important to me to understand the work I do in science class		**SA**	**A**	**N**	**D**	**SD**

Appendix 5.

Lesson on Lyme Disease: Before / After Survey

Check your current understanding of Lyme disease:

My level of confidence in being able to correctly identify a deer tick is

Not so good ← 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 →Great!

My confidence in being able to tell the difference between a deer tick and a wood tick is:

Not so good ← 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 →Great!

My awareness of the symptoms associated with Lyme disease is

Not so good ← 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 →Great!

My awareness of the ability to prevent Lyme disease is

Not so good ← 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 →Great!

My interest in participating in scientific research is

Not so good ← 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 →Great!

My interest in going to college is

Not so good ← 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 →Great!

My interest in pursuing science in college is

Not so good ← 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 →Great!

My interest in going to graduate school is

Not so good ← 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 →Great!

My interest in pursuing science in graduate school is

Not so good ← 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 →Great!

Please draw a picture of a deer tick and a wood tick next to each other and briefly point out differences between the two.

Please make a list of any and all symptoms you can think of that are associated with someone getting Lyme disease:

Appendix 6.

Parental Consent Form

DATE:

Dear Parent or Guardian,

I am conducting a research study entitled "A Lesson on Lyme Disease" with high school students at _____ High School.

Students are invited to participate in authentic field research in your area. Students who would like to take part in this opportunity will be asked to evaluate the effectiveness of this experience through surveys. The purpose of providing this opportunity is to promote enthusiasm for science, while increasing knowledge and understanding of scientific processes. With the permission of your student's principal and science teacher, we are requesting that you allow your child to participate.

Students of _____'s classroom will learn about Lyme disease with a power-point lecture on the topic given by a researcher on the topic. This lesson includes prevention techniques to ward against tick-borne illnesses and guides to identifying ticks that carry disease, and how to recognize symptoms of Lyme disease.

Students will additionally have an opportunity to conduct scientific field research. With the supervision of their teacher, and guidance of a visiting scientist, students would hunt for ticks, while learning about data collection and developing a scientific method. To gauge the success of this experience-based learning opportunity, students will be asked to fill out surveys and brief questionnaires, both before and after the lesson plan is executed. Neither names, nor personal information will be used to fill out any forms associated with this study, and all responses will be kept anonymous.

Participation in the field research aspect of this opportunity and any of the surveys are entirely voluntary, and students will not be penalized should they decide not to participate. Parental/guardian consent does *not* mean a student must participate in any aspect of this opportunity. Participants are free to stop taking part in the study at any time.

The opportunity will extend over multiple days, as determined by their science teacher. Students who opt to participate in the lesson and the fieldwork will do so during school hours, during the current semester. This lesson meets multiple state academic standards within your student's curriculum.

Please give your permission by signing the enclosed consent form and having your child return it to their science teacher tomorrow. Please keep this letter for your records. Should you have any questions about the study please contact my office 1.xxx.xxx.xxxx.

Sincerely,

Veronica A. Nelson
Masters Student, Integrated Biological Sciences
University of Minnesota-Duluth

University of Minnesota's Institutional Review Board (IRB) has approved this study.

Consent to Participate

I have read the attached informed consent letter and agree to allow my child to participate in the Lesson on Lyme disease citizen science project and the surveys associated.

Student's Name

Parent's or Guardian's Name (please print)

Parent's or Guardian's Signature Date

Appendix 7.

Lesson on Lyme disease power point lecture.

Catching Ticks

HOW TO TRACK LYME DISEASE

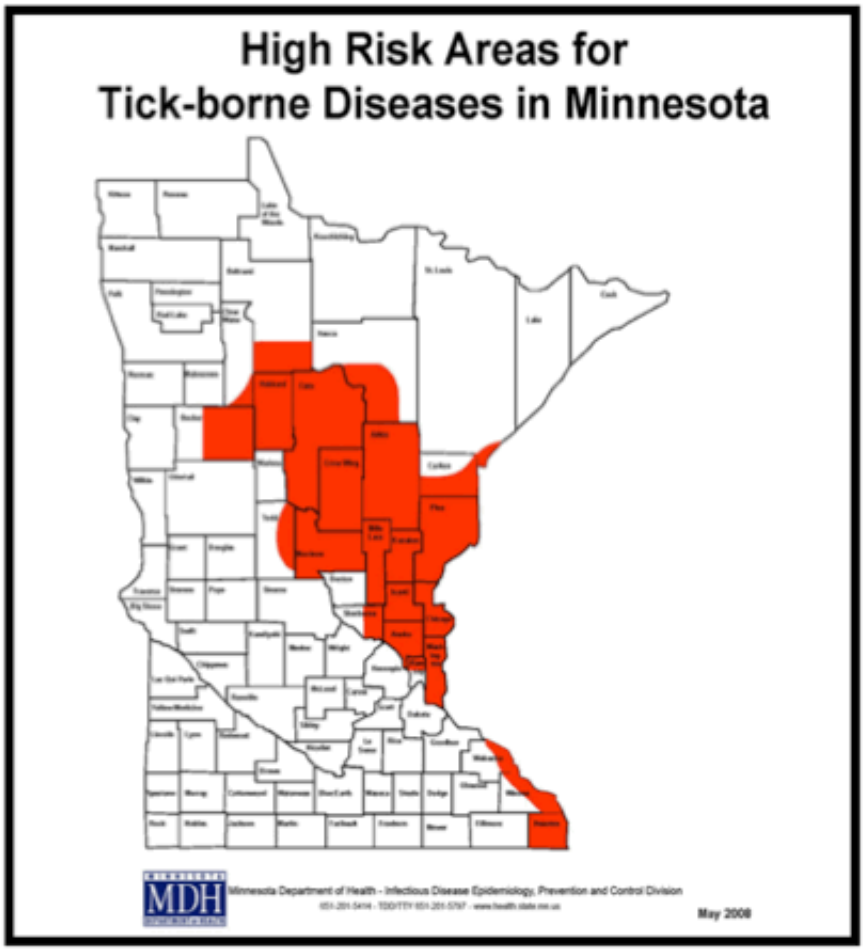
Outline

- Lyme disease Risk Factors
- Causes of Lyme disease
- Ecological overview of Lyme disease
  - Mice
  - Ticks
- Field experiment and data collection
- Symptoms of Lyme
- Prevention methods

Tick borne disease risks in Minnesota is highest in forested areas within the shaded zones.

Blacklegged ticks may also be found at lower levels in some forested areas outside this zone.

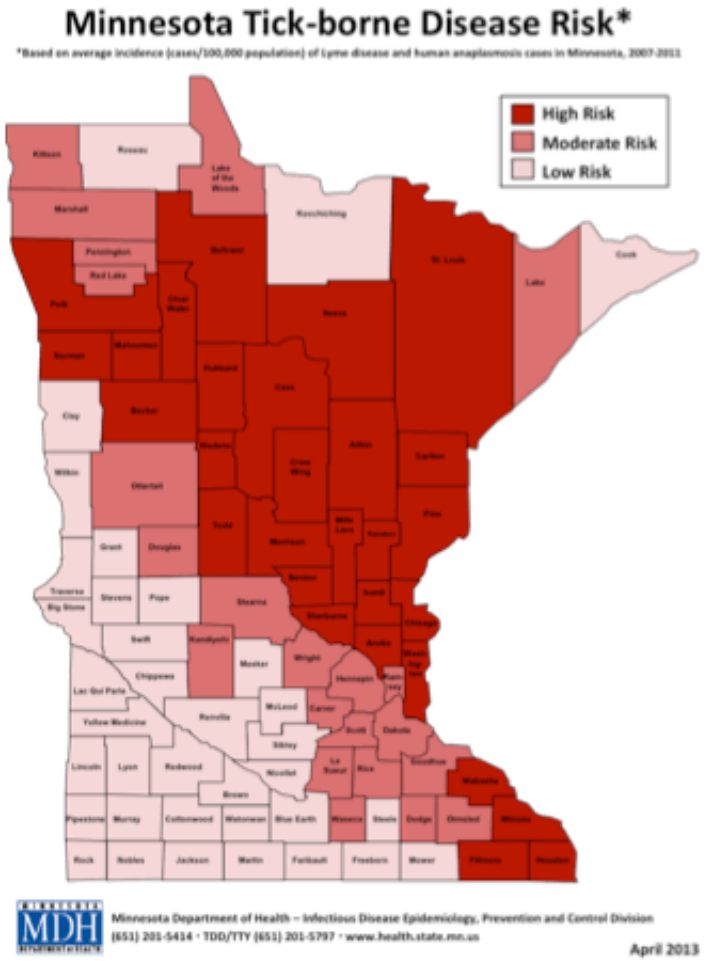
MDH. May 2008.



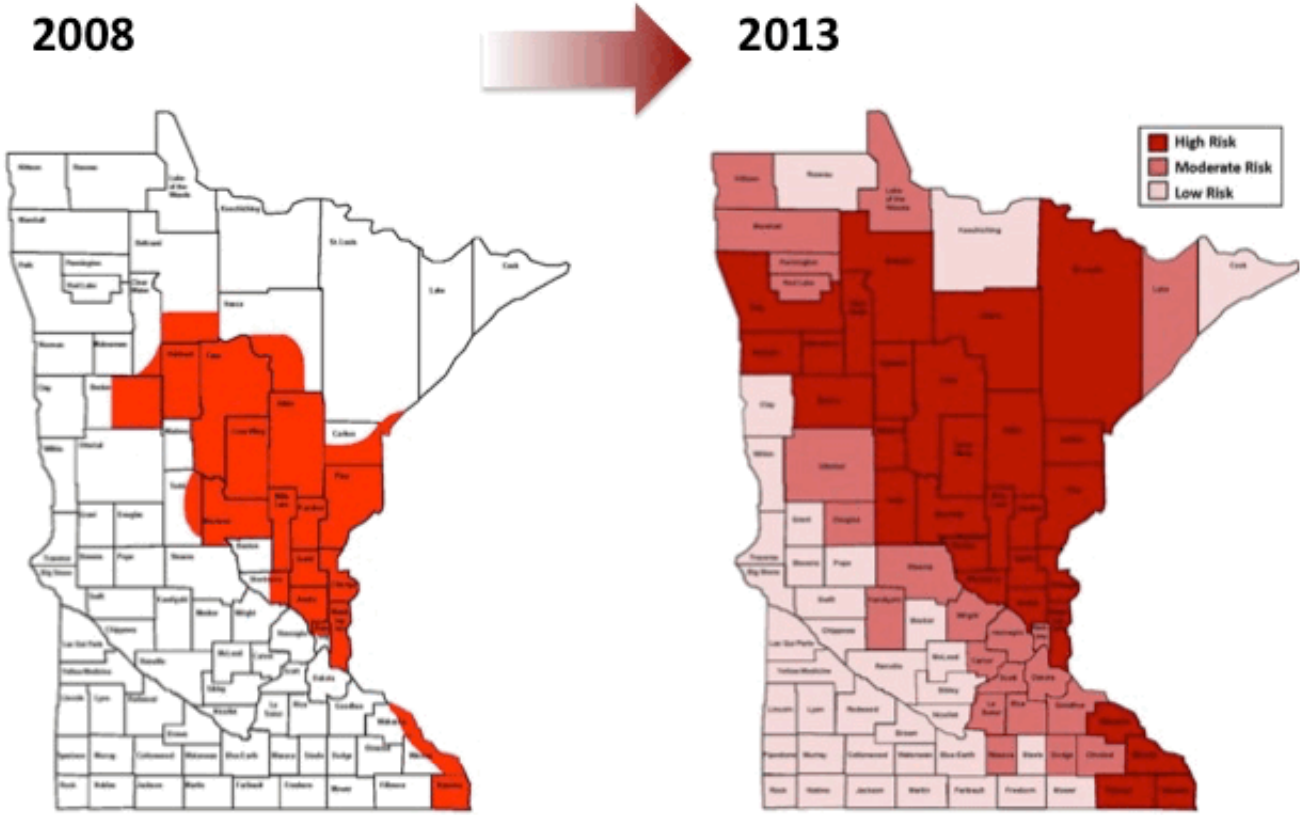
Tick-borne disease (TBD) risk is confined to forested areas throughout the state.

Take precautions to prevent TBD when visiting these areas.

MDH. April 2013.



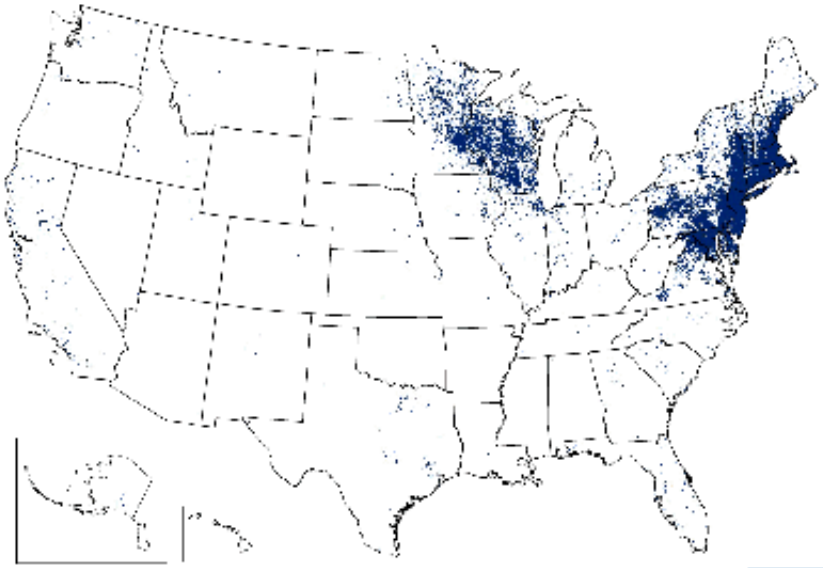
Minnesota Tick-Borne Disease Risk



MINNESOTA MDH DEPARTMENT OF HEALTH

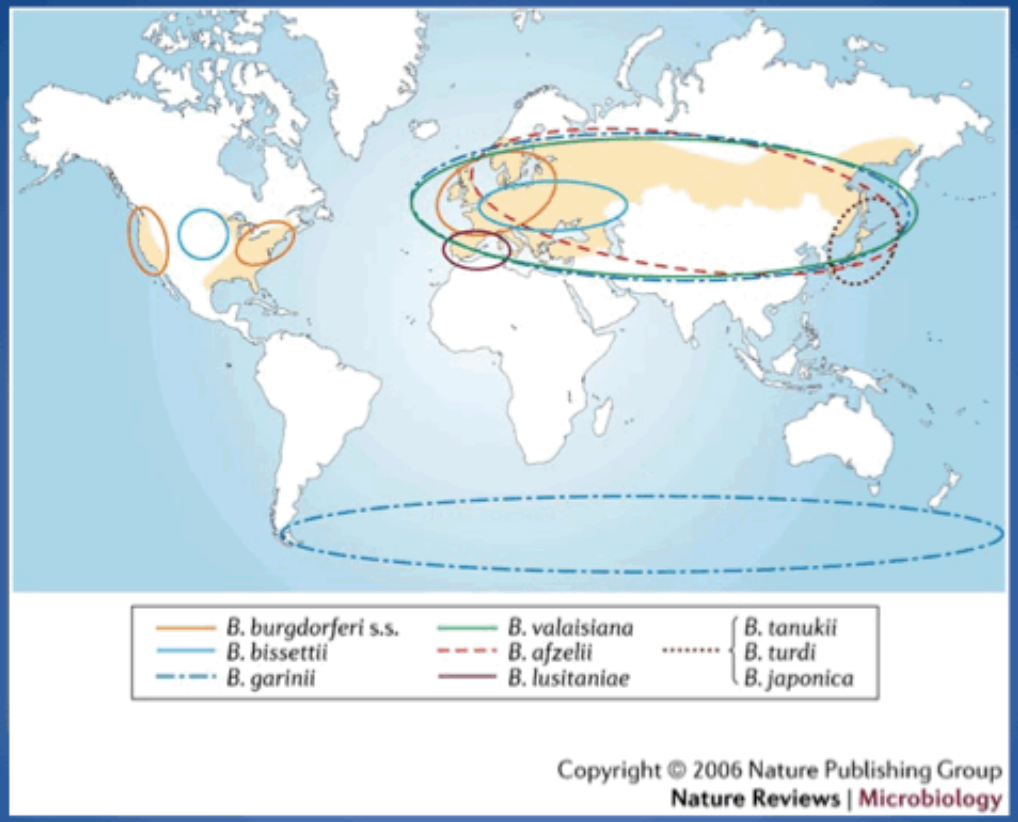
Reported Cases of Lyme Disease—United States, 2010

One dot is placed randomly within the county of residence for each confirmed case. Though Lyme disease cases have been reported in nearly every state, cases are reported based on the county of residence, not necessarily the county of infection.



National Center for Emerging and Zoonotic Infectious Diseases
Division of Vector Borne Diseases | Bacterial Diseases Branch

CDC

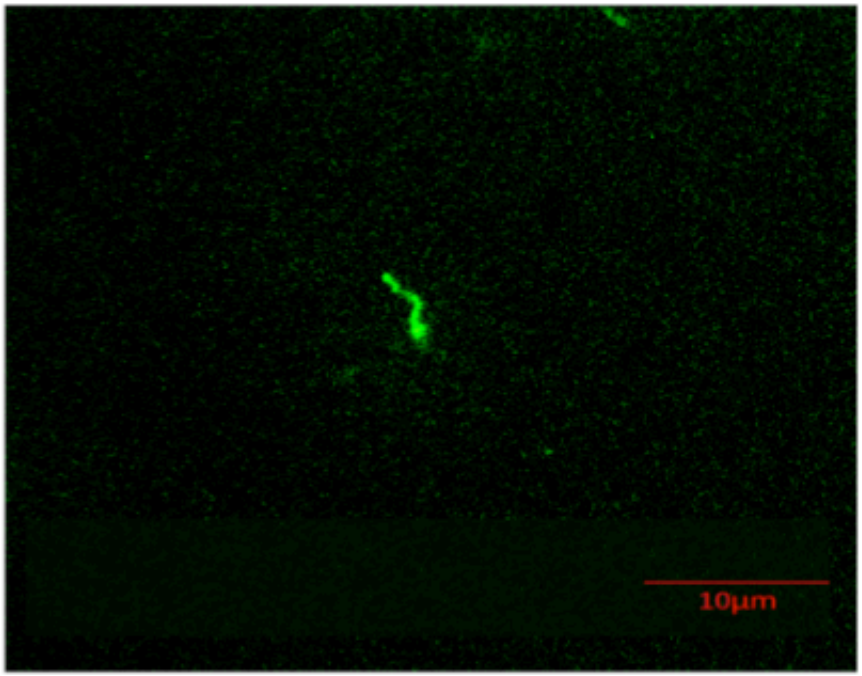


Worldview

Kurtenbach *et al. Nature Reviews Microbiology* advance online publication;
published online 07 August 2006 | doi:10.1038/nrmicro1475

Lyme Disease

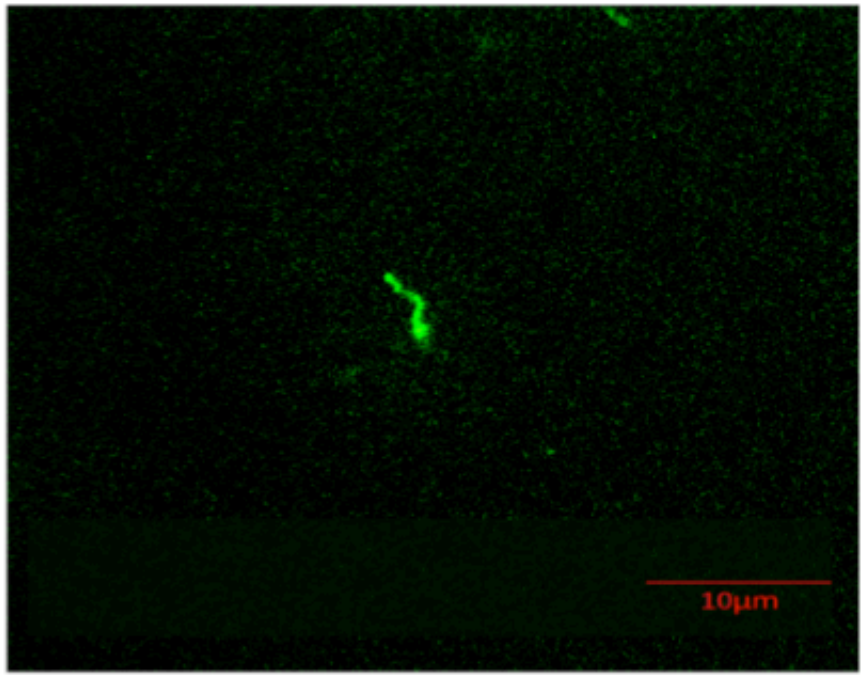
- *Borrelia burgdorferi*
  - Spirochete bacteria



Picture by Veronica A. Nelson and Samantha Toivenen.

Lyme Disease

- *Borrelia burgdorferi*
  - **Spirochete** bacteria



Picture by Veronica A. Nelson and Samantha Toivenen.

Bacterial Shapes:

- There are three main bacterial shapes:

- Sphere-shaped

- Rod-shaped

- Spiral-shaped
  - -

- Sphere-shaped
  - Coccus

- Rod-shaped
  - Bacillus

- Spiral-shaped
  - Spirochete*

SINGULAR

- Sphere-shaped
  - Coccus
  - Cocci

- Rod-shaped
  - Bacillus
  - Bacilli

- Spiral-shaped
  - Spirochete*
  - Spirochetes

PLURAL

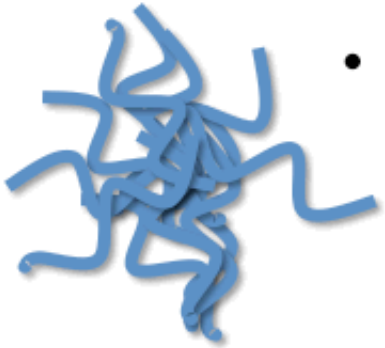
- Sphere-shaped
  - Cocci

- Rod-shaped
  - Bacilli

IN CHAINS

- Sphere-shaped: in clusters
  - Cocci

- Rod-shaped: in clusters
  - Bacilli



- Spiral-shaped: in clusters
  - Spirochetes

IN CLUSTERS

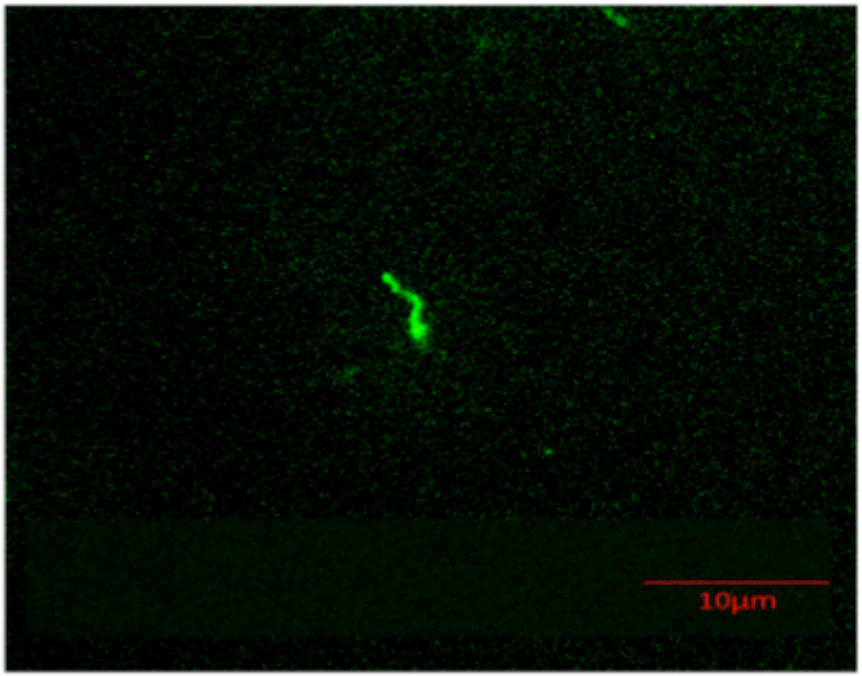
- Coccobacilus

- Vibrio

…and everything in between

Lyme Disease

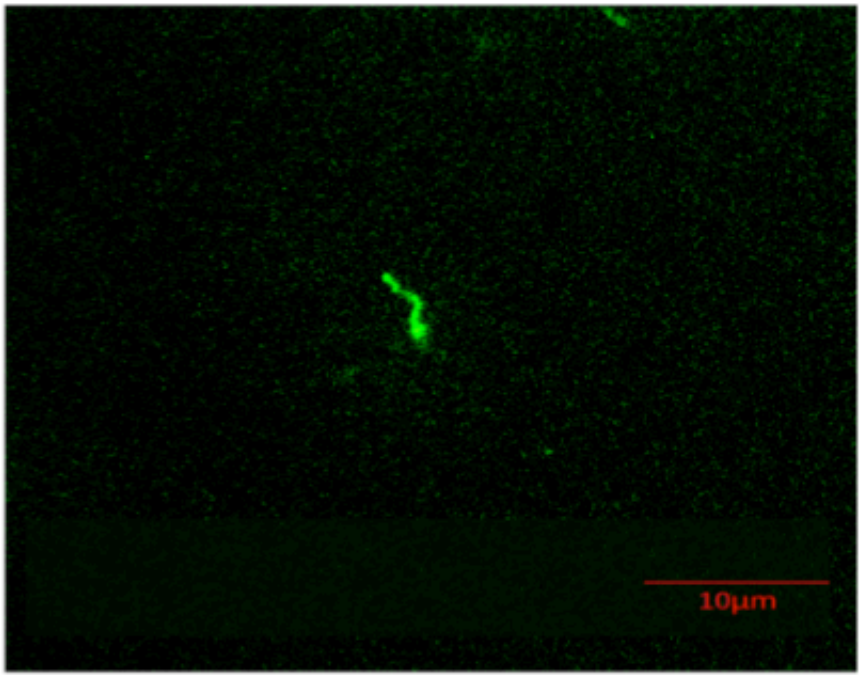
- *Borrelia burgdorferi*
  - Spirochete bacteria



Picture by Veronica A. Nelson and Samantha Toivenen.

Lyme Disease

- *Borrelia burgdorferi*
  - Spirochete bacteria
  - Zoonotic



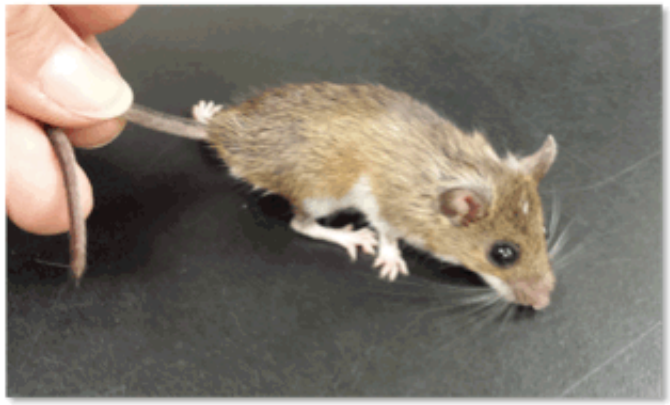
Picture by Veronica A. Nelson and Samantha Toivenen.

Zoonotics:
The Ecology of Disease

- Zoonoses depend on animal hosts
  - "Zoo" = *animal*
  - small and large vertebrates
- Ecology = study of [environmental] interactions and relationships.

Reservoir host

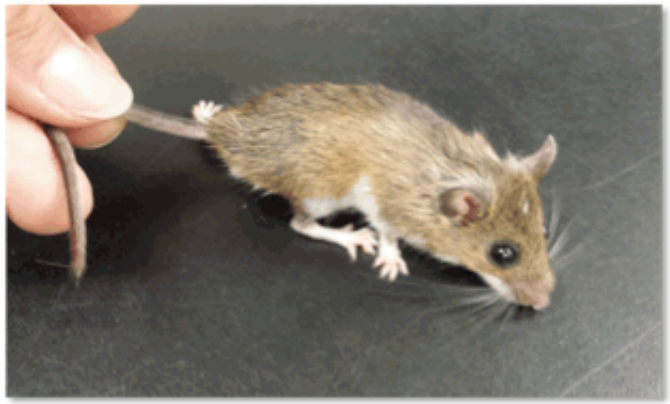
P. leucopus



White-footed mouse

Reservoir hosts

P. leucopus

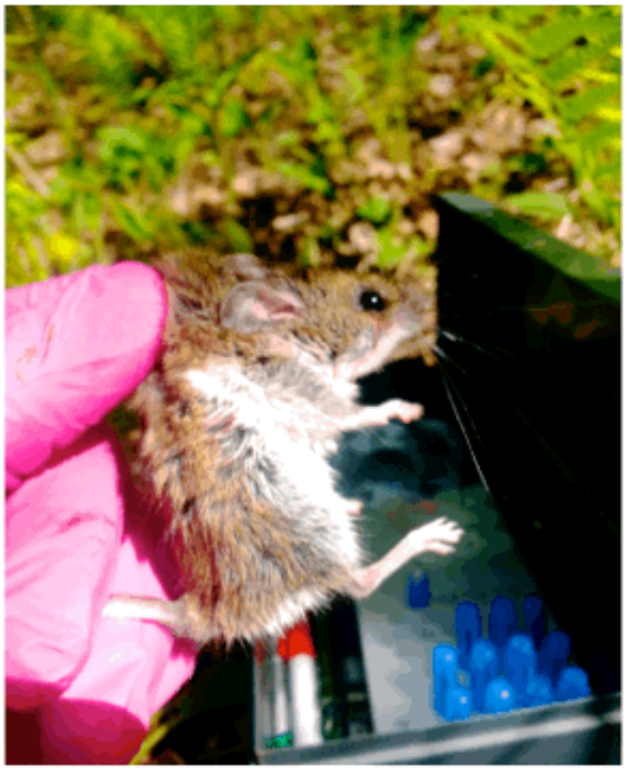


White-footed mouse

P. maniculatus

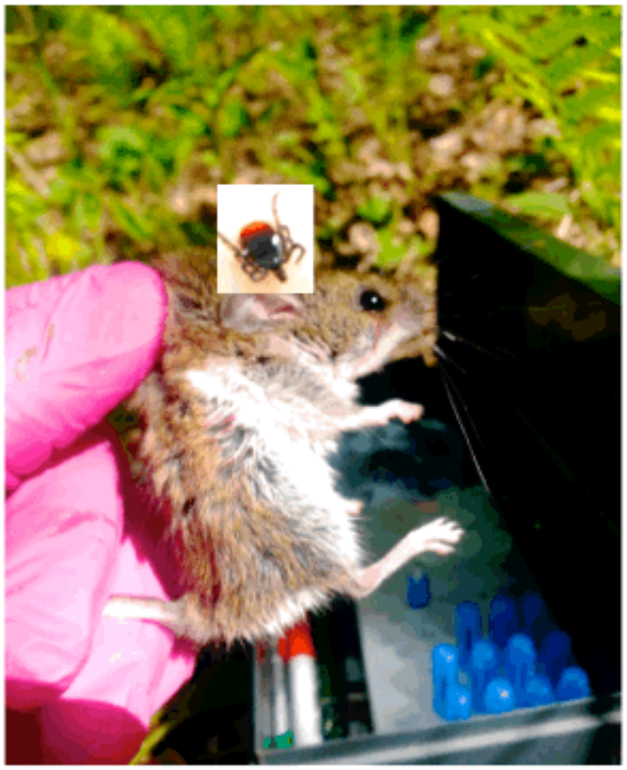
Deer mouse


the zoonotic triangle
tick. mouse. bacteria.



- The spirochete occurs in nature, living in small mammals.

Ticks get infected

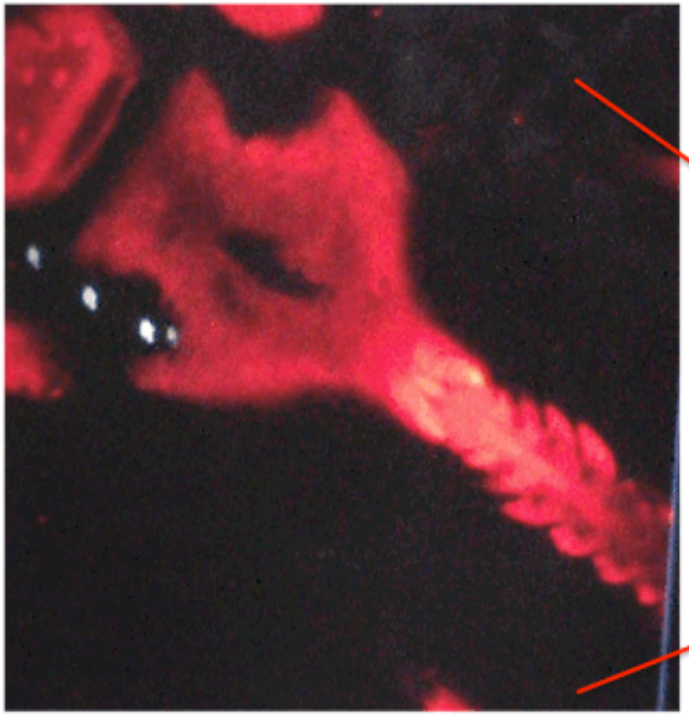


- The spirochete occurs in nature, living in small mammals.
- Deer ticks take blood from these small mammals and contract the bacterium.

…and infect us!

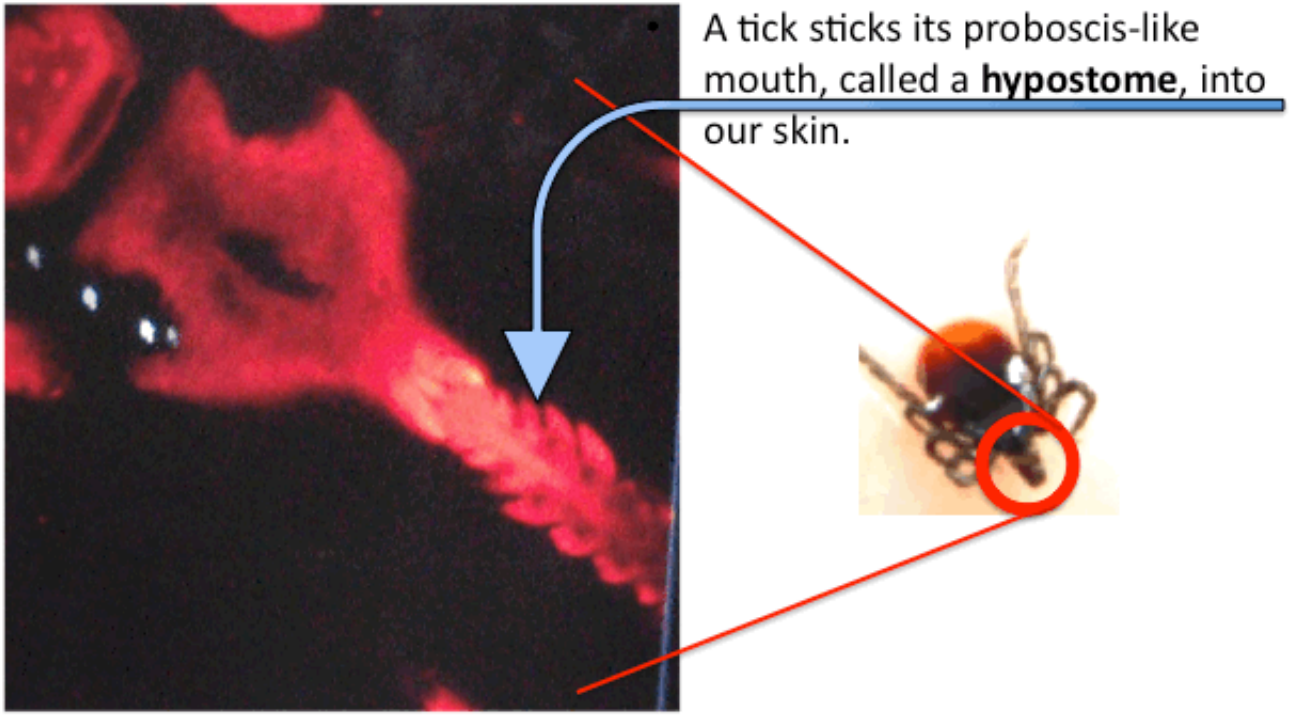
- A tick sticks its proboscis-like mouth, called a hypostome, into our skin.

…and infect us!

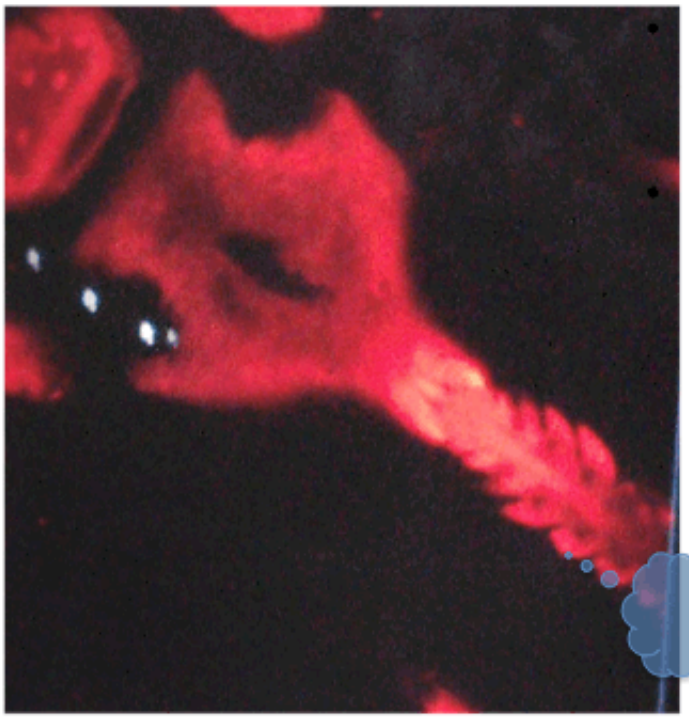


- A tick sticks its proboscis-like mouth, called a hypostome, into

…and infect us!

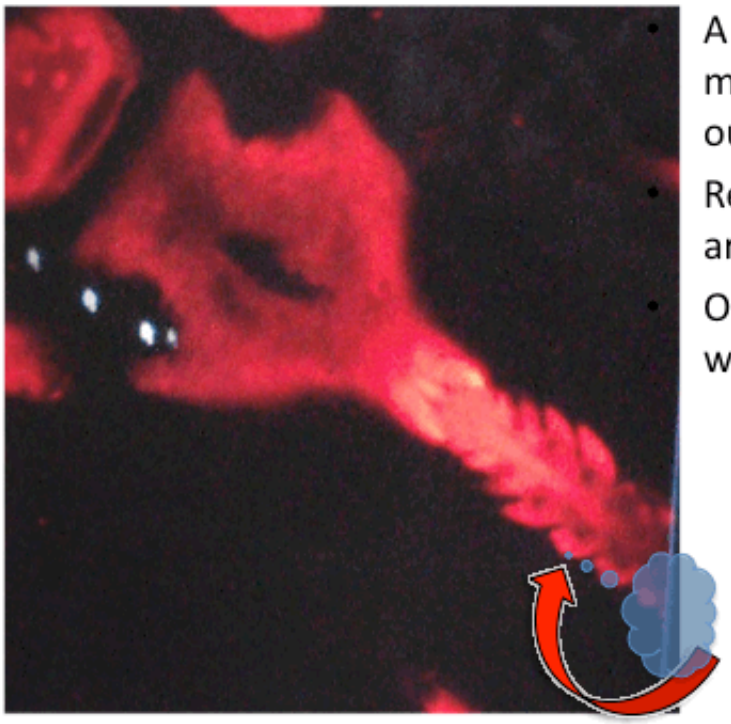


…and infect us!



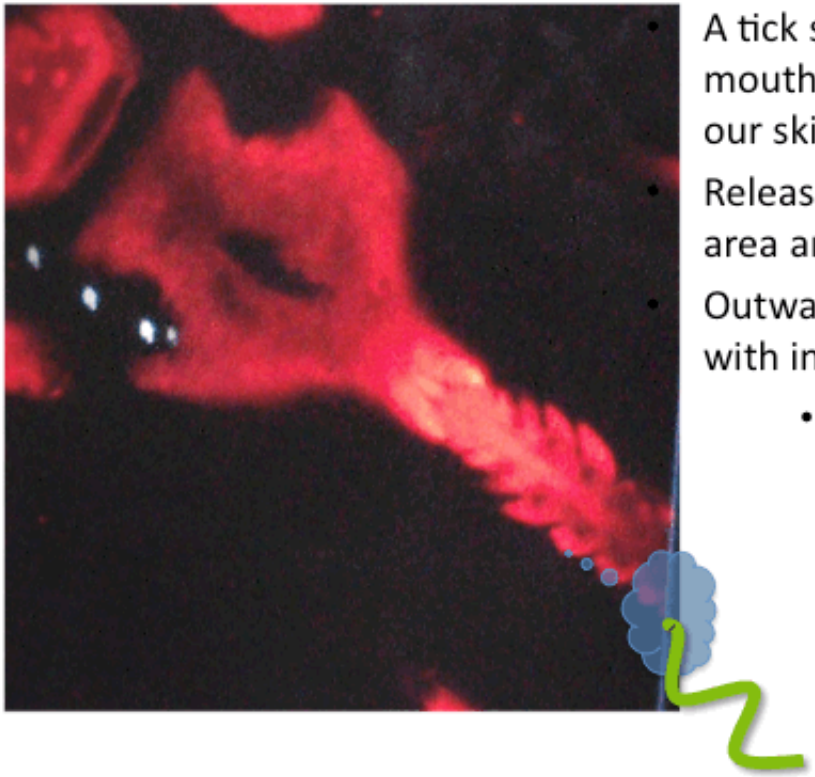
- A tick sticks its proboscis-like mouth, called a **hypostome**, into our skin.
- Releases saliva that anesthetizes the area and thins the blood.

…and infect us!



- A tick sticks its proboscis-like mouth, called a **hypostome**, into our skin.
- Releases saliva that anesthetizes the area and thins the blood.
- Outward-flowing saliva alternates with inward-moving blood:
  - it sucks your blood
  - while spitting into you

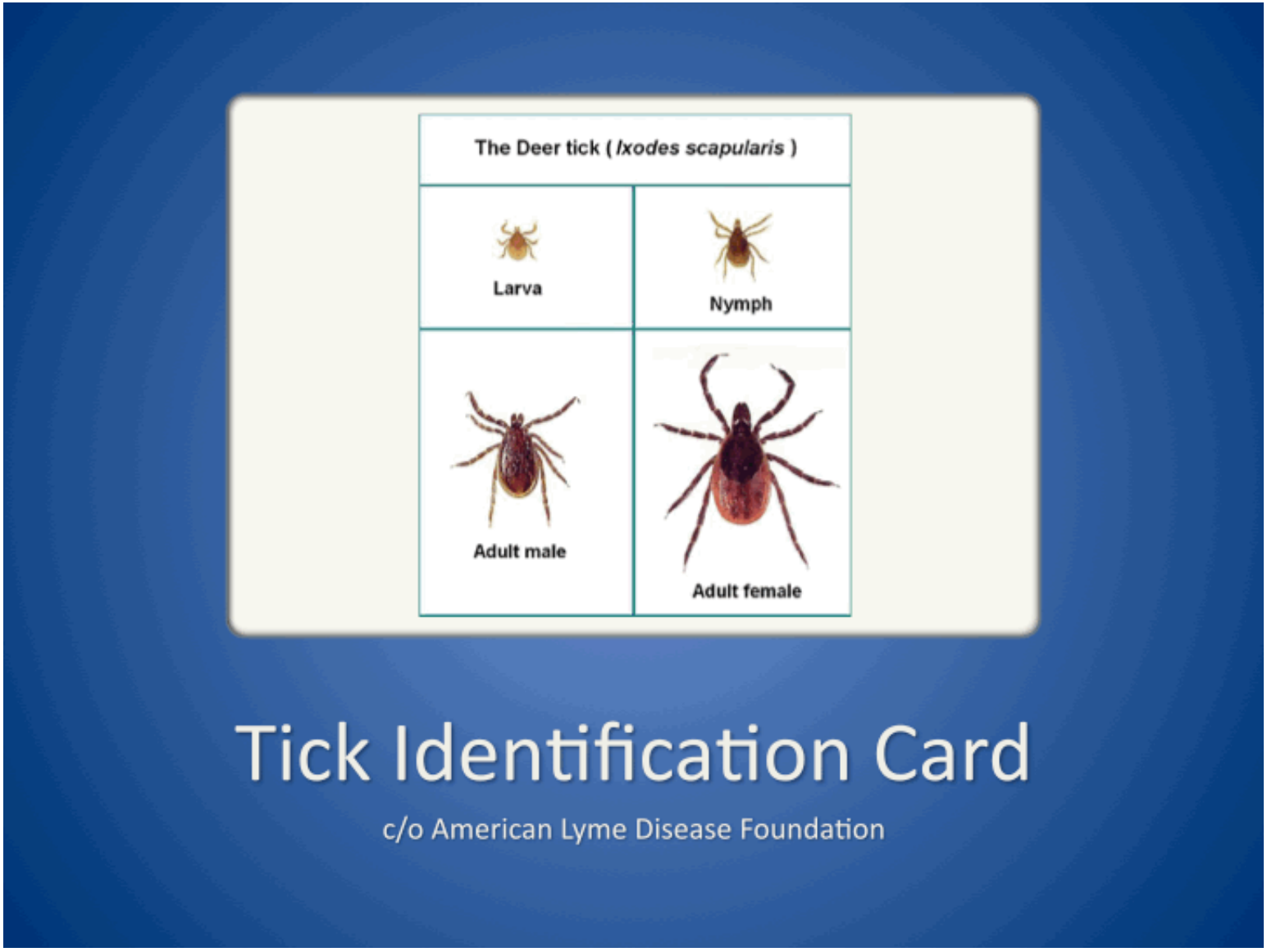
…and infect us!



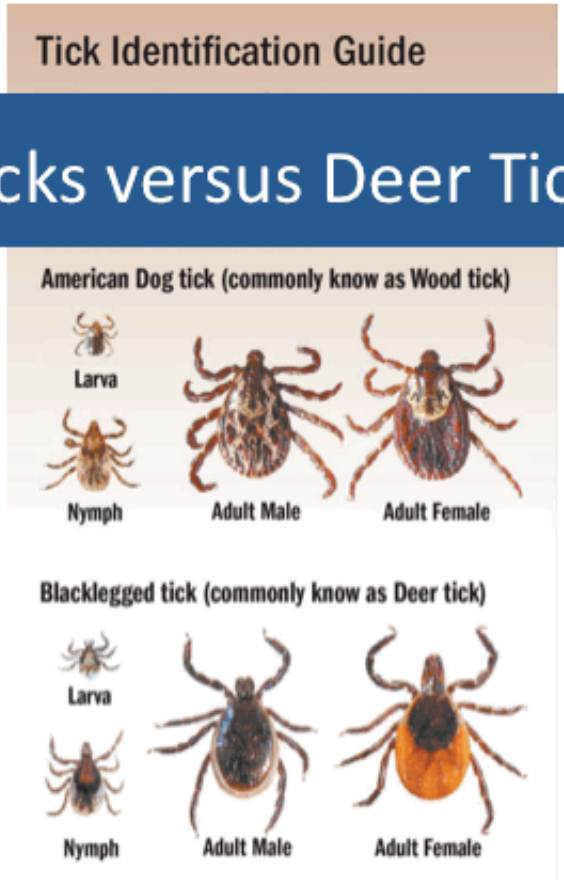
- A tick sticks its proboscis-like mouth, called a **hypostome**, into our skin.
- Releases saliva that anesthetizes the area and thins the blood
- Outward-flowing saliva alternates with inward-moving blood
  - Infection enters the body system with saliva

About Deer Ticks:


About Deer Ticks:
They are really small


The Deer tick (Ixodes scapularis)
Larva
Nymph
Adult male
Adult female
Tick Identification Card
c/o American Lyme Disease Foundation

Wood Ticks versus Deer Ticks



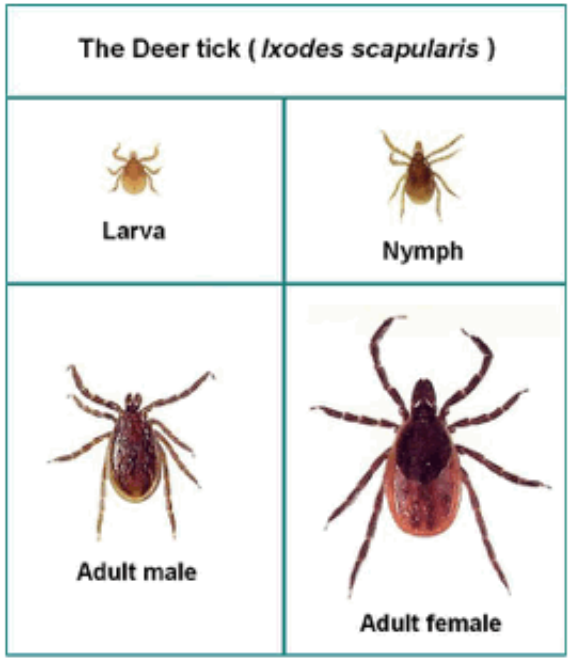
c/o University of Rhode Island

Deer Tick Season

Field seasons:

SPRING

FALL



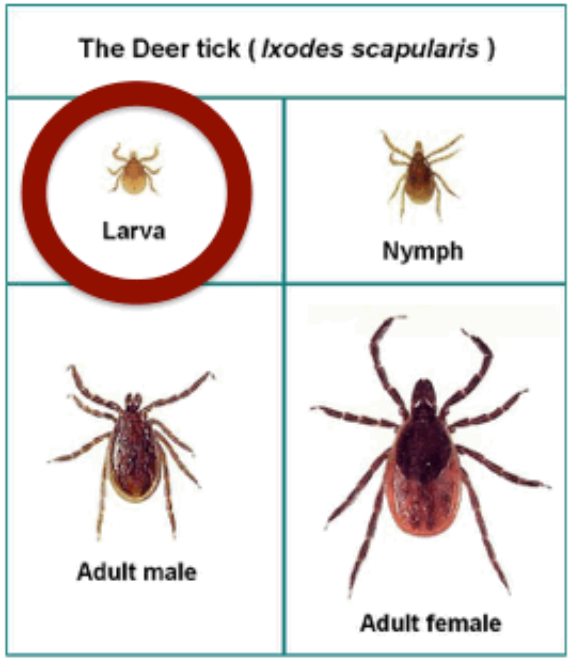
American Lyme Disease Foundation

Life Cycle: Year 1

Larvae

- hatch in the spring from eggs laid the previous fall
- first blood meal typically summer, on small mammals
- molt into nymph stage over the fall and winter

No risk of Lyme



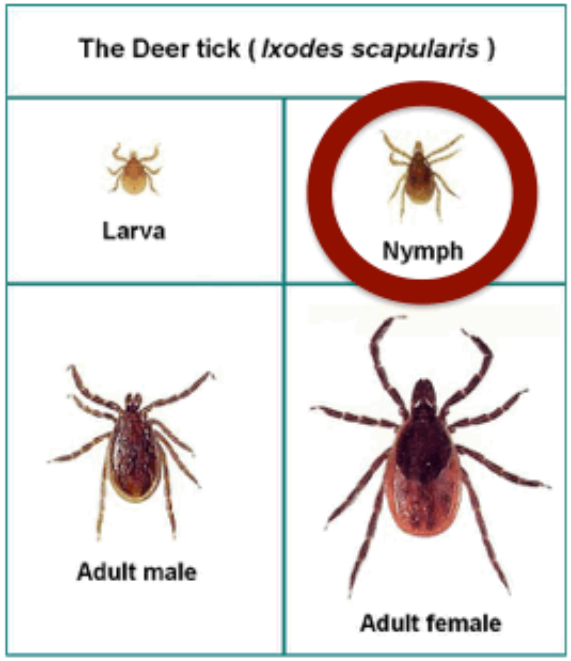
Life Cycle: Year 2

Nymph

- emerges from larval stage
- feeds in the **SPRING**
- molts into adult over the summer

Lyme risk!

Since this tick has had one blood meal, it has potentially been exposed to infection

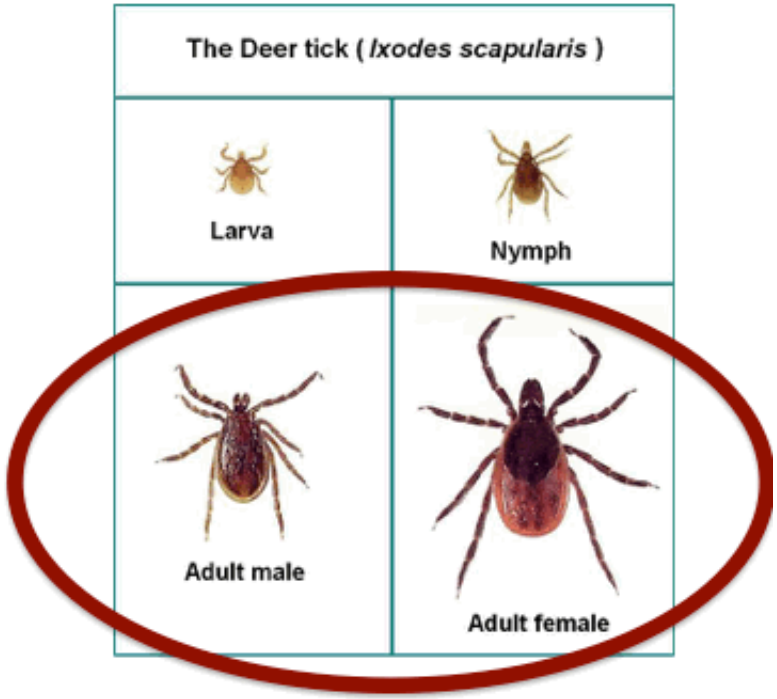


Life Cycle: Year 2

Adult

- feeds in the **FALL**
- after a blood meal, adult females spit eggs and die

Lyme risk!



Habitat

- Wooded areas, forests, over-grown grassy areas and trails
- Prefer moist and humid environments
- Frost does nothing to them
  - they burrow in leaves to overwinter
- Sit on tips of grass and “quest” for a host

How would you do it?

COLLECTING TICKS

How to make a drag

Materials:

- Light-colored nappy fabric
- Dowel
- Rope

The Dragger :

- One person performs the drag in a session
- Maintain consistency:
  - Height
  - Speed
- Keep the cloth low to the ground
- Check for ticks every 10 meters with The Collector.

The Collector :

- Helps The Dragger locate and pick ticks off the cloth.
- In charge of the vials
  - Labels: date, place
- Reports
  - How many
  - What kind
  - Age
  - Gender

Data Collection

- Report on ticks
  - How many
  - What kind
  - Age
  - Gender
- Vials
  - Labels: date, place
- Environmental factors

Sampling Data Sheet 1:

Data Sheet: *Ixodes Scapularis*

Name:	Program:	Date:
		Start Time:

GPS	Latitude	Longitude

Place	Nearest City:	State:

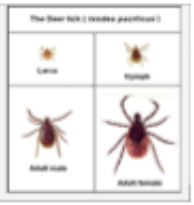
Weather Description:

Temp:	Humidity:

Soil Type	Sandy ☐ Sandy Loam ☐ Loam/Clay ☐ Clay ☐ Soil pH: Litter depth (mm):
	Soil Temp: Soil moisture:
Topology	

Describe Plants/Trees

Vial	Time	Deer Ticks		Wood Ticks	
#	Of Collection	Nymphs	Adult	Nymph	Adult
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					



The kit:


TICK IDENTIFICATION
Ixodes scapularis (deer tick, blacklegged tick)
Found: Northeast, Upper Midwest Transmits: Lyme, babesiosis, anaplasmosis, Powassan encephalitis, tick paralysis, tularemia, Bartonella
Dermacentor variabilis (dog tick)
Found: All U.S. Transmits: Rocky Mountain spotted fever, tularemia, human monocytic ehrlichiosis
Ixodes pacific (blacklegged tick)
Found: Far West
Bartonella

The kit:

- Tick Collection Methods
- Tweezers
- Lab gloves
- Vials
- Labels and a marker for each vial
  - Name. Date. Place.
- Data sheet
- Tick ID cards
- GPS
- Tick repellent

Of Lyme Disease

SYMPTOMOLOGY

Stage 1 Symptoms

Early infection

- Skin Rash
  - ~40 -75%
- Fevers
- Chills
- Swollen Lymph Nodes
- Headache
- Stiff Neck
- Muscle Fatigue

- These symptoms may appear from one day up to a month after infection.

Lyme Disease in humans

- Lyme is a multisystem inflammatory disease
  - affects the skin in the early stage
  - can spread to the joints, nervous system and may affect organ systems.

Long term Lyme…

- Bacteria burrows in body tissues, including vital organs.
- Bad news

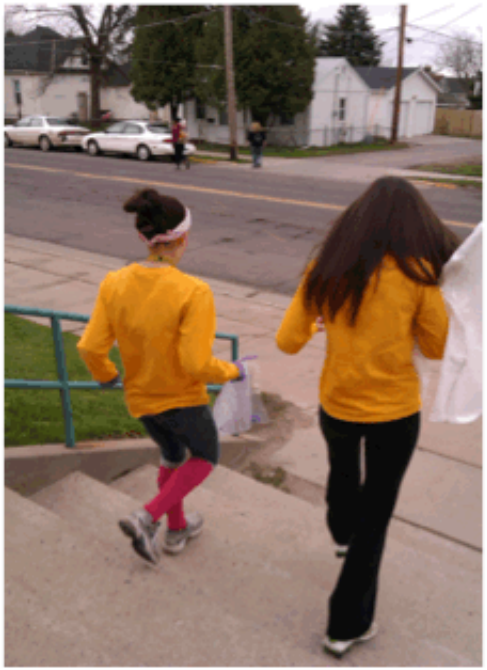
Lyme: It takes time

- A nymph or adult tick transmits Lyme in 36 to 48 hours
  - generation time of bacteria: ~ 12 hrs
- It takes several hours before a large enough dose can infiltrate the new host.

…is worth a pound of cure!

AN OUNCE OF PREVENTION…

Prevention:
it's a fashion trend!



- Wear clothing that covers the skin -all seasons!
- Socks over pants: to prevent ticks from crawling up under your pants.
- Wear bright and light colored clothing, ticks are more visible against the light color.
- Tick repellents:
  Deet, Promethrin.

Check yourself every day, every way.

and your pets!

If you find a tick…

- Be Careful How you Remove it
  - Don't use fire.
  - Don't use lubricants.
  - Do use tweezers –as close to the head as you can
    - So the mouth does not get stuck in your skin.
  - Do save the tick to give to your doctor
    - but don't expect a diagnosis based on testing the tick

…save it for research?

Where to send ticks:

- **DMED Biomedical Sciences**
  SMed 321
  1035 University Dr
  Duluth, MN 55812University of Minnesota