Tick and Lyme Disease Research at Mount Allison University, 2016-17

Annual Report
Canadian Lyme Disease Foundation

October 2017



Dr. Jeff Ollerhead, Provost and Vice President Academic & Research, Mount Allison University speaking at the Mount Allison Lyme Research Network Announcement, Sackville NB, May 5, 2017
https://www.mta.ca/Community/News/2017/May/Mount_Allison_establishes_Lyme_Research_Network/

Overview

On the 5th of 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 and to share findings. 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 ticks, tick borne-diseases and the human dimension of these diseases. The institution has a strong history of working with communities and so is well-positioned to form this network where Lyme patients, their caregivers, health care providers, and veterinarians have all been welcomed as key partners and have suggested important research avenues. Because we are doing it this way, it means the research we are doing is research the community of patients, caregivers, and medical professionals views as important. The translation of knowledge to the community is very good because they are involved at every stage.

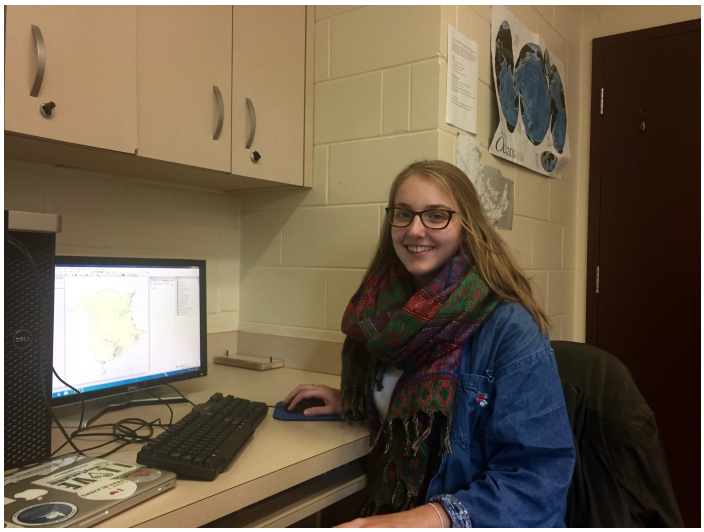
Thanks to the foundation's gift, five Mount Allison students spent their summer conducting independent, original Lyme-related research directly supported by the Foundation. A summary of these studies is provided and four of these studies are being continued for an additional eight months with the support of the university, supervisors and student researchers. An additional four students also performed Lyme-related research. Student research projects that took place during the summer of 2017 included: looking at Lyme disease diagnostics; identifying new tick hybrids; exploring potential tick repellants; studying the antibiotic properties of certain compounds and their effectiveness against the Lyme bacteria; assessing the impact of Lyme disease on performance horses; analyzing the ecology of wildlife reservoirs; examining spirituality and resilience among those dealing with chronic disease; and creating a way to share information online about the distribution of ticks in New Brunswick and the risk of Lyme disease.

The overall goal is to understand what is happening currently in Canada with Lyme disease and to move towards ameliorating the problem and reducing human and animal suffering from the disease. We are proud to present this report on our initial research directed towards this goal.

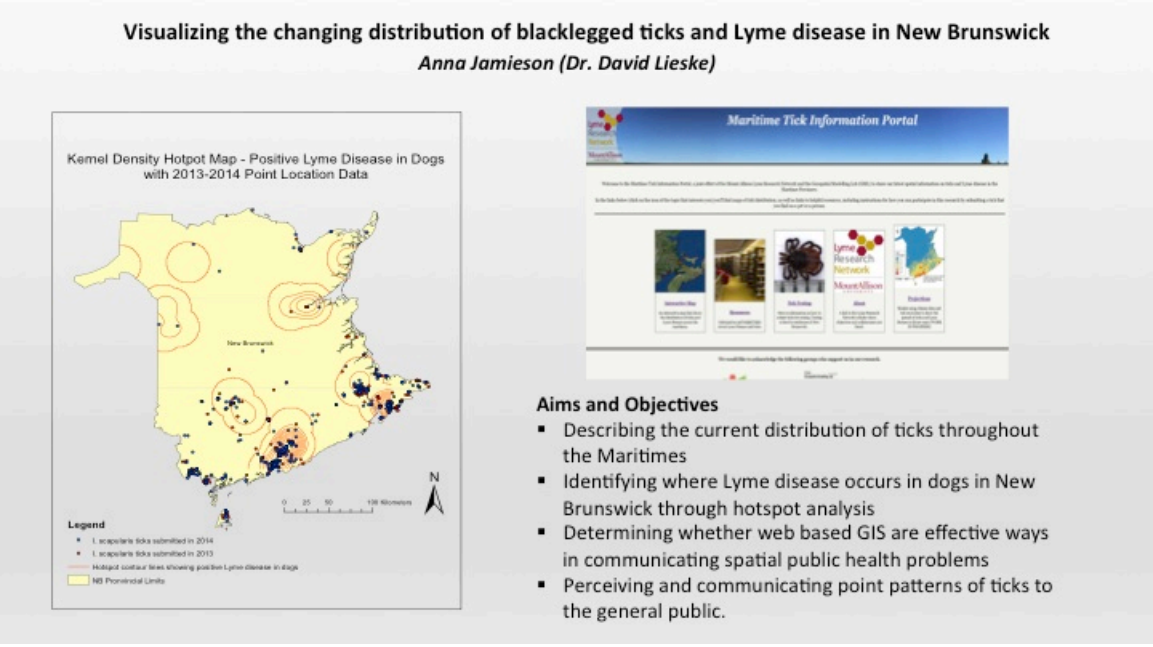
In the pages that follow, we provide summaries of the research completed by each five Mount Allison University undergraduate students whose work was directly supported by the Foundation. This is followed by a summary of additional funding support that was leveraged by the Foundation's contribution, and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

Anna Jamieson
Supervisor: Dr. David Lieske, Department of Geography & Environment

Creation of an accessible web-based geographic information systems (GIS), for public communication of New Brunswick black legged tick distribution and Lyme Disease risk



This project investigates the distribution and abundance of black legged ticks throughout the Province of New Brunswick, how that distribution is changing and expanding and how this relates to the occurrence of Lyme disease infection across the province. The goals are to create a comprehensive web portal for the public to be used to visualize tick distribution across the province. The resulting resource will be engaging, visually appealing, informative and interactive. The maps created will be on the scale of postal codes, allowing the concentration of tick populations to be clearly seen on the community level. The Mount Allison University tick bank holds a large collection of ticks sent from across the province through an ongoing public participatory surveillance program. This data will be geocoded and incorporated into the geospatial database to allow the public to perform customized search and display of the records, and to produce appealing and informative maps. Using a web- powered GIS is a tangible, visually appealing, and most importantly accessible way to portray spatial date for the everyday user. The system will be easy to use, and have various layers, displaying various years, and areas of distribution. This will help visualize changes by year and how distribution and infection are changing in relation to the changes in climate.

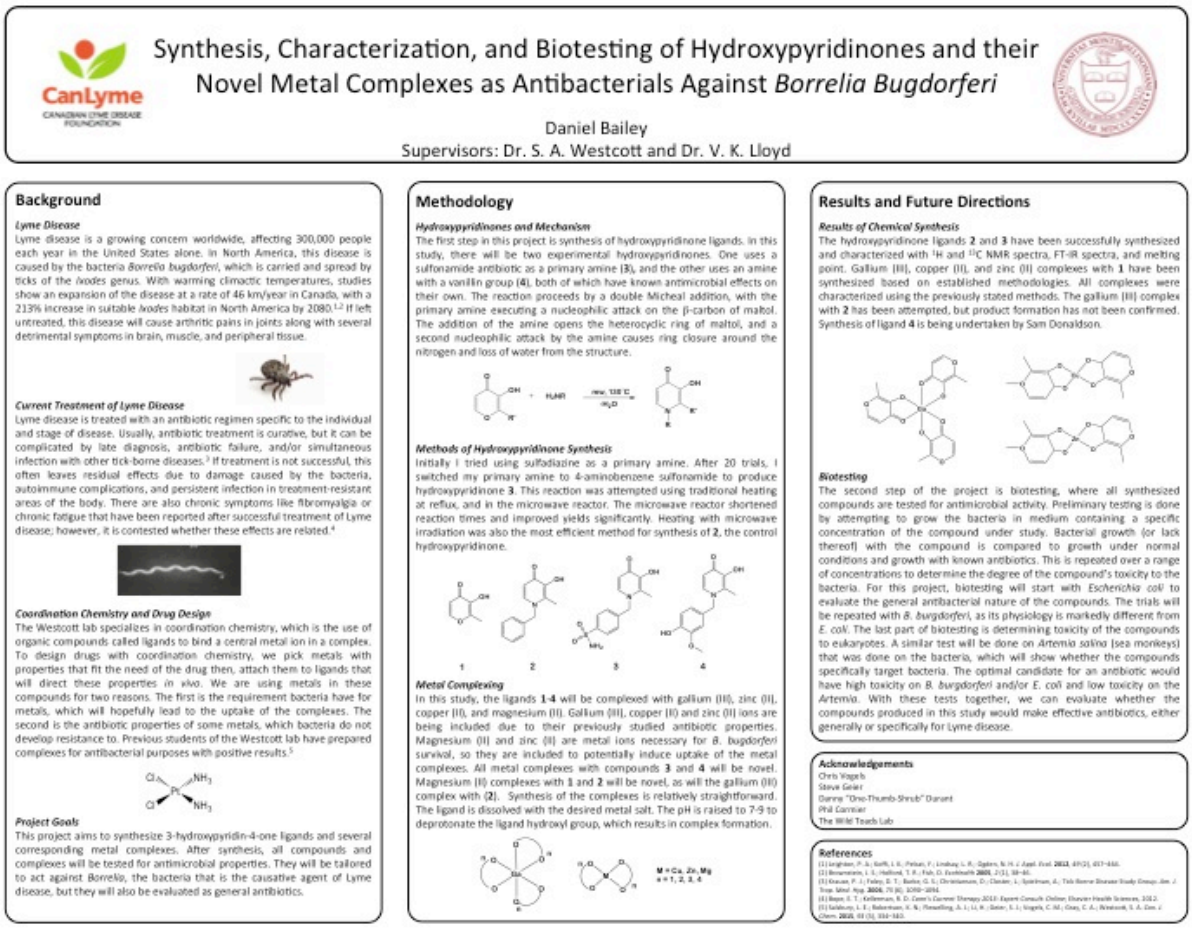


Daniel Bailey
Supervisor: Dr. Stephen Westcott, Department of Chemistry & Biochemistry

Synthesis, Characterization, and Testing of Gallium (III) Antibacterial Compounds



This project explores whether or not gallium (III) compounds will be effective bioactive agents and show potential for future therapeutic drugs against Lyme disease. Bioactive properties of gallium (III) have been noted as early as 1971, but little research into gallium-containing compounds has been done despite studies showing their antibacterial and antitumor activity with low toxicity. The mechanism of action of gallium (III) is based on its chemical similarity to iron (III), which is a key component in biochemical reactions necessary for cell growth. Gallium (III) replaces iron (III) in crucial enzymes of DNA and protein synthesis, where it renders the enzymes nonfunctional due to its inability to participate in redox chemistry like iron (III). The effects of gallium (III) arrest cell growth through iron starvation conditions, which is effective against rapidly proliferating tumor and bacterial cells that require large amounts of iron (III). The experiments will synthesize novel gallium (III) compounds 1-7 using established techniques to create Schiff-base ligands and complex the ligands to gallium (III). After synthesis, the compounds will undergo bio-testing where the compounds will be tested for effectiveness in dispatching ticks and their accompanying bacteria which cause Lyme disease.



Synthesis, Characterization, and Biotesting of Hydroxypyridinones and their Novel Metal Complexes as Antibacterials Against *Borrelia Bugdorferi*

Daniel Bailey
Supervisors: Dr. S. A. Westcott and Dr. V. K. Lloyd

Background

Lyme Disease

Lyme disease is a growing concern worldwide, affecting 300,000 people each year in the United States alone. In North America, this disease is caused by the bacteria *Borrelia bugdorferi*, which is carried and spread by ticks of the *Ixodes* genus. With warming climactic temperatures, studies show an expansion of the disease at a rate of 46 km/year in Canada, with a 213% increase in suitable *Ixodes* habitat in North America by 2080.[1,2] If left untreated, this disease will cause arthritic pains in joints along with several detrimental symptoms in brain, muscle, and peripheral tissue.

Current Treatment of Lyme Disease

Lyme disease is treated with an antibiotic regimen specific to the individual and stage of disease. Usually, antibiotic treatment is curative, but it can be complicated by late diagnosis, antibiotic failure, and/or simultaneous infection with other tick-borne diseases.[3] If treatment is not successful, this often leaves residual effects due to damage caused by the bacteria, autoimmune complications, and persistent infection in treatment-resistant areas of the body. There are also chronic symptoms like fibromyalgia or chronic fatigue that have been reported after successful treatment of Lyme disease; however, it is contested whether these effects are related.[4]

Coordination Chemistry and Drug Design

The Westcott lab specializes in coordination chemistry, which is the use of organic compounds called ligands to bind a central metal ion in a complex. To design drugs with coordination chemistry, we pick metals with properties that fit the need of the drug then, attach them to ligands that will direct these properties *in vivo*. We are using metals in these compounds for two reasons. The first is the requirement bacteria have for metals, which will hopefully lead to the uptake of the complexes. The second is the antibiotic properties of some metals, which bacteria do not develop resistance to. Previous students of the Westcott lab have prepared complexes for antibacterial purposes with positive results.[5]

Project Goals

This project aims to synthesize 3-hydroxypyridin-4-one ligands and several corresponding metal complexes. After synthesis, all compounds and complexes will be tested for antimicrobial properties. They will be tailored to act against Borrelia, the bacteria that is the causative agent of Lyme disease, but they will also be evaluated as general antibiotics.

Methodology

Hydroxypyridinones and Mechanism

The first step in this project is synthesis of hydroxypyridinone ligands. In this study, there will be two experimental hydroxypyridinones. One uses a sulfonamide antibiotic as a primary amine (**3**), and the other uses an amine with a vanillin group (**4**), both of which have known antimicrobial effects on their own. The reaction proceeds by a double Micheal addition, with the primary amine executing a nucleophilic attack on the β-carbon of maltol. The addition of the amine opens the heterocyclic ring of maltol, and a second nucleophilic attack by the amine causes ring closure around the nitrogen and loss of water from the structure.

Methods of Hydroxypyridinone Synthesis

Initially I tried using sulfadiazine as a primary amine. After 20 trials, I switched my primary amine to 4-aminobenzene sulfonamide to produce hydroxypyridinone **3**. This reaction was attempted using traditional heating at reflux, and in the microwave reactor. The microwave reactor shortened reaction times and improved yields significantly. Heating with microwave irradiation was also the most efficient method for synthesis of **2**, the control hydroxypyridinone.

Metal Complexing

In this study, the ligands **1-4** will be complexed with gallium (III), zinc (II), copper (II), and magnesium (II). Gallium (III), copper (II) and zinc (II) ions are being included due to their previously studied antibiotic properties. Magnesium (II) and zinc (II) are metal ions necessary for *B. bugdorferi* survival, so they are included to potentially induce uptake of the metal complexes. All metal complexes with compounds **3** and **4** will be novel. Magnesium (II) complexes with **1** and **2** will be novel, as will the gallium (III) complex with (**2**). Synthesis of the complexes is relatively straightforward. The ligand is dissolved with the desired metal salt. The pH is raised to 7-9 to deprotonate the ligand hydroxyl group, which results in complex formation.

Results and Future Directions

Results of Chemical Synthesis

The hydroxypyridinone ligands **2** and **3** have been successfully synthesized and characterized with ^{1}H and ^{13}C NMR spectra, FT-IR spectra, and melting point. Gallium (III), copper (II), and zinc (II) complexes with **1** have been synthesized based on established methodologies. All complexes were characterized using the previously stated methods. The gallium (III) complex with **2** has been attempted, but product formation has not been confirmed. Synthesis of ligand **4** is being undertaken by Sam Donaldson.

Biotesting

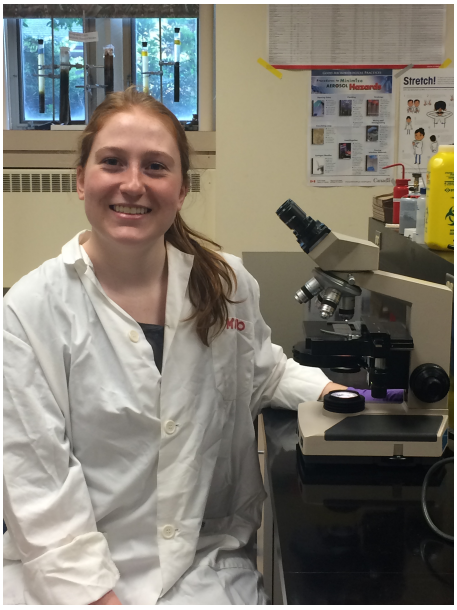
The second step of the project is biotesting, where all synthesized compounds are tested for antimicrobial activity. Preliminary testing is done by attempting to grow the bacteria in medium containing a specific concentration of the compound under study. Bacterial growth (or lack thereof) with the compound is compared to growth under normal conditions and growth with known antibiotics. This is repeated over a range of concentrations to determine the degree of the compound's toxicity to the bacteria. For this project, biotesting will start with *Escherichia coli* to evaluate the general antibacterial nature of the compounds. The trials will be repeated with *B. bugdorferi*, as its physiology is markedly different from *E. coli*. The last part of biotesting is determining toxicity of the compounds to eukaryotes. A similar test will be done on *Artemia salina* (sea monkeys) that was done on the bacteria, which will show whether the compounds specifically target bacteria. The optimal candidate for an antibiotic would have high toxicity on *B. bugdorferi* and/or *E. coli* and low toxicity on the *Artemia*. With these tests together, we can evaluate whether the compounds produced in this study would make effective antibiotics, either generally or specifically for Lyme disease.

Acknowledgements
Chris Vagelis
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Phil Cormier
The Wild Toads Lab

References

Laurabeth Gill
Supervisor: Dr. Vett Lloyd, Department of Biology

A potential treatment for Lyme disease



This project explores whether or not the chemotherapeutic drug dichloroacetate (DCA) will inhibit growth of the Borrelia spirochete. This drug inhibits pyruvate dehydrogenase kinase, which increases the proportion of active pyruvate dehydrogenase in mitochondria. Although the metabolism of Borrelia has not been fully elucidated, they are microanaerobes and rely solely on the glycolytic pathway for ATP production. It is therefore highly likely that Borrelia have some enzymes regulating pyruvate production and transformation that are structurally similar to pyruvate dehydrogenase kinase in eukaryotes. As DCA is a pyruvate analogue, it might act on these enzymes and inhibit Borrelia metabolism, inhibiting bacterial growth or possibly killing them. The effect of DCA on Borrelia growth will be tested by growing Borrelia burgdorferi in culture, determining the cell concentration, and then adding DCA to the cultures. After different periods of time, Borrelia cell concentration and morphologies will be assayed. Positive controls will be treated with the antibiotics conventionally used for Lyme disease, such as cefuroxime and doxycycline, and negative controls will not be treated. Combination therapies will be explored in vitro.

Dichloroacetate kills *Borrelia burgdorferi* B31 in culture

V.K. Lloyd, L. S. Gill
Mount Allison Biology Department

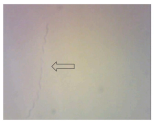


Figure 4. A *Borrelia burgdorferi* B31 spirochete seen under 400x magnification

Introduction

- Lyme disease is the most common vector-borne disease in North America and Europe
- caused by the *Borrelia burgdorferi* spirochete bacteria
- *Borrelia* infection can cause joint and muscle pain, meningitis, encephalomyelitis, and other central nervous system damage
- The complex metabolism of *Borrelia* bacteria limits the effectiveness of conventional antibiotics
- *Borrelia* can adjust its metabolic state and morphology to survive under harsh conditions and to protect itself against antibiotics
- Dichloroacetate (DCA) is a relatively non-toxic inhibitor of pyruvate dehydrogenase kinase
- Highly likely that *Borrelia* have enzymes involved in the pyruvate pathway that DCA could act on to inhibit bacterial growth

Objective

To determine whether dichloroacetate (DCA) is an effective inhibitor of *Borrelia* growth under laboratory conditions.

Methods

- *Borrelia burgdorferi* B31 was grown in medium for 2 weeks
- DCA was made first with dichloroacetate stock solution, then with a sodium dichloroacetate salt
- Different concentrations of DCA (0.025M, 0.0125M, 0.00625, 0.0025M, 0M) were added and growth and morphology was assessed after 14 days and every day over 14 days

Conclusions

- DCA is effective at killing *B. burgdorferi* in culture
- It is possible that DCA breaks down after 2 weeks in solution
- *Borrelia burgdorferi* might grow better in a strictly anaerobic environment

Results

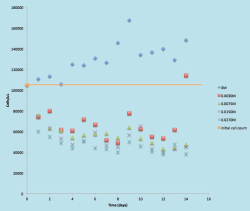


Figure 1. *Borrelia burgdorferi* B31 exposed to DCA and counted daily for a 14-day growth period

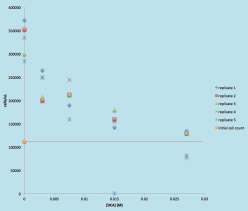


Figure 2. *Borrelia burgdorferi* B31 exposed to DCA, 14-day growth period

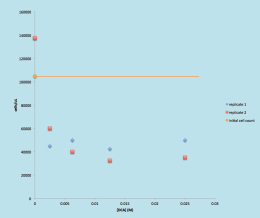


Figure 3. *Borrelia burgdorferi* B31 exposed to DCA, 14-day growth period

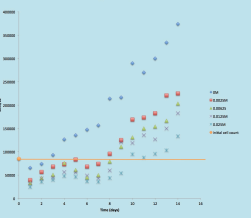


Figure 4. *Borrelia burgdorferi* B31 exposed to DCA and counted daily for a 14-day growth period (culture contaminated)

- *B. burgdorferi* grew considerably better without DCA
- Cell death was proportional to the concentration of DCA in culture
- Contaminated cultures grew considerably better, possibly due to oxygen depletion
- Newer starting cultures of *Borrelia burgdorferi* B31 showed more consistent cell death when exposed to DCA

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Acknowledgements

Dr. Lloyd and the Mount Allison Biology Department, the Canadian Lyme Disease Foundation, Wayne

Percephone Miller
Supervisor: Dr. Fiona Black, Department of Religious Studies

Alternative Faith Based Treatments and Lyme Disease

This project explores the question “what do people do when tradition health care and medicine fails them?” In other words, what are the ways in which people with Lyme disease seek their support for their illness, and how does faith or spirituality figure into treatment or support? More broadly, the idea is to develop a broader understanding of the ways in which people cope with the rejection of traditional health care. The project involves interviews with people with Lyme Disease, people with symptoms similar to Lyme Disease but not diagnosed as such, and with medical practitioners.



Researcher: Percy Miller
Supervisor: Dr. Fiona Black

The Impact of Religion and Spirituality on Coping with Lyme Disease

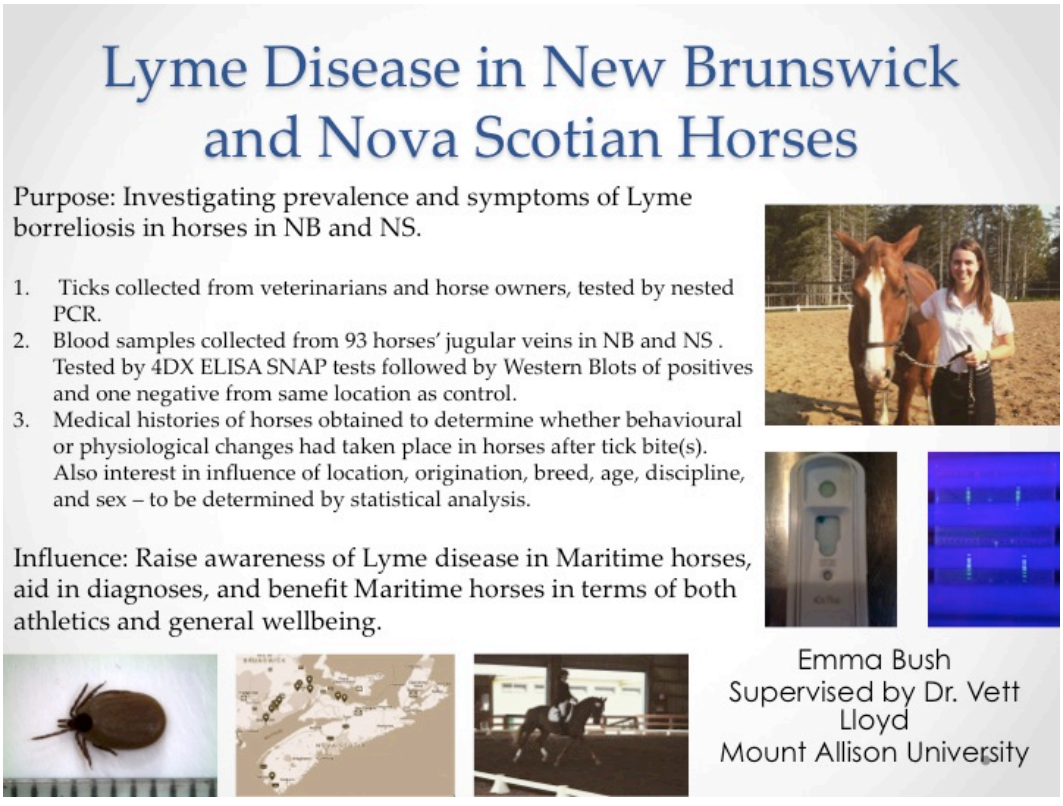
My central question concerned the use of religion and spirituality as a coping tool for people with Lyme disease. To investigate this relationship, I interviewed 12 participants from ages 40-65 originating from a variety of Canadian provinces (Ontario, Saskatchewan, Nova Scotia, New Brunswick). The interviews consisted of a combination of pre-prepared and open questions that concerned the following: the participants’ relationship to their faith/spirituality, how this factored into coping with Lyme disease, what material objects or rituals were involved in their coping, and other emergent questions brought about through the interview. Once I finished the interviews, the contents were analyzed in depth and major themes were determined. These themes include **Illness as part of God’s plan, Surrender, an Important Relationship to Nature, Isolation, Self-Improvement and Material Engagement**. Other findings of my project include an analysis of modification of R/S practices, how Lyme disease advocacy work factored into the participant’s process, and theoretical examination of issues like pain, social media, and stigmatization. My report also details the biblical verses, podcasts, books, media sources, and other material elements that were featured in participants’ coping process. From these results, I drew the conclusion that the support of spirituality/religion as a social infrastructure and coping tool is crucial for religiously/spiritually inclined people with LD. It provided a means of comfort, support (both individual and communal), increased hope, and alleviated some of the stress related with LD. In addition, my project also explored where religious and spiritual institutions may be lacking in their ability to support PWLD, further implications for the discipline of religious studies, and what these findings suggest for the betterment of Lyme disease treatment.

Emma Bush
Supervisor: Dr. Vett Lloyd, Department of Biology

Lyme Disease in New Brunswick Horses



This project explores the prevalence and symptoms of Lyme disease in horses in New Brunswick, the correlation between general horse health and Lyme disease, and the accuracy of two types of equine Lyme disease blood tests. New Brunswick has a large horse community with horses ranging from backyard pets to high performance animals. Anecdotal reports indicate that horses are becoming ill with Lyme disease, but there is no formal documentation of the presence, frequency, or effects of Lyme disease in New Brunswick horses. The study has three components: 1) Analysis of the infection status of ticks found feeding on horses; 2) Testing the blood of these horses, as well as control animals, for an immune response to disease; 3) Correlation of equine immune status to animal health and ability.



Leveraged / Matching Funds

The generous contribution of the Canadian Lyme Disease Foundation resulted in an additional $40,000 worth of research funds expended on Lyme Disease research, plus $4,000 worth of in-kind support.

- New Brunswick Environmental Trust Fund, $24,000 research project funds awarded to David Lieske and Vett Lloyd to develop an internet portal and web-based GIS system to bring together tick distribution and infection data.
- Mount Allison University (Department of Biology), $7,000 student stipend support for a research project on Lyme Disease.
- Mount Allison University (Department of Geography and Environment), $3,000 purchase of GIS software.
- Mount Allison University (research grants held by Vett Lloyd and Stephen Westcott), $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.

The announcement of the Mount Allison and Canadian Lyme Disease Foundation partnership garnered significant media interest, as noted below. The individual projects are also anticipated to receive attention as the projects are finalized.



Media requests (and subsequent interviews) related to the announcement:

CBC Moncton (radio)
CBC NB (TV)
Radio-Canada (radio)
CTV Atlantic (TV)
Global TV (TV)
Moncton Times and Transcript (newspaper)
Eastern Graphic (newspaper)
CHSJ Radio (radio)