

Tick and Lyme Disease Research at Mount Allison University, 2017-18

**Annual Report
Canadian Lyme Disease Foundation**

October 2018



The Mount Allison Lyme disease Research Network - <http://www.mtalymenetwork.ca/>

Overview

On May 2017, Mount Allison University announced the formation of the Mount Allison Lyme Research Network, formed with support of the Canadian Lyme Disease Foundation. This network consists of 14 researchers united to provide a comprehensive approach to Lyme disease research and respond to the research needs of the Lyme 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.

Multi- and interdisciplinary research is something that Mount Allison does very well and the institution has a history of work on veterinary, wildlife, indigenous and human health research. This includes work on ticks, tick borne-diseases and the human dimension of chronic health issues (<http://www.mtalymenetwork.ca/publications.html>). The institution also has a strong commitment to working with communities and is proud to partner with Lyme patients, their caregivers, health care providers, advocates. Because we welcome members of the community as equal partners in research, the research we are doing is research the community of patients, caregivers, and medical professionals views as important and knowledge is directly mobilized to the community because they are involved at every stage.

Thanks to the foundation's gift, seven Mount Allison students spent their summer conducting independent, original Lyme-related research directly supported by the Foundation. A summary of these studies is provided and five of these studies are being continued with the support of the university, supervisors and student researchers. An additional four students also performed Lyme-related research funded through university programmes. Student research projects that took place during the summer of 2018 included: expanding the sensitivity and ease of use of Lyme disease diagnostics for poorly studied animal species; assessing *Borrelia* infection in Maritime cows; screening ticks for rare *Borrelia* species; examining the impacts on families when children have Lyme disease; blockage mapping in Lyme disease-related health policy, looking at tick microhabitats and molecular detection of *Borrelia* in CFS and MS patients.

The overall goal is to further our understanding of Lyme disease in Canada and to move towards reducing human and animal suffering from the 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 7 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.

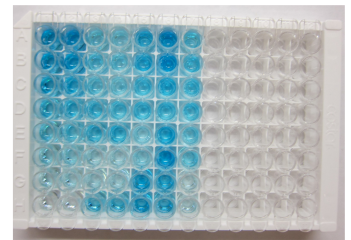
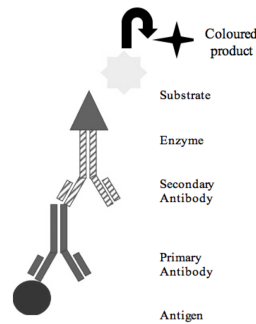
Julia Bland

Supervisor: Dr. Vett Lloyd, Dept. Biology

An ELISA Lyme disease test for determination of the infection status of horses, mice, quail and ducks and humans

The objective of this project was to develop a point-of-care C6 ELISA assay for use in diagnosing Lyme disease in non-traditional animal species. The approach is to modify the existing commercial canine SNAP test to

determine the infection status of horses, humans, mice, quail and ducks. Preliminary analysis has assessed the sensitivity of current equine testing by C6 ELISA, WSC ELISA and immunoblots. This will be followed by adaption of the existing canine test to provide sensitive point-of-care detection of seropositivity in horses. This process will be repeated for the other species.



An ELISA Lyme disease test for determination of the infection status of horses, mice, pheasant and ducks



Emilie Gaudet

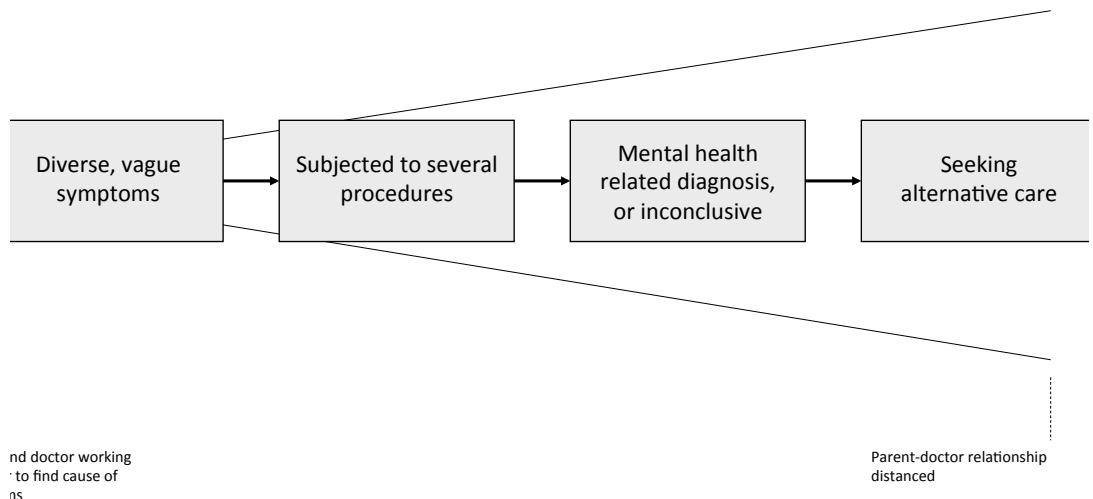
Supervisor: Dr. Odette Gould (Department of Psychology)

When Children Have Lyme Disease: Parent and Family Experiences

The aim of this project was to explore and make sense of the experiences of families in which children are affected by Lyme disease. We wanted to document and represent academically the experiences of Canadian families who struggle with Lyme disease. To achieve this, we asked for the help of a Lyme disease support group, LymeHope, in recruiting participants for the study. With their help, we contacted participants and asked them if they gave us permission to use a letter they had previously written on their family's experience with Lyme disease. A total of 21 letters were analyzed.

Results of this study support families' and past studies' concerns that doctors in the Canadian healthcare system are not properly trained in diagnosing and treating Lyme disease, and do not have much knowledge of the disease itself. The result of this lack of knowledge is strain to families who have children affected by Lyme, with hopelessness growing over time as resources become scarce for parents and support appears unavailable. These circumstances lead to family dynamics altering as finances are strained and relationships change from parenting to a growing sense of advocacy. Parents resort to seeking for alternate types of care, either from non-medical professionals or from other countries, and spend time and energy speaking out to gain justice for their children. Where at the beginning of the process parents trusted the Canadian healthcare system and trusted doctors to help, as the family navigates the system they grow weary and distrustful of medical professionals and the Canadian healthcare system, resulting in a strained working relationship.

A manuscript including a literature review, methodology, results, and discussion for the entire project has been prepared and will be submitted for publication in scholarly journals. A copy of the report will also be made available to the Lyme support groups who helped us with our project.



Caitlin McGowan

Supervisor: Dr. Vett Lloyd, Department of Biology

The prevalence of the Lyme disease bacteria, *Borrelia*, in NB cows and possible transmission into milk

The project involved collecting and analyzing cow blood for the presence of antibody response by ELISA and western blot to the Lyme disease causing bacteria, *Borrelia burgdorferi*. The goal was to collect blood samples from 250-300 cows from New Brunswick and Nova Scotia. While collecting these samples, information about the cow health was collected, such as age, and any characteristics that had been associated with bovine Lyme infection in papers read. The objective of the entire study is to gain a greater understanding of the incidence of *Borrelia burgdorferi* in New Brunswick and Nova Scotia cows. It was difficult to collect samples in the south of New Brunswick as it had been an eventful summer for the veterinarians with both flooding and a strangles outbreak; only 20 samples were obtained from this area, but 100 were obtained from Northern New Brunswick and 102 from Northern Nova Scotia. Testing is ongoing, with 75% of the 54 NS samples testing positive, and 50% of 10 Northern NB samples testing positive.

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The prevalence of the Lyme disease bacteria, *Borrelia burgdorferi*, in New Brunswick and Nova Scotian Cows

CanLyme

Caitlin McGowan
Supervisor: Dr. Vett Lloyd



Matthew Klohn

Supervisor: Dr. Mario Levesque, Department of Politics & International Relations

This project examines the potential blockages facing Lyme disease patients in the Maritime provinces and considers how government could address these issues. To do so, two literature reviews were conducted to cover both academic scholarship as well as media and government publications. These findings were then complemented by two surveys administered across the Maritimes targeting both medical professionals and decision makers in government. Together the literature reviews and surveys demonstrate that there is considerable debate surrounding the effectiveness of testing, treatment options, and the existence of Chronic Lyme disease. These debates often pit the medical community against Lyme advocates and thus far governments have been unable to produce policy that entirely pleases either side of this debate. To address these concerns, this study recommends that governments strike a Commission examining the best practice guidelines for Lyme disease. This would hopefully bring unbiased inquiry to the topic of treatment and diagnosis without completely alienating the medical community or Lyme advocates. While this would not necessarily solve the issue of Lyme disease it would hopefully foster a dialogue and allow stakeholders to take a critical look at the current protocol.

Madelaine Russell (Department of Biology)
Supervisor: Dr. Vett Lloyd, Department of Biology
Haptoglobin as a risk factor for MS, cancer and Lyme disease

This project was to determine if DNA from *Borrelia burgdorferi*, one of the *Borrelia* species responsible for Lyme disease and the most common causative agent in North America, could be isolated from blood cells of patients with suspected Lyme disease. The Haptoglobin project experienced delays in obtaining blood samples from patients at the partner hospital in New Brunswick so the analysis was conducted on blood previously obtained from patients with undiagnosed or unexplained chronic disease, included suspected Lyme disease. DNA was extracted from blood cells and tested by nested PCR (nPCR) amplifying a portion of the 23S intragenic spacer of *B. burgdorferi*. Amplicons of the correct size were by sequenced and the sequences analysed using the software FinchTV and running the nucleotide sequence through the nucleotide BLAST database available through NCBI. Samples with *B. burgdorferi* were then assessed by repeating the nPCR twice more to exclude spurious false amplifications. Most amplicons obtained were false priming that amplified human DNA. None of the amplicons that were *B. burgdorferi* were able to be re-amplified in a consistent manner. Although interpretation of this experiment suffers from the lack of a positive control, we conclude that direct PCR from blood cells is either not an efficient way to identify late stage disseminated *B. burgdorferi* infections or that none of the 165 individuals, many with positive *Borrelia* serology and a return to health following antibiotic treatment actually had a *Borrelia burgdorferi* infection.

Nested PCR Detection of *Borrelia burgdorferi* in blood from participants suffering from undiagnosed/unexplained chronic pain

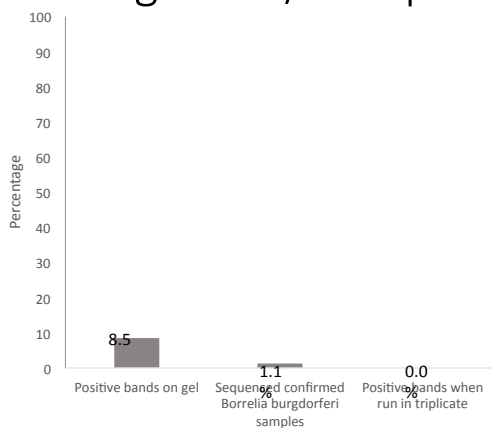


Figure 1: Percentage of positive bands on the gel electrophoresis, sequenced confirmed *Borrelia burgdorferi* and positive triplicate results (N=165).

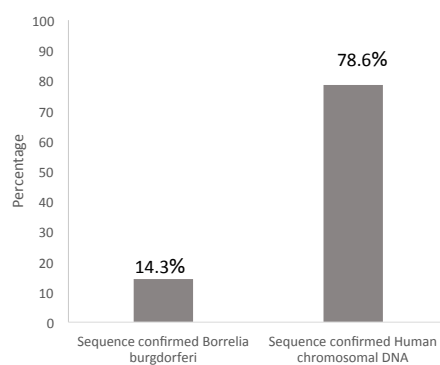
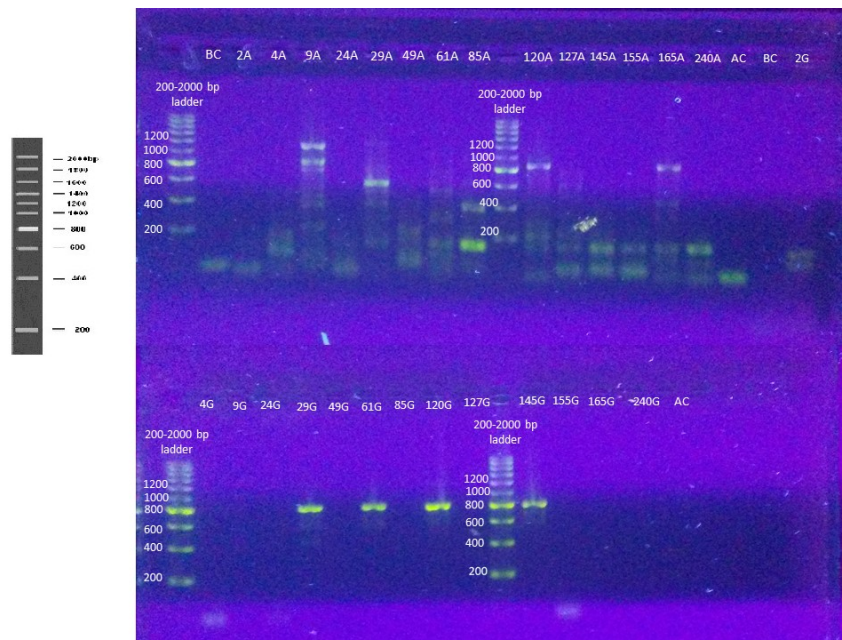


Figure 2: Percentage of positive bands that are sequenced confirmed *Borrelia burgdorferi* and Human chromosomal DNA (N=14).

Ellice Evans (Department of Biology)

Presence of unexpected *Borrelia* pathogens in ticks from the Canadian Maritime provinces

This project seeks to investigate the presence of unexpected *Borrelia* sp. bacteria within the Maritimes. Specifically, this project focused on the ability of *Dermacentor variabilis* to carry *B. miyamotoi*, a relapsing fever-like member of the *Borrelia* genus and the presence of “European” *Borrelia* species *B. afzelii* and *B. garinii* in *Ixodes scapularis* ticks. This project involved testing DNA of *D. variabilis* and *Ixodes scapularis* from the Maritimes that had been archived in the Mount Allison tick bank. Several sets genes were targeted in several hundred individuals of both tick species. Confirmation of results will rely on successful sequencing on promising amplicons. This step will be taking place in the forthcoming months. If time permits, a selection of New Brunswick ticks from the Mount Allison tick bank will be tested to determine baseline levels of co-infection with other common tick-borne pathogens such as *Babesia* and *Bartonella* in the region.



Cory LeBlanc (Department of Biology)

Supervisor: Dr. Vett Lloyd, Department of Biology

Questing microhabitat preference of *Ixodes scapularis*

The objective of this project was to answer the following question: Does *Ixodes scapularis*, the blacklegged tick, have a preferred microhabitat for questing in the Maritime provinces? The first part of the project focused on developing a standard operating procedure (SOP) for field sampling to maximize safety and efficiency when working with ticks in the field. This was done in conjunction with Rob Murray, Director, Canadian Lyme Disease Foundation. Tick dragging was performed using clothing and materials that are accessible to community members as part of the lab's commitment to encouraging tick surveillance that is both safe and community-based. Tick dragging was completed in southern NB, southern and western NS, and in parts of PEI. A total of approximately 280 *I. scapularis*, 252 *D. variabilis*, and 6 *H. leporispalustris* were obtained during this project. Data was obtained from 118 sampling sites, representing a total of 230 datasets. At least one tick was collected at 26.3% of sampled sites. The greatest catch per unit effort recorded was 45.52 *I. scapularis* ticks/hour. These results suggest that in areas where ticks are abundant, drag sampling can be used for sampling for questing tick species, although the sensitivity of the technique in areas of low tick abundance appears to be low. A detailed statistical analysis of the data obtained from the summer fieldwork is currently underway. Although we cannot make any claims about potential patterns in the data at this time, we hope to be able to determine associations between both *I. scapularis* and *D. variabilis* ticks and certain microhabitat qualities. This work will ultimately shed light on what features of environments common to the Maritime provinces are most likely to harbour questing ticks.



Leveraged / Matching Funds

The generous contribution of the Canadian Lyme Disease Foundation resulted in an additional \$64,000 worth of research funds expended on Lyme Disease research, plus \$4,000 worth of in-kind support. These projects are described below.

- Victoria Rust- Loewen Health Research Intern – **Genetic predisposition to chronic and inflammatory disease** (\$7,000 MTA, \$12,000 Magnotta Foundation)
- Ally and Emma Rogerson, Canadian Wildlife Service and Mount Allison University – **Cat behavior and predation of Lyme reservoir species and birds** (\$12000, Government of NB SEED grant, \$15,000 CWS)
- Sarah St.Pierre, Department of Psychology (Supervisor Odette Gould) – **A comprehensive survey of tick knowledge and tick bite prevention needs in the Maritimes.** (\$1,000)
- Leah Hagerman, Erika Graffunder, Odette Gould, Vett Lloyd, **Assessing motivations for health care seeking activity in Lyme disease patients.** (\$1,000, Lloyd research funds)
- Chris Roy – **antibiotic production by soil bacteria** –Mount Allison University (\$5,000)
- Michel Nadar – **LAMP amplification of Borrelia in ticks** – Mount Allison University (\$5000)
- Mount Allison University, research grants held by Vett Lloyd, (~\$6,000 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).

Victoria Rust- Loewen Health Research Intern

This summer I worked as a research intern at the Upper River Valley Hospital under the supervision of Dr. Gurpreet Singh-Ranger (URVH) and Dr. Vett Lloyd (MTA biology) on a collaborative project between the URVH, MTA and Dr. M. Wills at the University of Guelph. This opportunity was made possible by the Loewen Health Internships, which were recently established by the university's new chancellor, Lynn Loewen. Through this internship I was a part of two research projects; one evaluating biomarkers in chronic inflammatory diseases, which includes Lyme disease, and one an audit of patient consent forms. I also had the opportunity to write up a case report of medical interest. I took part in patient consults and observed minor surgeries which were not opportunities I expected to have with this internship and I am extremely grateful to the patients, nurses and doctors who allowed me this experience. In speaking with medical students over the course of the summer I came to better understand the sacrifices they had made getting into medical school and those they will continue to make moving forward into a helping profession. My goal of becoming a medical professional has not changed. If anything, I feel I am much more prepared for what is ahead having completed this internship.

