Tick and Lyme Disease Research at Mount Allison University, 2018-19

Annual Report
Canadian Lyme Disease Foundation

February 2020

The Mount Allison Lyme Disease Research Network - http://www.mtalymenetwork.ca/

Overview

In May 2017, Mount Allison University announced the formation of the Mount Allison Lyme Research Network in partnership with the Canadian Lyme Disease Foundation. This network consists of 16 researchers united to provide a comprehensive approach to Lyme disease research and respond to the research needs of the community. The researchers represent a variety of disciplines including biology, chemistry, geographic information systems, religious studies, English, commerce, political science, psychology, philosophy, economics, and computer science.

Mount Allison University has a strong interdisciplinary research environment that includes work on veterinary, wildlife, indigenous and human health research, and research on ticks, tick-borne diseases and the human dimension of chronic health issues (www.mtalymenetwork.ca/publications.html). Mount Allison also has a strong commitment to working with communities and is proud to partner with Lyme disease patients, their caregivers, health care providers, and advocates. Because we welcome members of the community as equal partners in research, our approach is to include patients, caregivers, and medical professionals at every stage of research, with our research grounded in respectful acknowledgement of lived experiences.

With the foundation's generous support, five Mount Allison students spent the summer of 2019 conducting independent, original Lyme disease-related research directly supported by the Foundation. A summary of these studies is provided. An additional five students also performed Lyme-related research funded through the university and other programs. Student research projects that occurred during the summer of 2019 included: characterizing media accounts of Lyme disease in the Maritimes post-2000; making transgenic ticks to develop tools to control tick-borne pathogens and better understand tick genetics; generating an improved serological test for Lyme disease in horses and assessing contact transmission in wild mice; studying the epigenetic effect of Borrelia in ticks; and mapping forest cover changes in New Brunswick to show areas of potential tick expansion.

The overall goal of the Mount Allison Lyme Disease Research Network is to further our understanding of Lyme disease in Canada and to move towards reducing human and animal suffering from this disease. We are proud to present this report on our continued research towards this goal.

In the pages that follow, we provide summaries of the research completed by each of the five Mount Allison University undergraduate students whose work was directly supported by the Foundation. This is followed by a summary of additional funding support that was leveraged by the Foundation's contribution.

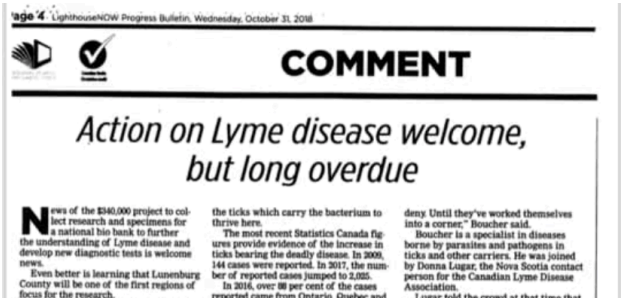
Margaret Cameron*
Supervisor: Dr. Mario Levesque, Department of Politics & International Relations
Characterizing Media Accounts of Lyme Disease in the Maritimes Post-2000

* Although Ms. Cameron was unable to complete her student research project due to medical reasons, Dr. Levesque is continuing this work.

This project examines media representations of Lyme disease in Canada's Maritime provinces. The media has an important role in framing emerging issues and news events. How an issue is framed can affect one's response to it, including government decision makers (Giasson & Small 2014). This is important for understanding the spread of ticks and increase in Lyme disease in the Maritime provinces in the last 20 years (Lieske & Lloyd 2018). Lyme disease can be debilitating and if proactively addressed, negative health outcomes can be minimized. Yet, debate within the medical community surrounding diagnoses, testing procedures and treatment have led many people to seek alternative health care including outside of Canada (Boudreau, Lloyd & Gould 2017). Citizens are increasingly contesting Lyme disease protocols for which policy makers do not seem to want to intervene (Klohn 2018). The media is increasingly reporting on this contestation between citizens (individuals with Lyme disease), the medical community and policy makers (Rankin 2019). Many questions arise out of this contestation that form the basis of this investigation including:

- How accurate is the tick and Lyme disease information that is being reported (e.g., spread, symptoms, prevention)?
- How are Lyme disease patients characterized: helpless victims, irrational and confused, poorly informed vis-à-vis medical practitioners (doctors) and decision makers (politicians, Medical Officers of Health)?
- Whose experiences are validated and why (patients, medical establishment)?
- How is the characterization of Lyme disease in media accounts connected to interpretations of disability?
- How do media accounts vary based on the media type (newspapers vs TV newscasts) over time?

A review of English daily and weekly newspapers (Moncton Times & Transcript, CBC news, Halifax Chronicle Herald, PEI Guardian) and nightly televised newscasts (CBC New Brunswick News, CTV News Atlantic) in Canada's Maritime provinces will be conducted. For newspapers, searches will be conducting using databases (Eureka, LexisNexis, ProQuest). Both databases and news stations themselves will be reviewed for televised coverage. The time period is post-2000 and will be broken into 5-year segments for analyses (2001–2005, 2006–2010, 2011–2015, 2016–present). News coverage will be broken down into its components and compared for analyses. From a political perspective, the analysis will be aided by the use of punctuated equilibrium theory which posits that a significant focusing event(s) is needed to enact changes in policy that deviate from long established and widely accepted practices (True, Jones & Baumgartner 2007, Lowry 2006).


age 4 LighthouseNOW Progress Bulletin, Wednesday, October 31, 2018

COMMENT

Action on Lyme disease welcome, but long overdue

News of the $340,000 project to collect research and specimens for a national bio bank to further the understanding of Lyme disease and develop new diagnostic tests is welcome news.

Even better is learning that Lunenburg County will be one of the first regions of focus for the research.

the ticks which carry the bacterium to thrive here.

The most recent Statistics Canada figures provide evidence of the increase in ticks bearing the deadly disease. In 2009, 144 cases were reported. In 2017, the number of reported cases jumped to 2,025.

In 2016, over 88 per cent of the cases

deny. Until they've worked themselves into a corner," Boucher said.

Boucher is a specialist in diseases borne by parasites and pathogens in ticks and other carriers. He was joined by Donna Lugar, the Nova Scotia contact person for the Canadian Lyme Disease Association.

Christopher Roy
Supervisor: Dr. Vett Lloyd, Department of Biology

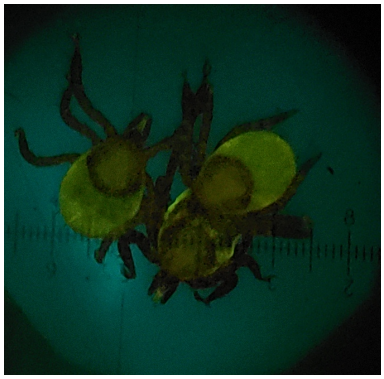
Using Agrobacterium-mediated gene transfer to make transgenic ticks expressing green fluorescent protein, as a proof of concept study to develop transgenic tick anti-Lyme disease strategies

Over the course of the summer, Roy met with individuals living with Lyme disease, Canadian organizations fighting this diseases' stigma, and studied both the science and human aspect of the effects of Lyme disease.

In 2015, Machado-Ferreira et al. demonstrated that genetic manipulation of tick larva was possible using *Agrobacterium tumefaciens*. Research has already been performed on mosquitoes examining the possibility of transgenic modification that could prevent the spread of malaria (Gantz et al. 2015). Roy's research focused on determining if this approach could be successful for ticks. *Agrobacterium tumefaciens* is a bacterial species that inserts transfer DNA in the form of a tumor-inducing (Ti) plasmid into host plant cells, resulting in growth of benign nodules in which the bacterium grow symbiotically with the plant host. Such strategies have recently been used to insert engineered plasmids into cells for the purpose of creating transgenic organisms (Sheng & Citovisky 1996).

The goal of this experiment was to alter the genome of larval ticks by using Agrobacterium with a TI-plasmid capable of generating green fluorescent protein (GFP) with eukaryotic promoters. The GFP would act as a visual and molecular marker to indicate successful gene-transfer. While ticks expressing GFP in their genome has little practical use, the ability to successfully alter the genome of ticks and other disease-carrying arthropods carries great potential to alleviate many serious diseases. If ticks could be engineered to selectively destroy Borrellia bacteria, Lyme disease would lose its primary vector.

Roy received engorged female Ixodes scapularis from public submissions to the Lloyd tick bank and the Oklahoma State University tick bank. The ticks were nurtured in plastic containers with damp cotton and incubated at 25 °C. Eggs were produced after ~2 weeks, and the eggs hatched after three additional months at constant temperature, providing larval ticks. Roy identified strains of Agrobacterium that had been used to successfully transform arthropod DNA and obtained the plasmid from Intact Genomics (Maximova et al. 2003). The plasmid was purified, transformed into E. coli for and verified. The next part of this project has been to transform the plasmid into the Agrobacterium, and then transform the ticks. After ~2 weeks green fluorescence was observed in the larval ticks. Confirmation of successful genome integration is now occurring as this project has been continued throughout the fall and winter. The success of this experiment would allow new avenues of tick-control through introduced and edited genes. If insertion of GFP into Ixodid ticks is possible, then it should also be possible to introduce genes coding for proteins antagonistic towards the Borrellia bacterium, the causative agent of Lyme disease and/or other tick-borne pathogens.



GFP-expressing transgenic ticks (Roy and Lloyd)

Julia Bland
Supervisor: Dr. Vett Lloyd, Department of Biology
An ELISA Lyme disease test for determination of the infection status of horses

The summer research project continued preliminary work completed as part of Bland's honours work on an ELISA-based serological test for Lyme disease in horses. The initial objectives included correlating equine symptoms with ELISA results, work on developing a solid-state ELISA on the IDEXX platform, analyzing immunoblot tests band-by-band, and writing a manuscript for subsequent publication.

Bland began the horse-focused study by completing a number of enzyme-linked immunosorbent assays (ELISAs) to confirm the results of her honours thesis and adapting and optimizing western blots for horses, developing a new standard operating procedure (SOP) for this work.. Meta-data collected as part of a previous study (i.e. horse breed, sex, activity level, location) were investigated statistically to determine the existence of a correlation between infection status via ELISA and these parameter and a draft manuscript for publication in *BMC Veterinary Research* was drafted. Bland also completed work on a mouse contact transmission study.

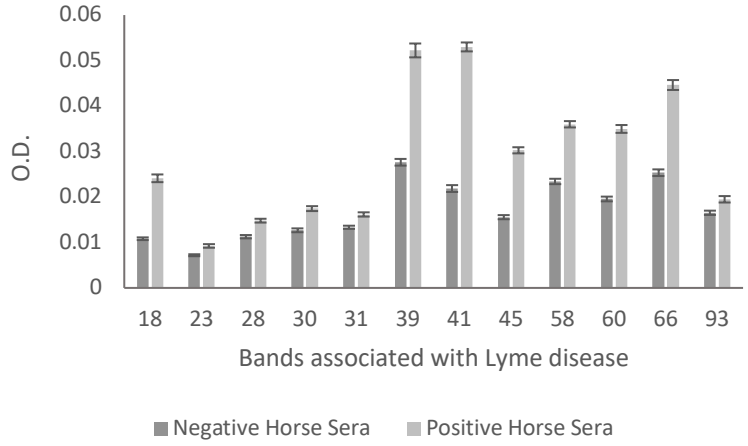


Figure 1. Optical density of immunoblot bands associated with Lyme disease diagnosis of immunoblot test strips tested with horse sera. Asterisks indicate statistical significance via ANOVA, $\alpha = 0.05$. *p=0.008, **p=0.010, ***p=0.011, ****p=0.004 *****p=0.012, ******p=0.009, *******p= 0.013.

Jessica Vickery
Supervisor: Dr. Vett Lloyd, Department of Biology

Epigenetic Variation between Borrelia-Infected and Un-Infected Ticks, and the Role This Plays in the Risk of Contracting Lyme Disease.

The objective of this study was to determine differences between the methylation patterns of DNA in ticks infected with the Borrelia bacteria and those not infected. Physiologically, these two types of ticks have been reported to differ such that those infected by the bacteria have a larger fat reservoir, enabling them to be less sensitive to desiccation. Infected ticks also experience reduced locomotion and are larger, which in turn aids in conserving their fat reserves. It is unclear what changes occur in the tick's genome that are responsible for these changes, but epigenetic mechanisms may be involved.

Epigenetics describes the process by which changes in an organism's gene expression occur due to modification of chromatin, as opposed to a change in the genetic code itself; DNA methylation is one of the inter-related epigenetic mechanisms. Methylation involves the addition of methyl groups to the C5 position of a cytosine base. This study aims to identify a difference in methylation patterns between these two types of ticks.

After receiving training in the necessary methodology including polymerase chain reaction (PCR), autoclave and DNA extraction techniques, the Diagenode MagMeDip qPCR kit was chosen to quantify the amount of methylated DNA. The target region chosen to pilot this projects was 6 tandem repeat regions within Ixodes scapularis

Primers were designed to target these regions, as well as for the SALP10 gene, which was used as a control. Following primer design, the primers were validated and this work is continuing through the academic year.

Connor Nickel
Supervisor: Dr. David Lieske, Department of Geography & Environment

Mapping forest cover changes in New Brunswick to show areas of potential tick expansion

This work shows the potential for ticks to expand into the province of New Brunswick by highlighting mixed land cover habitat enjoyed by deer. As deer are known to support reproducing populations of ticks, corridors where deer are free to move and concentrate are possible areas for tick colonization as they move northward with the warming climate. A mosaic of Landsat 8 imagery was constructed for the province and object-oriented classification was performed, demarcating the province into four categories: clearing, forest, road/urban, and water. It was found that there are ample areas for deer to roam within the province, with extensive forestry activity and further development inevitable. Areas of particular note are the western border with Maine and the south of the province. These areas indicate mixed land cover classification, due to urban areas, forestry operations opening up clear-cut areas, and agricultural activity. Therefore, these areas should be watched as potential areas where new populations of ticks can be supported. To provide insight into how patterns of colonization have the potential to change, future work could compare this analysis with imagery from 2013 with level 2 Landsat imagery from Fall 2019.

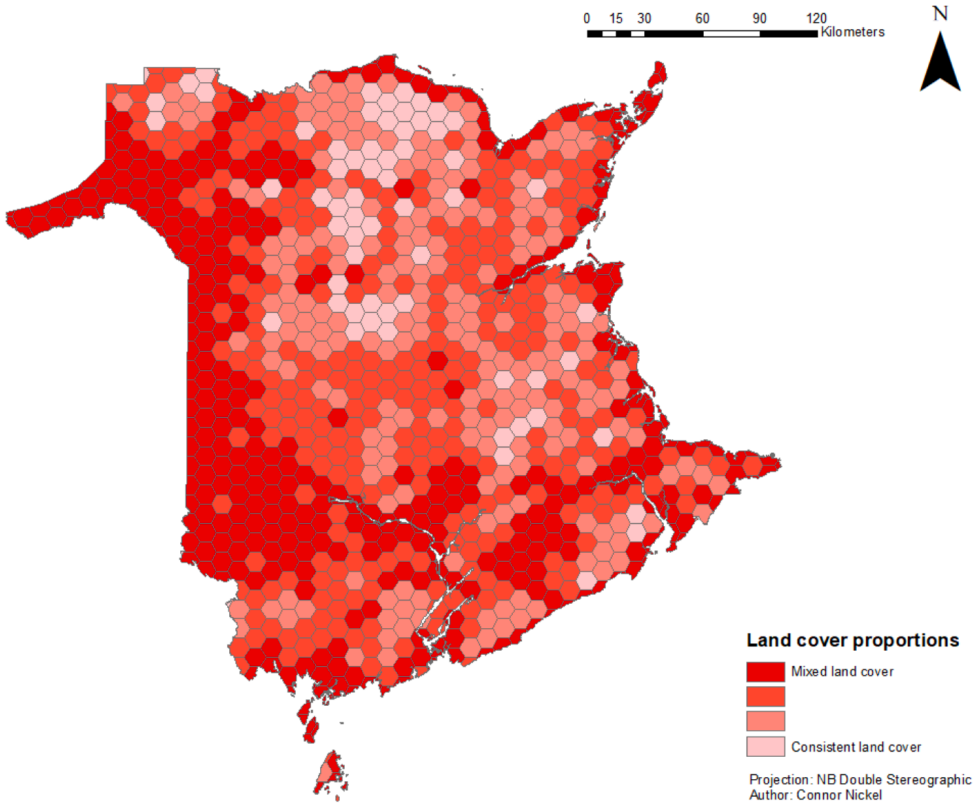


Figure 1. Standard deviation of proportions of classes within each hexagon, indicating either a mix of classes or a dominant class. Domination by one class would indicate consistent land cover and therefore little edge habitat, and vice versa.

Leveraged / Matching Funds

The generous contribution of the Canadian Lyme Disease Foundation resulted in an additional $37,000 worth of research funds expended on Lyme Disease research, plus $4,000 worth of in-kind support. These projects are described below.

- Yahya Farooqi- Greener Living Products (industrial grant) – **Human and in vitro testing of a natural product tick repellant for activity against ticks** ($7,000)
- Emma Rogerson, Experiential Learning Fund – **Health Studies** ($7,000)
- Connor Flynn (UNB) – **Borrelia diagnostics** ($1,000)
- Caeleagh Ryalls, **Primary care for Lyme disease patients** ($7,000, Donor)
- Kiana Gagnon – **Patient experience database** – SEED ($6,000)
- Mount Allison University, research grants held by Vett Lloyd, (~$9,000 purchase of research lab supplies including necessary reagents to support the student research projects funded by Canadian Lyme Disease Foundation).
- Mount Allison University, Office of Research Services, Financial Services, ($4,000 worth of management time to support the development of the Lyme Disease Research Node and the management of research grants and related funds).