

Lyme disease in Eastern Canada: Disease burden and serology

BY

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Abstract

Lyme disease is of growing concern in Canada and multiple countries across the world as climate change is creating an increasing number of favourable environments for ticks to migrate to. With this expansion in territory comes an increased risk of Lyme disease in many new areas. Despite this increase in risk, there remains a gap in knowledge both in the public and the medical field about diagnosis, testing, and treatment. Testing in Canada follows a two-tiered serology beginning with an enzyme-linked immunoassay followed by a western blot if the previous test is positive. However, these testing methods have proven to be flawed depending on the stage of infection, as well as the seroconversion ability of a person's immune system. All data were collected from a population of healthy and ill individuals who filled out a questionnaire with demographic information as well as symptom evaluations and provided blood samples to be tested. We first looked at the relationship between the ELISA and western blot serological results to determine whether there was a correlation. As they are both testing for antibodies against *Borrelia burgdorferi*, the expectation was that they would be positively correlated. There was no relationship found between the ELISA and IgM western blot but a significant positive relationship with the IgG western blot. This was followed by looking at the relationship between these serological methods and the disease burden of participants where we were expecting to see a positive correlation. The ELISA and IgG western blot results versus disease burden showed a significant negative correlation contrary to predictions, whereas the IgM western blot showed a non-significant slightly positive correlation. Further results were obtained by looking at the relationship between disease burden versus seroconversion class and the number of previous diagnoses. Where there was a significant difference in disease burden in the IgG and IgG+IgM classes compared to healthy individuals. Finally, the effect of other factors such as sex and treatment on disease burden were studied. There was a significant effect of sex on disease burden with women scoring higher than men and a significant difference between groups that had been treated or not, with those treated still presenting with higher disease burden. The results of this study demonstrate the inability of serological methods to be used as the sole diagnostic tool for Lyme disease and give insight to the ineffectiveness of current treatment methods in Canada.

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Table of Contents

Abstract	ii
Acknowledgements	iii
Introduction	1
1.1 Ticks and the history of Lyme disease	1
1.2 Pathogenesis	2
1.3 Lyme disease	3
1.4 Testing and diagnosis	4
1.5 Treatment	7
1.6 Prevention	8
1.7 Study	9
Methods	10
2.1 Participants	10
2.2 Questionnaire	10
2.3 Symptomology	11
2.4 Serology	11
2.5 Statistical analysis	12
Results	12
3.0 Goal of the study	12
3.1 Characterization of study population	13
3.1.1 Population demographics and symptoms.....	13
3.1.2 Disease Burden.....	14
3.1.3 Other diagnoses.....	15
3.2 Correlation between ELISA serology and western blot	17
3.3 ELISA and WB serology as symptom predictors	19
3.5 Treatment	23
Discussion	24
4.0 Goal of the study	24
4.1 Characterization of study population	24
4.1.2 Disease burden.....	25
4.1.3 Other diagnoses.....	25
4.2 ELISA and western blot serology correlation	26
4.3 ELISA and western blot as symptom predictor	27
4.4 Seroconversion and Symptomology	29

<i>4.5 Treatment and sex</i>	30
<i>4.6 Implications and future directions</i>	31
<i>Literature cited</i>	33
<i>Appendix</i>	39
<i>5.1 Participant questionnaire</i>	39

List of Figures

Figure 1.1.1 <i>Lyme disease risk areas in Canada</i>	1
Figure 1.2.1. <i>Pathogenesis of Lyme disease from tick to human</i>	3
Figure 1.4.1. <i>The two-tiered serological testing system currently used in Canada</i>	5
Figure 3.1.1. <i>Normalized symptom count of participants across organ systems</i>	14
Figure 3.1.2. <i>Normalized disease burden for each organ system</i>	15
Figure 3.1.3. <i>Other diagnoses participants have received and the number of participants that have received each diagnosis</i>	16
Figure 3.1.4. <i>Relationship between total symptom scores and the number of other diagnoses participants have received</i>	17
Figure 3.2.1. <i>ELISA serology results versus western blot serology</i>	18
Figure 3.3.1. <i>Correlation between disease burden and ELISA serology</i>	20
Figure 3.3.2. <i>Correlation between disease burden and IgG western blot serology</i>	21
Figure 3.3.3. <i>Correlation between disease burden and IgM western blot serology</i>	21
Figure 3.4.1. <i>Effect of seroconversion on disease burden</i>	22
Figure 3.5.1 <i>Effect of treatment on disease burden</i>	23

List of Tables

<i>Table 1. Characterization of study population.</i>	13
<i>Table 2. Correlation between serological results and disease burden.</i>	19
<i>Table 3. Significant differences between treated and untreated groups when accounting for sex.</i>	24

Introduction

1.1 Ticks and the history of Lyme disease

Climate change is of growing concern for many reasons; one of them being the facilitation of population expansion and dispersion of arthropods (Gage et al., 2008). Ticks are no exception and their habitat in Canada is quickly expanding due to more favourable conditions (Figure 1). Increasing temperatures allow the ticks to move further north as well as increase their active period (Bouchard et al., 2019). Many tick species can be found around the world that transmit numerous viral, bacterial, and protozoan pathogens (Bouchard et al., 2019). In Canada, there are two primary vectors: the black-legged tick (*Ixodes Scapularis*) and the Western black-legged tick (*Ixodes pacificus*). These ticks are the primary vectors of Lyme disease in the eastern and western regions of Canada, respectively. They are also carriers of Anaplasmosis, Babesiosis, Powassan virus, and *Borrelia miyamotoi* (Bouchard et al., 2019).

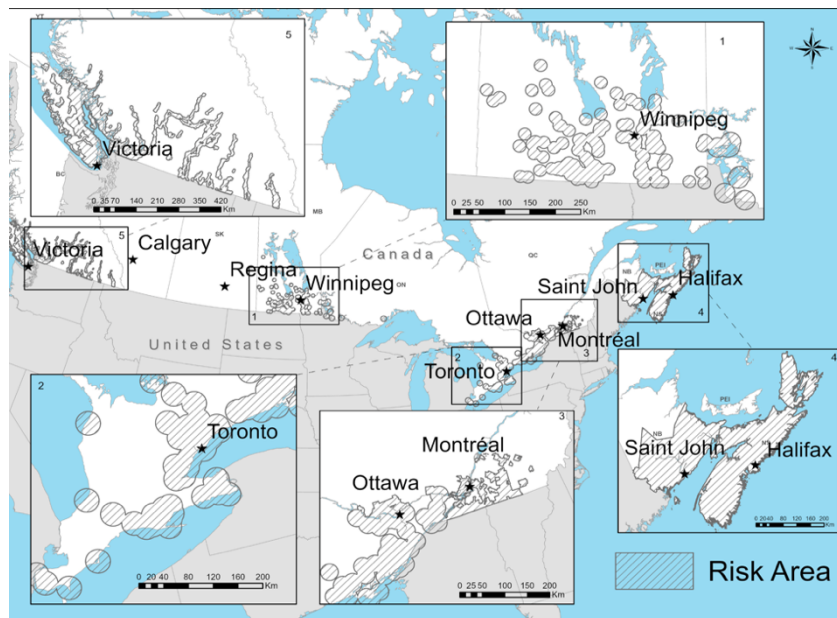


Figure 1.1.1 Lyme disease risk areas in Canada. From Canada Public Health. <https://www.canada.ca/en/public-health/services/diseases/lyme-disease/surveillance-lyme-disease.html>

Research around Lyme disease remains a small field in Canada creating an overall lack of expertise about the disease in the medical field as well as a lack of knowledge in the general population. The disease was first discovered in Lyme, Connecticut when two

mothers noticed increasing rates of rheumatoid arthritis in their children and surrounding families. They then involved scientists Allen Steere and Stephen Malawista from the rheumatology department at Yale. These families lived in rural, wooded areas and many of the infected people presented with a bull's eye rash. It was believed that ticks were the vector of the disease when the scientists observed a larger incidence of infections on one side of the river where deer and deer tick populations were larger than the other and this vector was later confirmed (Elbaum-Garfinkle, 2011).

1.2 Pathogenesis

Lyme disease is caused by the spirochete bacteria *Borrelia burgdorferi*. The bacteria originate in small rodents and are acquired by ticks during feeding periods. The bacteria can then be transmitted to mammals and humans during subsequent feedings. A tick's life cycle is characterized by four stages: egg, larval, nymphal and adult (Radolf et al., 2012). The tick may become infected during any of these stages, however, it is in the nymphal stage that the transmission of *B. burgdorferi* occurs from reservoir animals to ticks (Dennis and Hayes, 2002).

When a tick feeds on a human, it is during this period that the bacteria may be transmitted (Piesman et al., 2001). The likelihood of a person getting Lyme disease is reduced if the tick is promptly removed, or if infection occurs, it is recognized and treated quickly (Mazori et al., 2015). However, this does not always occur, and it is in these cases that the bacteria begin to replicate and disseminate throughout the body. It is commonly stated that the risk of infection is significantly decreased if the tick is removed within the first 48h, however, this timeline varies across studies. Moreover, evidence has shown the possibility of transmission within the first 16h of attachment in animal models (Kahl et al., 1998). During the transmission process, the bacteria must alter its gene expression to adapt to its new environment within the human host. It must adapt to increasing temperatures, a new pH, and different nutrients as well as defend itself from the host's immune system (Radolf and Samuels, 2021). *B. burgdorferi* has many mechanisms that allow it to adapt to these changing environments and that allow it to evade the host's immune system (Coburn et al., 2021). Once the tick has successfully transmitted the bacteria into the host, the bacteria begin to replicate and spread through the skin sometimes causing a rash around the

site of the bite. The bacteria will continue to spread through the blood and/or lymph while continuing to replicate moving into tissues leading to initial symptoms. If the bacteria are successful in crossing the blood-brain barrier they will initiate an inflammatory response throughout the brain (Coburn et al., 2013) (Figure 1.2.1).

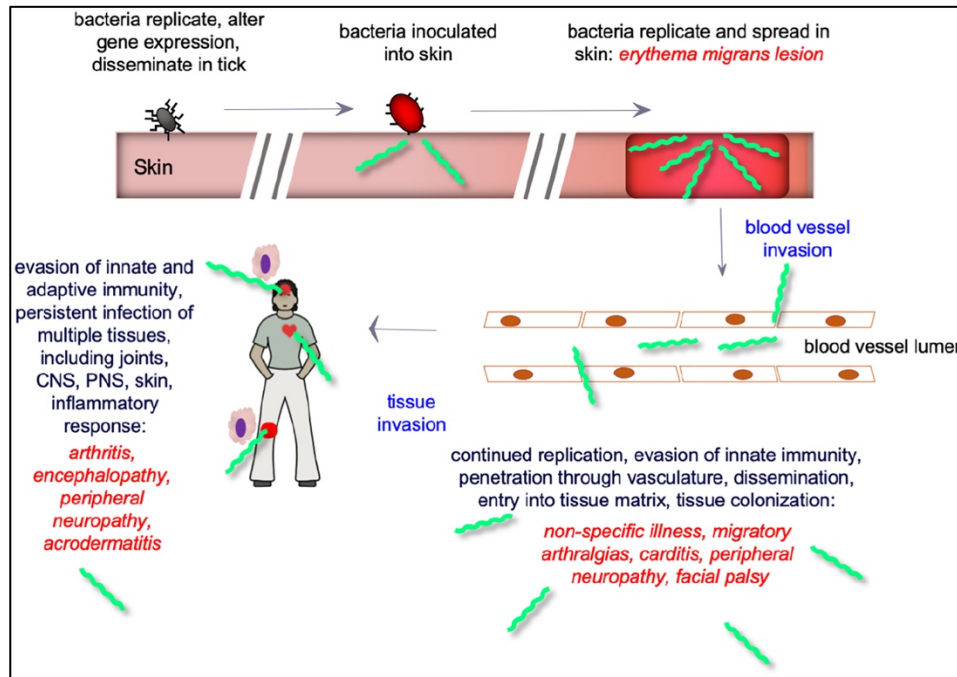


Figure 1.2.1. Pathogenesis of Lyme disease from tick to human (black text) and the symptoms associated with each stage (red text). This figure has been adapted from Coburn et al. (2013).

1.3 Lyme disease

The progression of Lyme disease in the human body can be separated into three stages. The first is the early localized stage which occurs after a tick bite, which may be characterized by an erythema migrans at the site of the bite and with flu-like symptoms such as a fever. If present, the rash will appear in the weeks following the transmission of the bacteria and may appear as a bullseye (Cardenas-de la Garza et al., 2019). The second stage is the early disseminated stage where the bacteria disseminate throughout the body by way of the bloodstream or lymphatic system. Symptoms present at onset will begin to worsen and cardiac and arthritic symptoms will begin to appear due to the invasion of bacteria in the vascular system and connective tissue. Finally, there is the late disseminated stage at which point joint pain has become a recurring and consistent symptom along with

many neurological manifestations including memory loss, mood swings, fatigue, and brain fog. At this stage, the bacteria have successfully invaded most organs and tissues and crossed the blood-brain barrier affecting the central nervous system (Steere et al., 2004).

Patients may receive treatment at any point during these stages, however, the bacteria may persist, or people may continue to experience symptoms due to treatment failure which can occur in approximately 10-35% of patients who fail to receive treatment at the appropriate time (Johnson et al., 2018). In some cases, people do not receive treatment due to difficulty in diagnosing the disease and finding a Lyme literate doctor, who is someone with experience and knowledge of Lyme disease. If a person has been treated but symptoms persist, this is commonly referred to as Post-treatment Lyme Disease Syndrome (PTLDS), which remains somewhat of a mystery. However, there are some hypotheses as to why symptoms persist. These include the persistence of symptoms due to the resistance of the bacteria to antibiotics, as well as the continuing activation of the immune and inflammatory responses (Bobe et al., 2021; Sapi et al., 2011). These patients are likely to experience declines in their social, work, or family lives diminishing their overall quality of life (Geebelen et al., 2022). PTLDS is commonly used interchangeably with the term chronic Lyme. However, it is important to note that the progression of the disease can either occur due to failure of treatment or lack thereof. Chronic Lyme disease more commonly refers to cases of untreated patients where the bacteria have successfully evaded the immune system and moved into the late disseminated stage as patients face the issue of receiving timely diagnoses (Mead, 2015). Moreover, whether it be through lack or failure of treatment the persistence of *B. burgdorferi* has been found to alter immune function leading to autoimmune illnesses like Lyme arthritis (Gutierrez-Hoffmann et al., 2020).

1.4 Testing and diagnosis

Serological testing for diseases can either occur directly where the bacteria/virus is measured or indirectly by measuring antibodies for the bacteria/virus (Branda and Steere, 2021). The common testing method involves a two-tier serology including an enzyme-linked immunoassay (ELISA) followed by a western blot if the ELISA presents as positive or equivocal. These tests are indirect detections of *B. burgdorferi* as they are measuring

antibodies and not the bacteria itself. Direct testing methods include culture and PCR of tissue and/or blood samples, however, they are less sensitive in the early and late stages (Dumler, 2001; Liveris et al., 2012). The diagnosis of Lyme disease is rendered difficult due to many factors. The first being the serological testing required for a diagnosis. The current method in Canada, the United States and Europe involves the two-tiered test of an ELISA followed by a western blot (Figure 1.4.1).

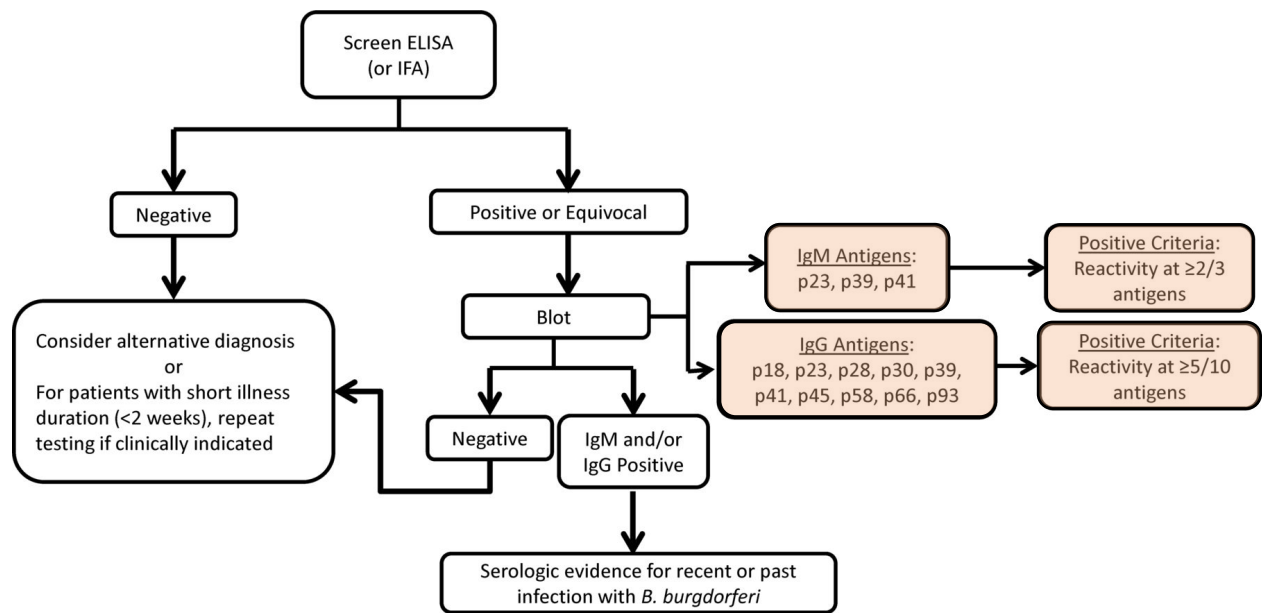


Figure 1.4.1. The two-tiered serological testing system currently used in Canada. On the far right (in orange) is the interpretation of IgM/IgG western blot criteria for a positive result. This figure is adapted from Theel (2016).

One issue with detecting antibodies is that they take time to be produced by the body. Therefore, during early infection, the tests may not be sensitive enough to detect the antibodies and may present as a false negative (Bobe et al., 2021). Once antibodies have been produced in larger amounts, the tests become more accurate, however, the bacteria have then had the opportunity to disseminate within the host (Bobe et al., 2021). The tests have also shown a lack of sensitivity following short-term antibiotic treatment and they are limited when it comes to detecting active versus past infections (Bobe et al., 2021). This means that anyone that may have been treated for Lyme disease or other illness, but not necessarily enough to limit infection, may not present with a positive test despite the bacteria being present since the treatment may affect antibody production (Pachner et al.,

2001). Therefore, relying on serology alone is not an effective method of diagnosis and false negatives are likely to occur in situations such as early infection and immune exhaustion. Immune exhaustion can occur in cases of chronic Lyme disease where the chronic activation of the immune response, specifically T cells, may lead to their unresponsiveness to antigen stimuli (Khaitan and Unutmaz, 2011). As the immune response decreases due to dysfunctional T cells, there will be fewer antibodies produced and therefore may not reach the threshold for serological testing to be positive.

Finally, the interpretation of the test results can vary between medical groups and physicians. The Western Blot results produce bands when the bacteria are present, however, results depend on the number of bands present and which of the bands are present. The current interpretations of bands from western blots are largely guided by CDC recommendations. According to the CDC, for a case to be positive, two of three bands in the IgM blot and five of ten bands in the IgG blot must be present (CDC, 1995). When it comes to the IgG blot, another problem arises since some of the bands can also represent other binding antibodies from a different illness, however, this problem is largely eliminated by the requirement of many bands to be present which has driven the criteria to be highly specific but with decreased sensitivity. Although specificity is increased, there is always the possibility of false positives as other diseases may cross-react with the serological tests (Waddell et al., 2016).

With testing not always being accurate, physicians required to heavily rely on other diagnostic methods such as presenting symptoms, as well as tick exposure, to clinically diagnose a patient (Tatum & Pearson-Shaver, 2015). This clinical diagnosis typically relies on the presence of an erythema migrans at the site of the bite. However, using the erythema migrans as a form of diagnosis can be misleading as it does not always appear (Cameron et al., 2014). In Canada, if a person presents with an erythema migrans and has been recently exposed, they may be diagnosed without serologic tests to ensure prompt treatment, however, they must seek and/or have access to proper care (Government of Canada, 2022). If a person does present with flu-like symptoms without the erythema migrans it does not mean that they are guaranteed to not be infected. However, the recommendation is to send in an acute and convalescent sample for serological testing to confirm the presence of Lyme disease, yet this rarely occurs (Government of Canada, 2022).

Another issue that arises when serological testing is not done or not sensitive enough is misdiagnosis. Patients suffering from Lyme disease typically receive many other diagnoses prior and are commonly misdiagnosed. Some misdiagnoses include early ALS, fibromyalgia, chronic fatigue syndrome, MS, and Parkinson's (Dressler et al., 1993; Nettleton et al., 2005). This misdiagnosis allows the bacteria the time to further disseminate within the host's body and trigger further symptoms. Finally, it is important to consider the possibility of co-infection when testing for Lyme as there is a chance, they may also be infected with a second bacteria. Since ticks are known to be carriers of many pathogens, co-infections are likely and being aware of proper treatment for all possible bacteria will ensure a better outcome for the patient (Berghoff, 2012).

1.5 Treatment

When treated promptly after exposure, dissemination of the bacteria within the body can usually be prevented (Johnson et al., 2018). While this would be the ideal situation, there remains a gap in knowledge within the medical community in Canada leading to higher rates of undiagnosed patients and therefore lower rates of treatment for Lyme disease (Ferrouillet et al., 2015; Sperling et al., 2012). The provision of proper treatment of Lyme disease in Canada is not only hindered by flawed testing but also due to the current testing protocol, which is supported by older, lower-quality evidence.

Two treatment guidelines have been developed by professional organizations: the International Lyme and Associated Diseases Society (ILADS), and the Infectious Diseases Society of America (IDSA). These guidelines differ in recommended duration of treatment and acknowledgment of persistent (chronic) infection.

Following a tick bite, the IDSA recommends that the tick be sent for species identification but does not recommend testing for *B. burgdorferi* in the tick since this information is not reliable for predicting whether a person will be infected. It is also important to note that testing the tick will not necessarily predict if a person will have Lyme disease as there are other determinants such as how long the tick has fed. The IDSA does not recommend testing for Lyme disease in asymptomatic people who have had a known tick bite and only recommends antibiotic prophylaxis to those who have been bit by a tick of the Ixodes species, from an endemic area and that was attached for more than 36

hours. Those who do not meet the three criteria are recommended to continue watching for symptoms. However, all these considerations, as well as the stress involved in waiting for an illness to arise can be problematic in achieving the best health outcomes for individuals. In contrast, the ILADS guidelines strongly recommend that diagnosis of Lyme disease be done on a clinical basis as the common serological testing, ELISA and western blot, are unreliable. Although they do not recommend using serological results as the primary diagnostic tool, they suggest using it to support clinical evaluation. As Lyme disease symptoms can imitate those of many other diseases, ILADS recommends a comprehensive evaluation of patients to ensure proper diagnosis and course of treatment (International Lyme and Associated Diseases Society, 2022).

A major difference between the two organizations is the guidelines for the duration of treatment. The IDSA believes in a short-term treatment such as a 10-day course of doxycycline, whereas the ILADS proposes a minimum of 4-6 weeks of treatment. An explanation for these opposing recommendations comes from beliefs about the *B. burgdorferi* bacteria persistence within a person. The ILADS guidelines accept that the bacteria can survive short-term treatment whereas the IDSA argues that persistent symptoms are caused by factors other than the bacteria and refer to the ongoing illness as Post-Treatment Lyme Disease Syndrome.

The long-term antibiotic treatment proposed by ILADS has been a controversial topic with debate on whether it is beneficial. The ILADS argues that fewer than 20 days of treatment when an erythema migrans is present, is curative in 52.2% to 84.4%, indicating that between 16-48% of patients have failed treatment which may lead to the infection progressing further to become chronic (Cameron et al., 2014). Although, they recommend long-term antibiotic treatment and adjusting treatment to each individual. More research must be done to understand the benefits and pitfalls of both short-term and long-term antibiotic therapy in patients with Lyme disease.

1.6 Prevention

While there may be a lack of consensus on diagnostic and treatment protocols, prevention protocols remain similar across organizations. The first step in the prevention of any disease is educating the general population as well as the medical staff. It is important

that practicing physicians are educated on the proper recognition, diagnosis, and treatment protocols for Lyme disease in Canada so that they can provide effective care to possible patients. It is also important to educate individuals on tick habitats, how to avoid tick bites and how to search for ticks. Simple prevention methods include wearing repellents when going into wooded and grassy areas as well as checking for ticks after returning from those same places.

1.7 Study

Lyme disease only became a nationally notifiable disease in Canada in 2009, contributing to an overall lack of research. Cases have increased from 144 in 2009 to 3147 in 2021 and while this is a dramatic increase, under-detection is significant, estimated at approximately ten-fold nationally (Lloyd and Hawkins, 2018). Lyme disease research presents large gaps as well as controversy and as its rates increase further research and education must be done on the disease.

Within the Canadian healthcare system there is a lack of consensus when it comes to Lyme disease diagnostics and treatment. Many people presenting with possible Lyme disease have gone outside of Canada in hopes of receiving an accurate diagnosis and/or treatment (Boudreau et al., 2018). People have looked elsewhere for treatment believing they have been misdiagnosed or their condition has not improved despite multiple consultations with various specialists (Boudreau et al., 2018). Acquiring treatment and diagnosis for Lyme disease is not made easy by a lack of knowledge in the area and the persistent debate about effective treatment methods.

This study examines the diagnostic component of Lyme disease and its correlation to disease burden reported by participants. This study aims to determine the correlation between disease burden and serological test results. Symptoms were gathered from both healthy and ill people through a questionnaire. After completing the questionnaire people also provided blood samples used for ELISA testing as well as western blots. The hypothesis is that there will be a positive correlation between disease burden and serological test results since increased serological results indicate an increased infection which would then mean increased disease burden. Furthermore, I will be exploring the interaction between sex, other diagnoses, seroconversion as well as treatment with disease

burden. The results will provide more insight into the value of Lyme disease serology and promote further research in the area to better patient recognition and diagnosis.

Methods

2.1 Participants

Participants were recruited from a variety of groups including Lyme disease support groups that include people with current Lyme disease, treated individuals, those who think they might have Lyme disease but have not had treatment, family members of those with current, past, or suspected Lyme disease, and healthy individuals without likely tick exposure and healthy people occupationally exposed to ticks. Participants were asked to fill out a questionnaire and donate a vial of blood. The blood was taken by a qualified phlebotomist or nurse. There was a total of 153 participants who partially or filled out the questionnaire. There were 157 participants that provided blood samples. Most participants were from the province of New Brunswick, but others were from Nova Scotia and Quebec. For anonymity and confidentiality, data was entered using a code for each participant. These codes were DA-001 through DA-162 and were used consistently across the questionnaire and blood tests. This study was approved by the Mount Allison Research Ethics Board (REB # 103265/2014-028). All participation was voluntary, and participants were made aware that their care would not be impacted if they chose to participate or not. All personal identifying information was stored separately from the survey results and blood samples. The blood samples were identified only by participant code.

2.2 Questionnaire

The first part of this study asked participants to fill out a five-part questionnaire (Appendix 5.1). The first part consisted of demographic information such as age, gender, and the communities in which participants currently live and have lived. The second part was about possible *Borrelia* exposure; this section looked at their occupation, the types of neighbourhoods they have lived in (urban, suburban, or rural), their recreational exposure, possible animal exposure, and finally their sexual exposure. The third section asked participants whether they remembered a tick bite followed by a rash, where on their body a

tick bite occurred, as well as the geographical location of occurrence. Following these questions participants were given a list of symptoms to rate as either none, mild, or severe. Questions from the fourth section of the questionnaire related to their diagnostic history. Participants were asked if they had received a previous diagnosis that shares similar symptomology with Lyme disease, such as fibromyalgia and multiple sclerosis. They were then asked how they were diagnosed and whether it was clinically or serologically, and by whom the diagnosis was made. Participants were then asked questions about Lyme disease. These questions included asking the participant whether they had been diagnosed with Lyme disease, by whom it was done, and whether it was clinically or serologically diagnosed. The final section asked participants to report on whether they have received treatment for Lyme disease. If participants responded yes to having received treatment, they were then asked to specify the treatment received and whether the treatment had any effect on their condition.

2.3 Symptomology

Symptoms were collected across 11 different organ system categories where participants patients rated their symptoms as none, mild, or severe. These results were coded as none = 0, mild = 1, and severe = 2 to provide quantitative data. There were four yes or no questions that were coded as no = 0 and yes = 2. By coding the results, it was then possible to sum the results of each organ system and in total to give the overall disease burden. If a participant failed to respond to an entire section or to a symptom within a section, the answer was coded as 0 and did not contribute to total disease burden. There is a bias in the total disease burden as certain categories include more symptoms, however, for this study, the goal is to obtain results looking at overall disease burden. Therefore, the bias only applies when comparing disease burden of each individual organ system, but results can be normalized to compare each system. The data was normalized to compare each individual organ system.

2.4 Serology

After filling out the questionnaire, participants were asked to provide blood that was used for Lyme disease serological tests. From the participants ($n = 157$) who provided

blood samples, both enzyme-linked immunoassay (ELISA) and immunoblot/western blot (WB) tests were done. The immunectics C6 ELISA kit was used. As whole cell sonicate western blots were used, results were obtained for both the number of bands identified by patient antibodies as well as the intensity of each band, which was summed as the total intensity of all bands detected. There was a total of 20 bands that were tested for the IgG WB and 17 for the IgM WB. Band intensity was measured on a scale of 1-3 where 1 represented a visible band but weaker than the standard, 2 represented a visible band at a similar intensity to the standard, and 3 represented a band that was visible at a greater intensity than the standard. Seroreactivity to multiple pathogens was tested in certain participants ($n = 56$) using the Tickplex Plus to determine the seroconversion class of each participant.

2.5 Statistical analysis

All statistical analyses were performed using R (“R Core Team”, version 4.1.3, 2022). As assumptions of normality and homogeneity of variance were not met across all data, non-parametric statistical analyses were performed. Kendall’s rank test was used to determine the correlation between both types of serologies as well as the correlation between serological results and disease burden of participants. A Kruskal-Wallis test, followed by a Monte Carlo simulation posthoc test, was used to assess the effects of various factors on disease burden. These factors included: sex, seroconversion class, and treatment. Finally, a two-way ANOVA was used to determine the interaction between sex and treatment, and their effect on disease burden. The data was transformed using a square root transformation and was analyzed with a Tukey HSD post-hoc test.

Results

3.0 Goal of the study

The overarching goal of the study is to investigate the relationship between disease burden in Lyme disease patients and the ELISA and western blot serological results. This was achieved by assessing the number of symptoms for each participant as well as their

overall disease burden. Secondary goals included investigating the effects of certain factors such as sex, other diagnoses, seroconversion, and treatment on disease burden.

3.1 Characterization of study population

3.1.1 Population demographics and symptoms

Demographic information about the participants was obtained from the questionnaire (Table 1). The average participant age was 58.42 years old ($SD = 15.10$ years). Participants also indicated whether they remembered a tick bite and if so whether a rash appeared at the site of the bite. A Kruskal-Wallis test showed a significant effect ($p = 0.027$) of sex on the number of symptoms where females presented, on average, with a higher disease burden than men, despite more women having received treatment.

Table 1. Characterization of study population.

Sex	Average number of symptoms	Average disease burden	Remember a tick bite	Rash at site of bite	Received treatment
Female (n = 78)	28	38.55	23	20	20
Male (n = 70)	18	24.53	25	8	7
Unknown (n = 5)	22	31.80	1	1	2

There were 11 organ symptoms for which participants could rate their symptoms: Head, face, and neck; Vision; Hearing; Digestive; Musculoskeletal system; Respiratory and circulatory; Neurologic; Psychological; Mental capability; Reproduction; General well-being. The total number of symptoms were obtained for each category and normalized according to the number of symptoms within each organ system (Figure 3.1.1). The musculoskeletal system presented with the highest normalized total symptom count with other systems showing similar levels of symptoms across participants.

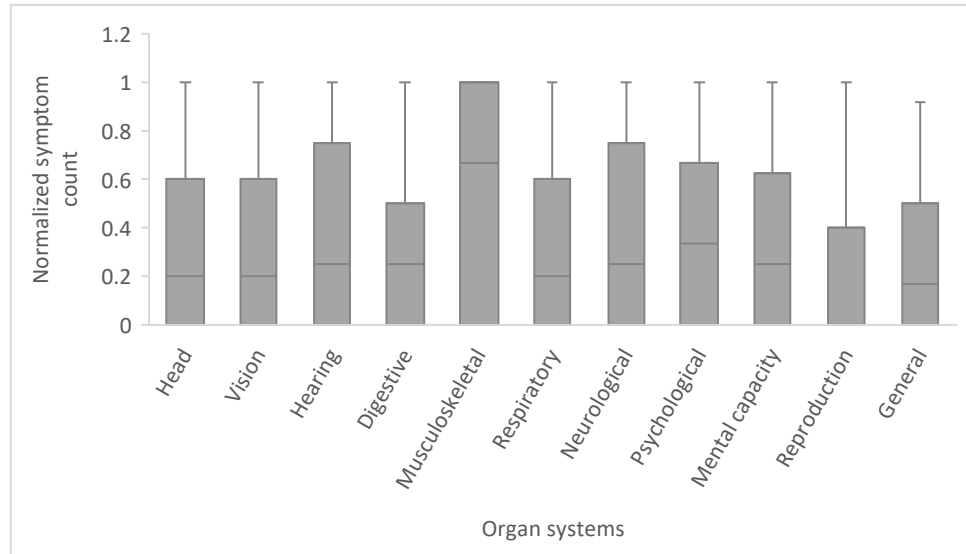


Figure 3.1.1.

Normalized symptom count of participants across organ systems. The grey line in each box represents the median. The lines above the boxes represent the standard deviation.

3.1.2 Disease Burden

Symptoms were scored according to severity to provide insight into the overall disease burden. The lowest disease burden score was 29 and the highest was 133. While the lowest overall score was 29, some participants scored 0 within certain categories.

One area of interest was to determine whether there was one symptom category that generally scored higher than others. For this to be possible, scores across organ systems were once again normalized according to the number of symptoms within each group (Figure 3.1.2).

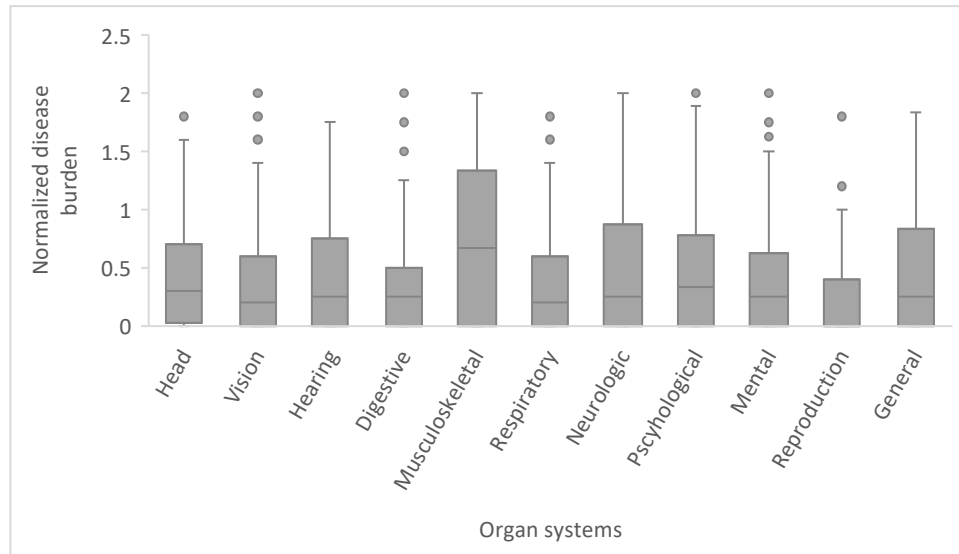


Figure 3.1.2. Normalized disease burden for each organ system. Grey line indicates the median score. The extended lines indicate the standard deviation, and the circles indicate the outliers.

The graph demonstrates that the musculoskeletal system has the highest median and the highest score across all categories of symptoms. The reproductive category showed no evidence of high disease burden with the lowest median and score of all categories. This result is consistent with what was observed when simply looking at symptom count.

The Kruskal-Wallis test showed that there was a significant difference between organ systems for disease burden. The post hoc test showed significant differences between all categories and the musculoskeletal category. The results also showed a significant difference between the reproductive category and the neurological, head, and psychological categories.

3.1.3 Other diagnoses

Of the 153 participants that answered the questionnaire, there were 71 participants who reported having been (mis)diagnosed with one or more Lyme-like illnesses. Many of these participants reported only one other diagnosis but one person reported having received 5 diagnoses. The most common diagnosis was fibromyalgia followed by chronic illnesses labelled as other such as postural orthostatic tachycardia syndrome (POTS) and other syndromes with similar symptomology to Lyme disease (Figure 3.1.3). There were 40 participants that reported having received one other diagnosis, 13 reported two previous

diagnoses, 10 reported 4 diagnoses and only one person reported 5 diagnoses. The hypothesis was that the number of previous diagnoses would correlate with the disease burden.

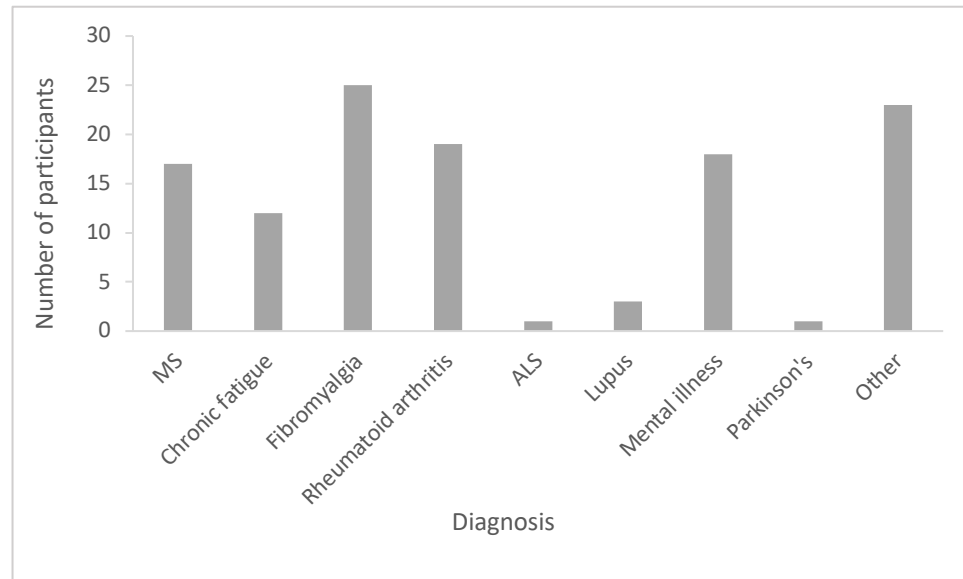


Figure 3.1.3. Other diagnoses participants have received and the number of participants that have received each diagnosis. Fibromyalgia was the most common and ALS, Lupus and Parkinson's were the least common.

When plotting the disease burden versus the number of other diagnoses, a linear trend could be observed, however, there was a lot of variance that lead to the failed assumption of homogeneity of variances. With the use of the non-parametric Kendall correlation test, a significant positive relationship was found between the total number of symptoms and the number of other diagnoses (Figure 3.1.4).

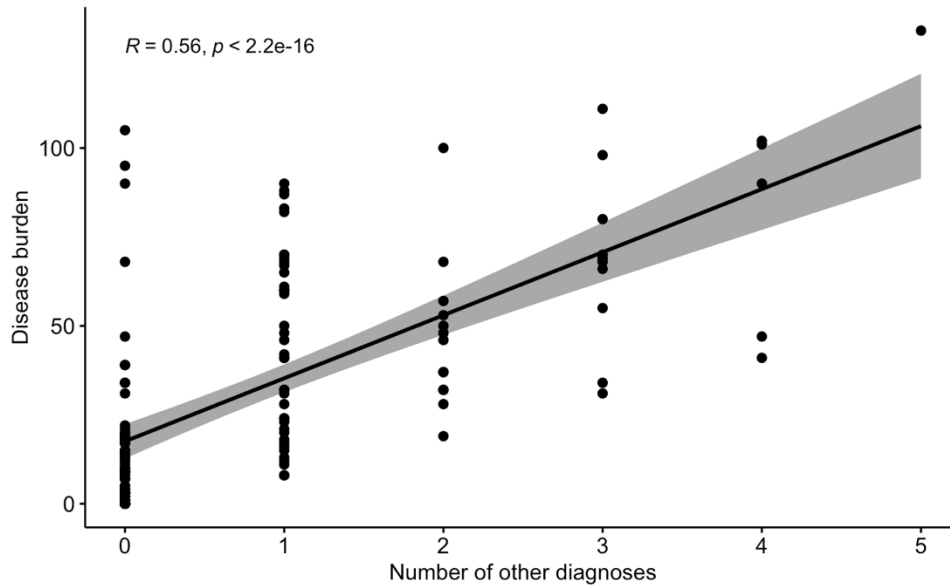


Figure 3.1.4. Relationship between disease burden and the number of other diagnoses participants have received. The black line represents the trendline and the grey shadowing represents the 95% confidence interval. A significant positive relationship was found ($R = 0.56$, $p > 0.001$).

3.2 Correlation between ELISA serology and western blot

For serological tests to be used clinically for diagnosis, it is important that they are accurate. As the current detection method requires both the ELISA and western blot to be positive for a case to be positive, it is important that there be a correlation between the two testing methods indicating that they are in fact giving the same results. Western blot results were quantified as band number and band intensity. Kendall correlation tests were done for both band number and intensity and showed similar results. The relationship between ELISA serology and western blot number of bands for both IgG and IgM can be observed in Figure 3.2.1.

A significant positive relationship was found between ELISA and IgG western blot serology ($z = 4.896$, $df = 155$, $R = 0.28$, $p < 0.001$). A negligible and non-significant relationship was found between the ELISA serology and the IgM western blot using the Kendall correlation coefficient ($z = 1.782$, $df = 155$, $R = 0.1$, $p = 0.075$). These results indicate that there is no correlation between IgM western blot serology and ELISA results, but that as IgG western blot results increase so do ELISA results. These results indicate that ELISA and western blot serology are not always detecting the same levels of infection

across participants. Although, there is some correlation occurring between the ELISA and the IgG indicating there may be similar detection between these two tests, but it remains unpromising.

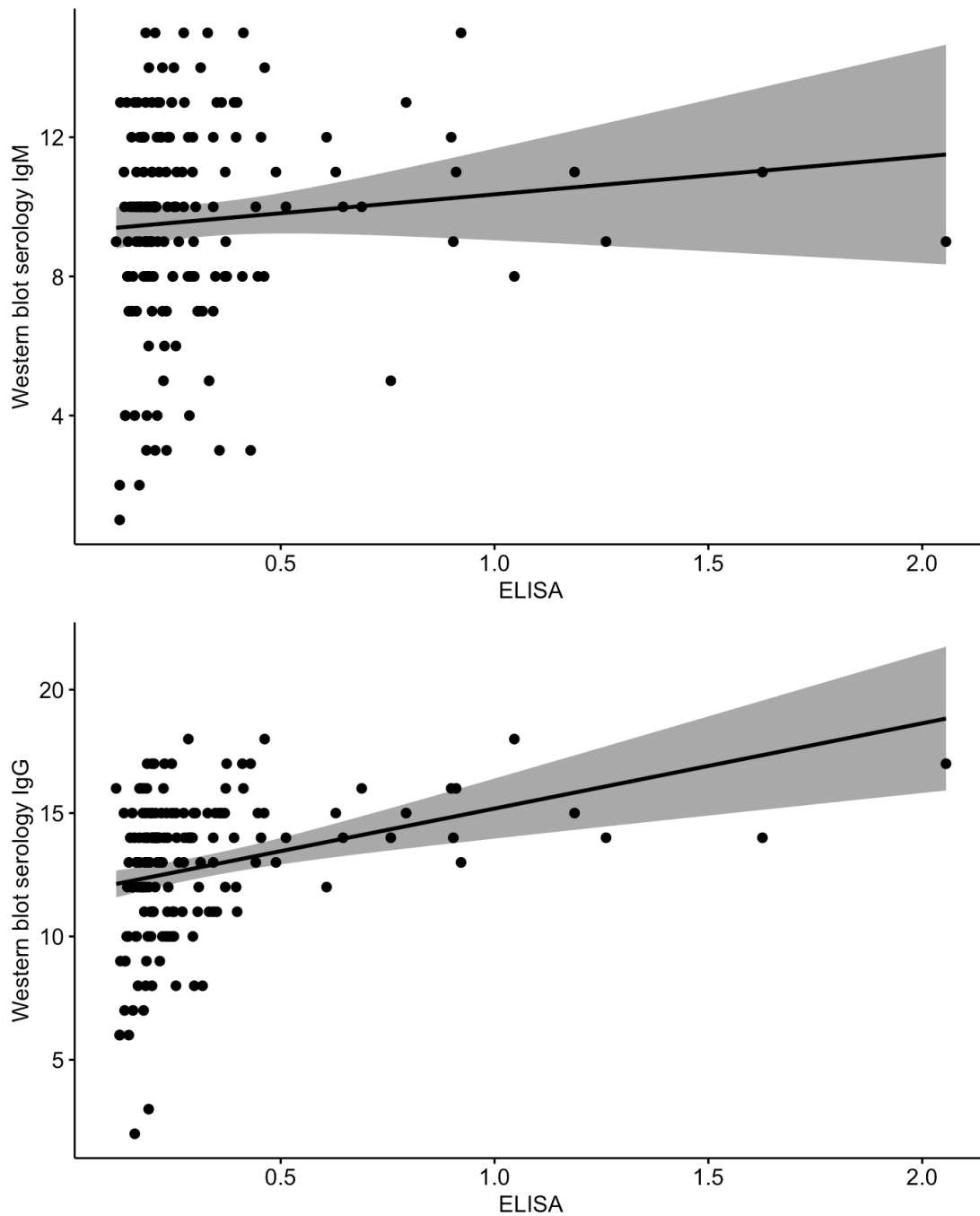


Figure 3.2.1. ELISA serological results versus western blot serology. Grey areas represent the 95% confidence intervals associated with each Kendall correlation test. Top graph: A non-significant relationship was obtained for ELISA versus IgM western blot ($R = 0.1, p = 0.075$). Bottom graph: A significant positive relationship was found between ELISA and IgG western blot serology ($R = 0.28, p < 0.001$).

3.3 ELISA and WB serology as symptom predictors

For serological results to be clinically useful, they should correlate positively with disease burden. Meaning that when serological results increase there should also be an increase in disease burden. Since increased serological results signify a higher level of antibodies and therefore infection, it would be expected that increased infection would lead to a higher disease burden.

As a slightly better correlation was found between the western blot band numbers and the ELISA, these western blots were used for further analysis rather than the results for band intensity. A significant relationship was found between both western blot IgG serological methods and disease burden but not in the IgM serological methods (Table 2). The correlations between the IgG serologies and disease burden were negative while those of IgM serologies showed a positive correlation (Figure 3.3.1.; 3.3.2; 3.3.3). These results indicate that as IgG serological results increase there is a decrease in overall disease burden whereas when IgM serological results increase, overall disease burden increases.

Table 2. Correlation between serological results and disease burden.

	ELISA	Western blot IgG	Western blot IgM
df	151	151	151
z	-2.79	-3.39	1.55
R	-0.15	-0.20	0.14
p-value	< 0.01	< 0.001	0.09

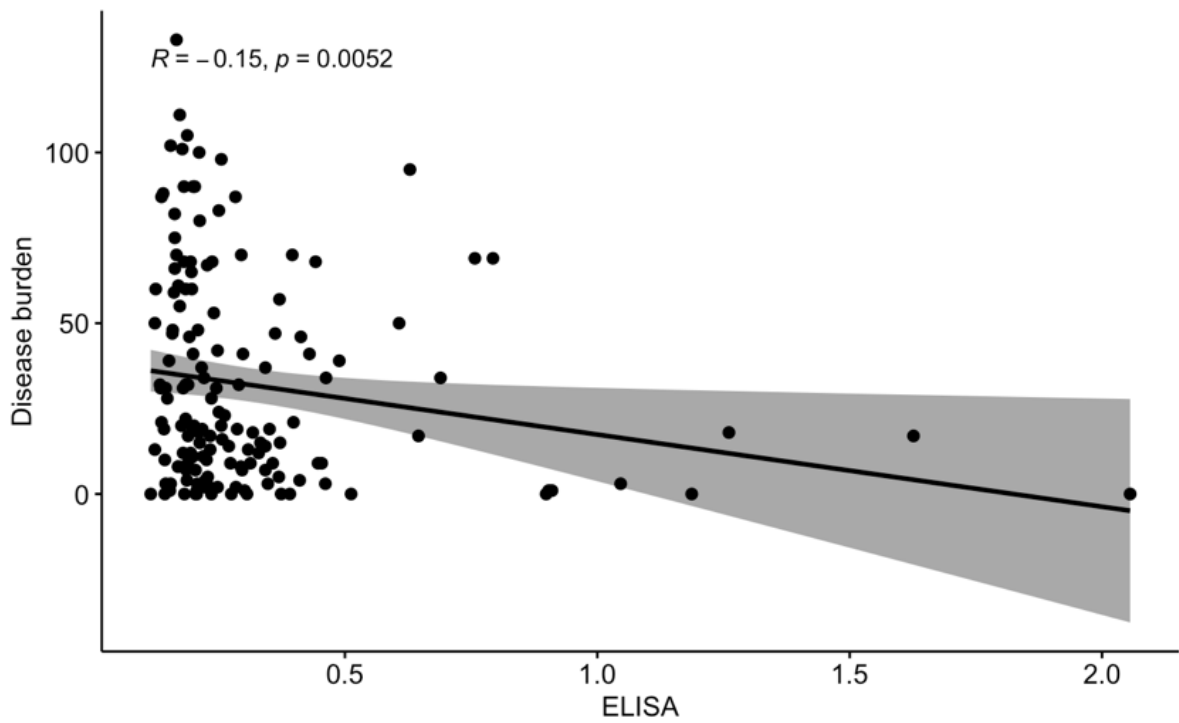


Figure 3.3.1. Correlation between disease burden and ELISA serology. Kendall correlation coefficient was used. Grey areas represent the 95% confidence intervals. A significant negative correlation was found ($R = -0.15, p < 0.01$).

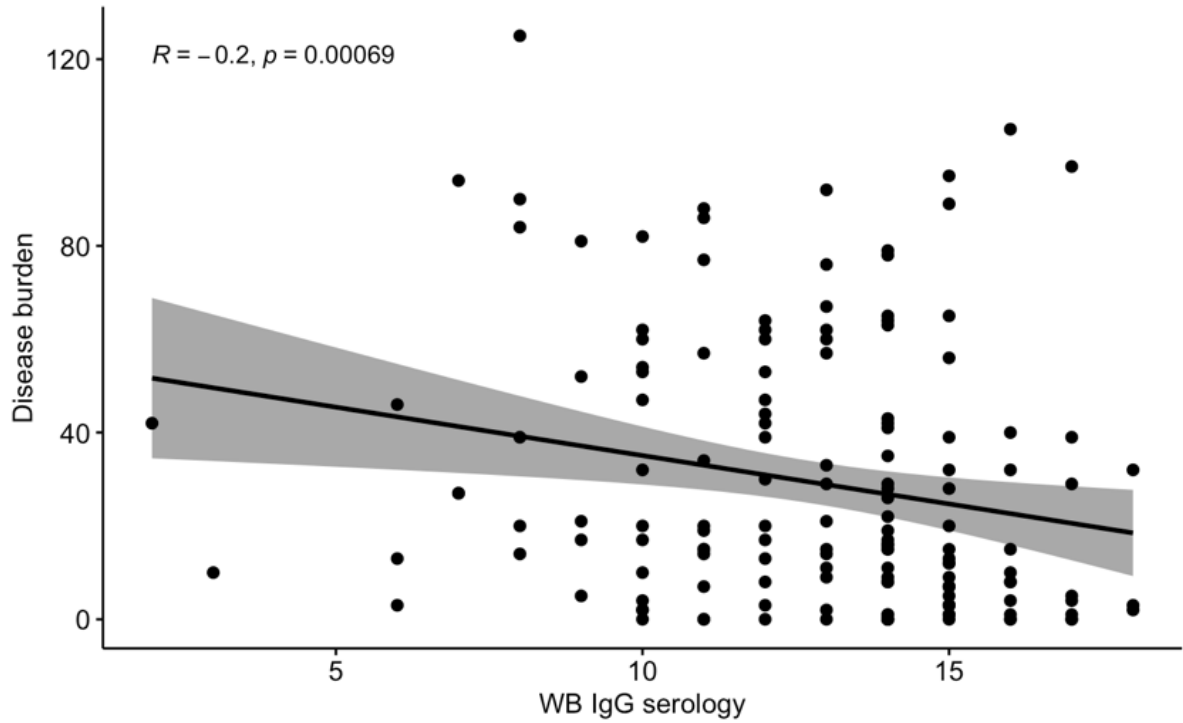


Figure 3.3.2. Correlation between disease burden and IgG western blot serology. Kendall correlation coefficient was used. Grey areas represent the confidence intervals. A significant negative correlation was found ($R = -0.20$, $p < 0.01$).

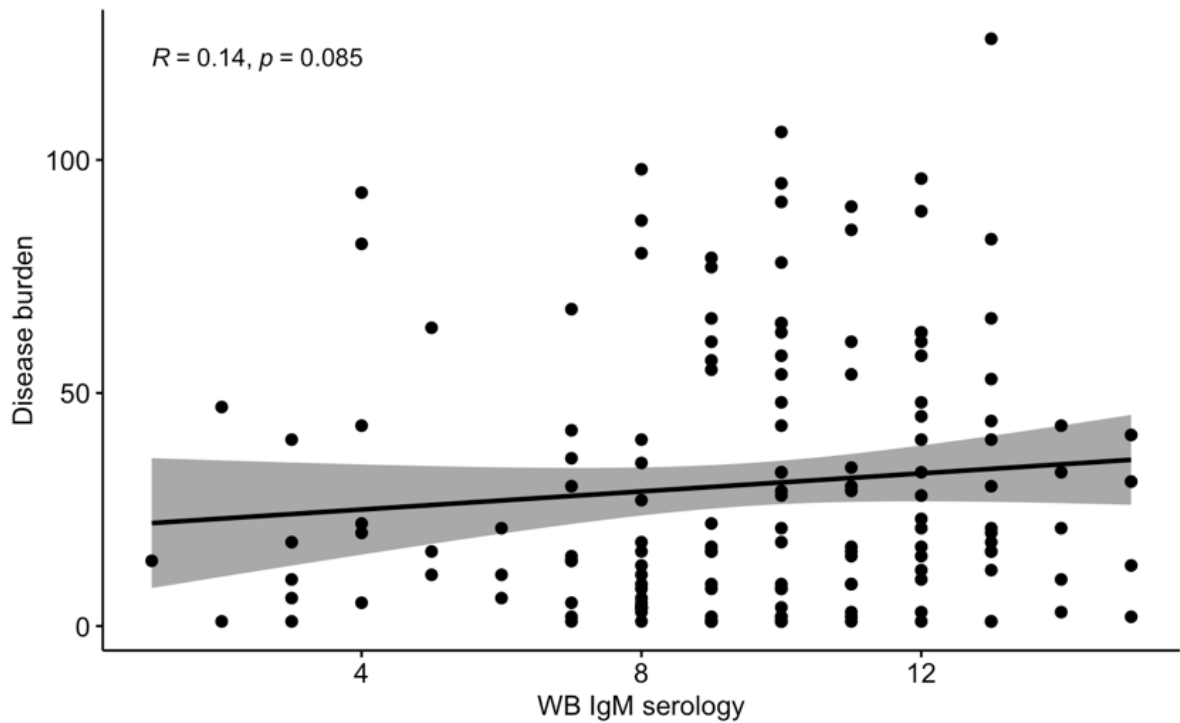


Figure 3.3.3. Correlation between disease burden and IgM western blot serology. Kendall correlation coefficient was used. Grey areas represent the 95% confidence intervals. A non-significant positive correlation was found ($R = 0.14$, $p = 0.085$).

3.4 Seroconversion- IgG class switching

The antibodies produced in response to infection vary, for example the IgM immune response is usually replaced by the IgG immune response over time, a process known as seroconversion. People's ability to produce these classes of antibodies can vary; some people do not transition between the IgM and IgG response, a problem known as impaired class switching. We were interested in knowing if class switching affects symptomology. A subset of the study population (n=56) was tested for IgM and IgG responses to multiple pathogens and participants were grouped according to the antibodies present. There were five possible categories: Healthy, IgG, IgM, IgG+IgM, and non-reactive. A Kruskal-Wallis test was performed to determine whether there was any effect of the ability to class switch on disease burden and there was a significant effect ($p = 0.002$). Post-hoc testing was done to figure out where the differences lay between groups. Significant differences were found between the healthy group and the IgG group, as well as between the healthy group and those who expressed both IgG and IgM antibodies (Figure 3.4.1).

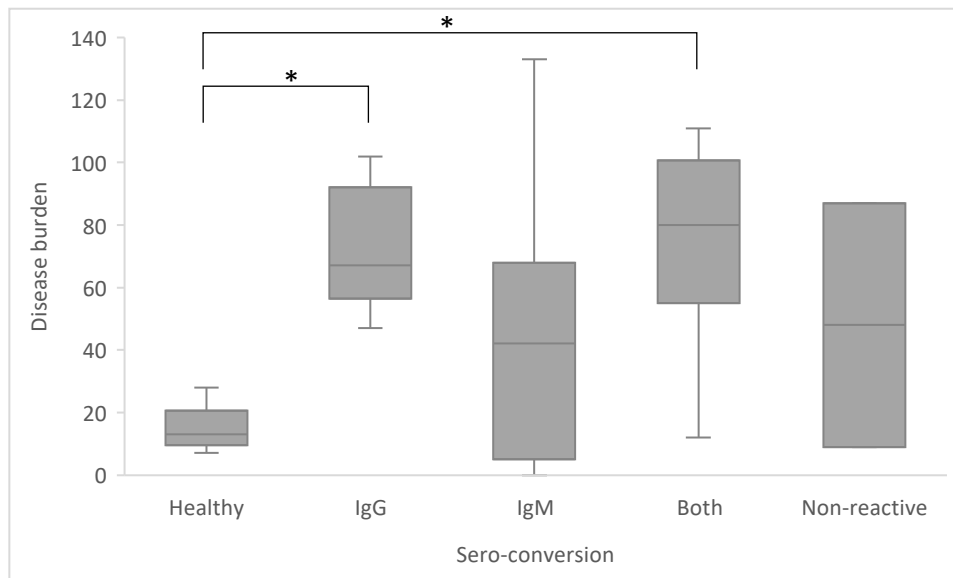


Figure 3.4.1. Effect of seroconversion on disease burden. A significant difference is marked by the * and was found between the healthy group and the IgG group as well as the group presenting with both IgG and IgM. The grey lines across the boxes represent the median whereas the lines out of the boxes represent standard error.

3.5 Treatment

Finally, disease burden was compared between those who had been treated and those untreated to determine whether there was a significant difference (Figure 3.5.1). There was a total of 29 patients that had received treatment. The results indicated that treatment had not successfully decreased disease burden in many participants as there remained a significant difference between those who had received treatment and those who had not ($p < 0.001$).

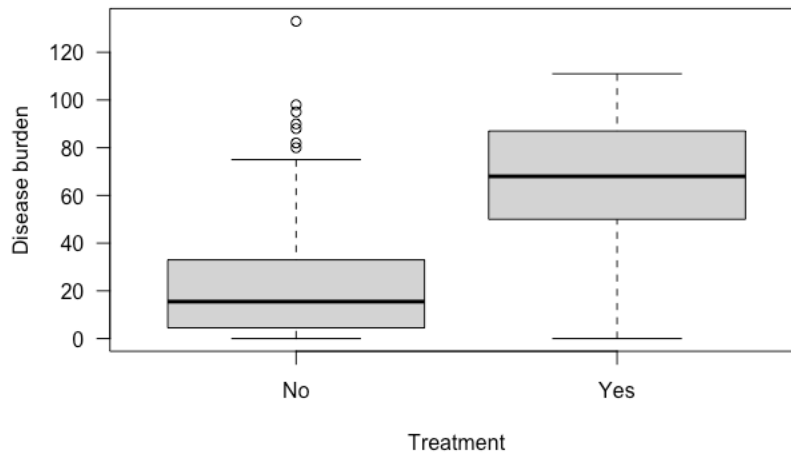


Figure 3.5.1. Effect of treatment on disease burden. The black line represents the median. The lines extending from the boxes represent standard error and the circles represent outliers. A significant difference was found between the two groups ($p < 0.001$).

Next, the interaction between sex and treatment were investigated as well as their effect on disease burden. The two-way ANOVA showed that there was no significant interaction between sex and treatment ($F = 2.18, df = 2, p = 0.12$). However, significant effects of sex ($F = 5.29, df = 2, p = 0.006$) and treatment ($F = 48.7, df = 1, p = < 0.001$) on disease burden were found. Post hoc testing showed that the significant effect of sex occurred between males and females. Despite there being no overall significant interaction between sex and treatment certain significant interactions were found with the post hoc testing (Table 3). These results further demonstrate the discrepancy in disease burden between treated and non-treated participants.

Table 3. Significant differences between treated and untreated groups when accounting for sex.

	Female treated	Male treated	Unknown treated
Female not treated	> 0.001	0.022	
Male not treated	0	> 0.001	
Unknown not treated	> 0.001	0.0013	0.0077

Discussion

4.0 Goal of the study

The main goal of this study was to establish a relationship between serological testing methods and disease burden in Lyme disease. The expectation had been that serological results would demonstrate a positive correlation with disease burden as they are measuring level of infection and increasing infection should indicate increased symptoms. However, this hypothesis was unsupported by the results obtained. Furthermore, the effects of factors such as sex, other diagnoses, seroconversion, and treatment on disease burden were investigated. These factors all demonstrated a significant effect on disease burden.

4.1 Characterization of study population

Lyme disease is known to affect multiple systems within the body, leading to a variety of symptoms. In the early stages, patients will present with general infection symptoms such as fatigue, fever, and muscle aches that are imitative of the flu. If treated promptly, further progression of the disease can be prevented, however, when treatment is not administered soon after infection a progression of the infection, and a higher likelihood of eventual treatment failure, occurs (Johnson et al., 2018). As the disease progresses, the infection spreads into several organs and eventually crosses the blood-brain barrier affecting the central nervous system.

4.1.2 Disease burden

While Lyme disease affects numerous systems, they are not necessarily affected equally. Understanding the organ systems that are most impacted by Lyme disease is important as it can provide guidance when clinically diagnosing the disease. The questionnaire used in this study assessed 11 categories of organ systems with a varying number of more specific symptoms scored within each. The normalized scores from each category were compared to assess whether one organ system was more compromised than others. The results indicated that compromise of the musculoskeletal system, with attendant pain and loss of mobility, was the most problematic for the study participants. This result is consistent with previous research that has demonstrated that joint pain is a common symptom among patients with Lyme disease (Arvikar and Steere, 2015; Halperin, 2013). On the other hand, reproductive symptoms appeared to be the least affected, so caused the least amount of distress. Other symptoms appeared equal in their contribution to overall disease burden. It is important to note that there was a lot of variance across organ systems, indicating considerable person-to-person variability in the way their health was compromised.

4.1.3 Other diagnoses

Diagnosing and treating Lyme disease can be complicated due to the overlap in symptoms with many other diseases (Dressler et al., 1993; Nettleton et al., 2005; Patrick et al., 2015). Finally, it is important to look at serological testing as a guide to further support previous evidence, however, in the case of Lyme disease, it should not be the final deciding factor. This delay in diagnosis and the misdiagnoses of possible Lyme disease patients lead to a delay in treatment allowing the disease to progress further and increase in severity (Bobe et al., 2021). Therefore, when considering whether a person may have Lyme disease, it is important to take many factors into account. This includes looking at demographic factors such as the area in which they live and the daily activities that may have exposed them to ticks. It is also important to consider past medical history along with the present symptoms and whether previous diagnoses have been made that fit better. Many participants in this study have received at least one prior diagnosis that was not

Lyme disease. A significant positive relationship was found between the number of prior diagnoses and disease burden. The relationship showed a strong correlation ($R = 0.56$) indicating that with an increasing number of diagnoses, there is an increase in disease burden among the participants. Although a relationship was found, further research needs to be done into whether these diagnoses were upheld or were simply a misdiagnosis along the way to diagnosing Lyme disease.

It is crucial to recognize that these results are demonstrating a flaw in the medical system and the lack of knowledge on Lyme disease. These results are not encouraging as the number of diagnoses did not reduce symptoms. Ideally, more diagnoses would mean more contact with the healthcare system and better patient outcomes. However, this is not the case. While it is no fault of their own, physicians must acknowledge that there is a gap when it comes to understanding Lyme disease in many communities and that to provide optimal care to patients, this gap needs to be addressed. Studies have shown that this knowledge gap exists and has led to inadequate practices regarding Lyme disease treatment (Ferrouillet et al., 2015; Gasmi et al., 2017). However, physicians have been found to be open to receiving more education on the disease (Ferrouillet et al., 2015). Therefore, programs to provide support and education are valuable when accurate information is provided.

4.2 ELISA and western blot serology correlation

Serological testing is commonly used in the diagnosis of Lyme disease as patients can present with diverse symptoms making it somewhat difficult to make a conclusive diagnosis based on symptoms alone (Coburn et al., 2021). The enzyme-linked immunoassay (ELISA) and western blot both measure antibodies against the most common species and strain of the bacteria that causes Lyme disease (Branda and Steere, 2021). The current protocol in Canada requires that both these tests be positive to receive an official diagnosis (Lindsay et al., 2014). However, it is well documented that the sensitivity of these tests is variable depending on the stages of infection (Aguero-Rosenfeld et al., 1993; Brown et al., 1999). As these tests are both measuring antibodies against *B. burgdorferi*, the assumption is that there would be a correlation between these two serological tests.

A significant positive relationship was found between the ELISA serology and the IgG western blot. Although this relationship was found, it must be interpreted with caution as there are many data points scoring low on the ELISA with a large variance in scores on the western blots, and there are many fewer data points on the higher end of the ELISA scoring. These results are supported by previous research demonstrating concordance between ELISA and western blot serology in cases where results were positive or negative (Branda and Steere, 2021).

In this study, no relationship was found between the ELISA and the IgM western blot, with the correlation being close to zero. The IgM antibody response typically varies significantly over the course of infection; IgM antibodies are typically present during the first 30 days of infection after individuals should seroconvert to IgG (Branda et al., 2011). Therefore, testing for the presence of IgM more than a month after the onset of the illness is not thought to give informative results. However, it has been found that those suffering from chronic Lyme disease present with an atypical perduring IgM response indicating that testing beyond the initial month of infection for IgM antibodies could prove to be beneficial in improving patient outcome (Kalish et al., 2001).

4.3 ELISA and western blot as symptom predictor

The purpose of the serological tests is to guide treatment by assisting in diagnosis of the underlying cause of illness. However, the sensitivity of serological tests can vary significantly depending on what stages of the disease a person is in and a person's ability to produce antibodies (Hinckley et al., 2014). The major focus of this study was to determine whether Lyme disease serological tests demonstrated a correlation with disease burden in Lyme and related diseases; low serological values would be expected to correlate with low symptom burden, and vice versa.

This pattern was not observed between the ELISA results and the disease burden of participants. Instead results showed a negative correlation between the serology and total disease burden. Although a significant relationship was found, the data was very clustered at low ELISA levels with a large variance in disease burden, so the significance is most likely driven by the fewer high value ELISA data points. These results are contradictory to what was expected, however, there may be some explanations for the observed results.

Firstly, the clustered data points at low ELISA serological scores may not all be associated directly with Lyme or may not be due to Lyme disease as there are many illnesses that share common symptoms that could be mistaken for Lyme disease (Dressler et al., 1993). It is also possible that a participant may have comorbidities which is likely as ticks are vectors for many diseases and are known to cause co-infections (Berghoff, 2012).

The same pattern was seen when looking at the correlation between both IgG band number and band intensity versus symptoms. This result is consistent with the previously reported positive correlation between the western blot IgG serology and the ELISA. Both presented with a significant negative correlation indicating that as serological results, so presumably IgG antibody levels, increased, disease burden decreased.

The IgM western blot results were positively correlated with disease burden; however, the correlations were not found to be statistically significant. This positive correlation could have been explained by the initial onset of symptoms causing an increase in disease burden during those initial weeks before seroconversion occurs where people will experience a range of flu-like symptoms, however, this does not apply to the participants in this study as they have been chronically ill (Johnson et al., 2018). In some people, their lymphocytes are unable to convert from IgM antibodies to IgG antibodies due to gene dysregulation (Vaillant and Qurie, 2021). Therefore, it may also be that participants who scored higher on the western blot may not be seroconverting and therefore their bodies are unable to produce the antibodies necessary to clear the infection.

For the data points presenting with higher serological results but low disease burden, this may be due to prior treatment that was effective in decreasing symptoms, yet the antibodies remain present at high levels. However, this is only likely to have occurred in a few participants as the disease burden remained significantly higher in those who had been treated versus those who had not been indicating that treatment had not been successful at decreasing symptoms to similar levels as healthy individuals. Another reason may be that they have remained asymptomatic throughout the infection, however, there is limited knowledge on asymptomatic infections with studies reporting between 0 and 50% possibility of being asymptomatic (Fahrer et al., 1991; Hanrahan et al., 1984; Steere et al., 2003, 1986). Finally, immune exhaustion could explain some of the participants with high disease burden but low serological scores. If the person has been infected for a long period,

either due to lack of or failure of treatment, it is possible that their immune system no longer responds to the bacteria (Khaitan and Unutmaz, 2011). Further investigation is needed to determine the factors driving the correlation between serological testing and disease burden.

4.4 Seroconversion and Symptomology

In the early stages of infection, IgM antibodies develop as initial protection against bacteria, but, typically, after a month or so these antibodies decrease production, and the immune response produces IgG antibodies. However, seroconversion does not always occur or may not have occurred within the timeframe of testing (Branda et al., 2011). This is not of concern for the participants of this study as they all presented with chronic illness and were not in early stages of infection. It was hypothesized that those unable to switch from IgM to IgG class would present with the largest disease burden as they are unable to develop the antibodies necessary to help decrease disease burden. However, this is not consistent with the results. Instead, I saw that those who showed a predominantly IgG response and those who had both an IgG and IgM response showed significantly more disease burden from healthy controls. While the IgM response showed no significant difference from the healthy group, there was a lot of variability within the data, and it is important to note that it is within the IgM group that the highest disease burden was scored. The higher variability seen in the IgM group than the IgG group may be due to more participants being part of that group. Future research will need to be done across similar-sized groups to see if similar results are obtained.

This result is interesting as it is contradictory to the results found when looking at the correlation between the western blots and disease burden. It was found that IgM results were positively correlated, although non-significant, while the IgG results were negatively correlated to symptoms. So, it would be expected that the IgM class would show higher disease burden compared to the IgG class. The discrepancy in results may be due to the number of participants that were tested for class-switching ($n = 56$) which only represented a portion of the entire sample size. It is necessary to further investigate why these differences are showing and what may be driving them.

Looking at seroconversion in Lyme disease patients is important as this can impact their diagnosis. It is currently recommended that patients who have been ill for more than 30 days should not be tested for IgM antibodies as the antibodies can persist for some time after the infection has cleared (Centers for Disease Control and Prevention (CDC), 1995). However, this does not account for those who are unable to seroconvert despite the appropriate time having passed. In some cases, it can take up to eight weeks for the required seroconversion needed for a positive test to take place (Branda et al., 2011). Finally, some people may never seroconvert and therefore will not test positive for IgG antibodies (Vaillant et al., 2022).

4.5 Treatment and sex

As early results demonstrated that the females from this study had increased disease burden compared to men, it was of interest to determine whether there were significant differences between the sexes. The results showed that women experienced significantly higher disease burden than men. This is consistent with previous research showing that women develop more cytokines than men leading to increased inflammatory response. Further investigation into whether sex had any effect on serological testing is necessary as previous studies have demonstrated that the current testing methods show a bias towards men where they test positive more so than women despite women being infected. This is most likely due to differences in immune response between sexes (Rebman et al., 2015; Schwarzwald et al., 2010).

There was a total of 29 participants that received treatment for Lyme disease. As treatment failure is known to occur, the effects of treatment on disease burden were investigated to assess whether those who had been treated showed a similar disease burden as those who are healthy. This is not what the results showed. In fact, the disease burden was significantly higher in those who had been treated indicating that treatment had likely failed which is known to occur in Lyme disease patients (Johnson et al., 2018). An interesting result that was observed was that the number of participants that had received treatment, had noticed a rash at the site of their tick bite. This indicates that there may be some influence of the erythema migrans on whether people will seek out treatment. This could be problematic if people are only seeking out treatment based on this symptom as it

is not always present (Cameron et al., 2014). These results are not promising as they are demonstrating the inefficiency of current Lyme disease treatment protocols in Canada. When it comes to Lyme disease treatment there is controversy as to the appropriate treatment and duration of treatment with different organizations such as ILADS and the IDSA recommending different protocols. These results indicate that research and consensus are necessary if improvements to patient outcomes are expected.

4.6 Implications and future directions

The overarching aim of my study was to assess the relationship between serological diagnostics and disease burden to better understand the reliability of the current serological testing methods in Canada. Ideally, there would be a linear correlation between the two variables indicating that the serological methods are showing internally consistent results and that testing is predictive of patient disease burden. However, this was not observed. Despite the contradiction to the initial hypotheses, and indeed because of this contradiction, these results provide insightful feedback about the current Lyme disease testing methods and their efficacy.

This study has provided a groundwork to assess the additional information provided by the participants. It will be important for future work to investigate how other variables such as potential tick exposures may be interacting with the observed relationships. These variables also include accounting for other diagnoses or possible co-infections that may be driving disease burden rather than Lyme disease or may add to the severity of symptoms. It will also be important to look at the demographic information provided by participants such as the community they live in, whether it is urban, suburban, or rural, and their exposure risk through outdoor activities and occupational risk.

This study provided good information on the relationship between serological methods and between serological methods and symptoms. However, further research should focus on other factors that may be influencing serological results and symptoms. Further research into Lyme disease testing is critical as the pitfalls of testing lead to an increased risk of developing chronic Lyme disease. For the past years, Lyme disease has been increasing in prevalence throughout Canada. Despite the large increase in cases, there has not been a lot of effort devoted to improving diagnostic methods, whether it be

serological or clinical and there also remains large gaps in knowledge that need to be addressed when it comes to appropriate treatment for the disease. These issues should be of serious concern as they further promote increasing chronic illness in the population.

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Appendix

5.1 Participant questionnaire

The questionnaire consists of 5 parts – the latter 2 can be omitted for healthy participants

Part 1 – Demographics

Part 2 - Borrelia Exposure

Part 3 – Symptoms

Part 4 – For those who are chronically ill – diagnoses history

Part 5 – For those who are chronically ill – treatment history

Part 1 - Demographics:

In what year were you born?

What gender are you?

In what community/region do you live?

Part 2 - Borrelia exposure:

1. Occupational

a. Traditional exposure professions:

- Do you work outside? (Ie in the forest or walking in fields etc?) *Yes/no*
- *If yes:* What is your job? _____
- How many hours a day or week are you directly exposed to natural areas*?
_____ hours/

*This means times when your body is directly exposed to vegetation. For example, walk through a field counts, sitting in the cab of a machine working in the forest should not be counted.

b. Medical exposure.

- Do you work in a medical/veterinary/dental/etc profession? *Yes/no*
- If yes, what is your job? _____
- Are you exposed to human or animal body fluids? *Yes/no*
- Have you had a blood transfusion? *Yes/no*

2. Residential

- Do you live in a rural, suburban or urban area?
Please circle: rural/suburban/urban
- How often are you outside your house (hrs/day)? _____ *hours*
- Are there deer in the immediate vicinity of your house? *Yes/no*
- Do you have pets? *Yes/no*
- If so, how many and what kind? _____
- Do you have livestock? *Yes/no*
- If so, what and how many? _____

3. Recreational

- Do you engage in outdoor recreational activities? (I.e. hiking, gardening, camping, hunting, golfing etc.) *Yes/no*
- If so, which activities do you engage in?

- Approximately how many hours do you engage in outdoor recreational activities (per day/week/ month) as appropriate? _____ *hours/*_____

4. Animal exposure:

- Are you in contact with animal blood, or large amounts of untreated animal products? *Yes/no*
- If yes, what is the nature of your exposure? _____
- Is this exposure part of your work? *Yes/no*

5. Sexual exposure

Important: Please note that some of the recent scientific literature suggests that Lyme disease may be transmitted sexually and that we have included the following section based solely on this literature. The questions in this section are only applicable to you if you are sexually active. If you do not feel comfortable answering any or all of the questions in this section, please feel free to say you would like to skip any or all of these questions.

- Approximately how many sexual partners do you have per year? _____ */year*
- If you have a long term chronic illness, approximately how many sexual partners did you have per year before you got ill? _____ */year*
- Do you have a sexual partner with chronic neurological, immunological or arthritic symptoms? (I.e Parkinsons, MS, CFS, Fibromyalgia, Lupus) *Yes/no*
- Do you have protected or unprotected intercourse?
Please circle: protected/unprotected

Part 3 – Symptoms -

A. Do you remember one or more tick bites?
B. Do you remember a rash at the site of the bite?
C. If so, describe the rash (plain/EM) and size (approx.)
D. Have you had rashes at other sites on our body? If so, describe appearance.

Other symptoms of Lyme disease	Please circle answer:
Head, Face, Neck	
Unexplained hair loss	none/mild/severe
Headache, mild or severe, seizures	none/mild/severe/seizures
Pressure in head, white matter lesions in brain (MRI)	none/mild/severe Circle applicable: pressure/white matter lesions
Twitching of facial or other muscles	none/mild/severe
Facial paralysis (Bell’s Palsy, Horner’s syndrome)	none/mild/severe
Tingling of nose, (tip of) tongue, cheek or facial flushing	none/mild/severe Circle applicable: tip of tongue/ cheek/facial flushing
Stiff or painful neck	none/mild/severe
Jaw pain or stiffness	none/mild/severe
Dental problems	none/mild/severe
Sore throat, clearing throat a lot, phlegm (flem), hoarseness, runny nose	none/mild/severe Circle applicable: throat clearing, phlegm,

	hoarseness, runny nose
Eyes/Vision	
Double or blurry vision	none/mild/severe
Increased floating spots	none/mild/severe
Pain in eyes, or swelling around eyes	none/mild/severe
Oversensitivity to light	none/mild/severe
Flashing lights, peripheral waves or phantom images in corner of eyes	none/mild/severe Circle applicable: flashing lights/peripheral waves/ phantom images
Ears/Hearing	
Decreased hearing in one or both ears, plugged ears	none/mild/severe Circle applicable: one ear/both ears/one plugged ear/ both plugged ears
Buzzing in ears	none/mild/severe
Pain in ears, oversensitivity to sounds	none/mild/severe Circle applicable: Pain/oversensitivity to sound
Ringing in one or both ears	none/mild/severe
Digestive and Excretory Systems	
Diarrhea	none/mild/severe
Constipation	none/mild/severe
Irritable bladder (trouble starting, stopping) or interstitial cystitis	none/mild/severe Circle applicable: irritable bladder/ interstitial cystitis
Upset stomach (nausea or pain) or GERD (gastroesophageal reflux disease)	none/mild/severe Circle applicable: Upset stomach/ GERD
Musculoskeletal System	
Bone pain, joint pain or swelling, carpal tunnel syndrome	none/mild/severe Circle applicable: bone pain/joint pain/joint swelling/carpal tunnel
Stiffness of joints, back, neck, tennis elbow	none/mild/severe Circle applicable:

	joints/back/neck/tennis elbow
Muscle pain or cramps, (Fibromyalgia)	none/mild/severe
Respiratory and Circulatory Systems	
Shortness of breath, can't get full/satisfying breath, cough	none/mild/severe
Chest pain or rib soreness	none/mild/severe
Night sweats or unexplained chills	none/mild/severe
Heart palpitations or extra beats	none/mild/severe
Endocarditis, heart blockage	none/mild/severe
Neurologic System	
Tremors or unexplained shaking	none/mild/severe
Burning or stabbing sensations in the body	none/mild/severe
Fatigue, Chronic Fatigue Syndrome, weakness, peripheral neuropathy or partial paralysis	none/mild/severe Circle applicable: fatigue/ Chronic Fatigue Syndrome/ weakness/ peripheral neuropathy/partial paralysis
Pressure in the head	none/mild/severe
Numbness in body, tingling, pinpricks	none/mild/severe Circle applicable: numbness/tingling/pinpricks
Poor balance, dizziness, difficulty walking	none/mild/severe Circle applicable: poor balance/dizziness/difficulty walking
Increased motion sickness	none/mild/severe
Light-headedness, wooziness	none/mild/severe
Psychological Well-being	
Mood swings, irritability, bi-polar disorder	none/mild/severe Circle applicable: mood swings/irritability/bi-polar disorder
Unusual depression	none/mild/severe
Disorientation (getting or feeling lost)	none/mild/severe
Feeling as if you are losing your mind	none/mild/severe
Over-emotional reactions, crying	none/mild/severe

easily	
Too much sleep, or insomnia	none/mild/severe Circle applicable: too much sleep/insomnia
Difficulty falling or staying asleep	none/mild/severe
Narcolepsy, sleep apnea	none/mild/severe Circle applicable: narcolepsy/sleep apnea
Panic attacks, anxiety	none/mild/severe
Mental Capability	
Memory loss (short or long term)	none/mild/severe
Confusion, difficulty thinking	none/mild/severe
Difficulty with concentration or reading	none/mild/severe
Going to the wrong place	none/mild/severe
Speech difficulty (slurred or slow)	none/mild/severe
Difficulty finding commonly used words	none/mild/severe
Stammering speech	none/mild/severe
Forgetting how to perform simple tasks	none/mild/severe
Reproduction and Sexuality	
Loss of sex drive	none/mild/severe
Sexual dysfunction	none/mild/severe
Unexplained menstrual pain, irregularity	none/mild/severe
Unexplained breast pain, discharge	none/mild/severe
Testicular or pelvic pain	none/mild/severe
General Well-being	
Phantom smells	none/mild/severe
Unexplained weight gain or loss	none/mild/severe
Extreme fatigue	none/mild/severe
Swollen glands or lymph nodes	none/mild/severe
Unexplained fevers (high or low grade)	none/mild/severe
Continual infections (sinus, kidney, eye, etc.)	none/mild/severe
Symptoms seem to change, come and go	yes/no
Pain migrates (moves) to	yes/no

different body parts	
Early on, experienced a “flu-like” illness, after which you have not since felt well	yes/no
Low body temperature	yes/no
Allergies or chemical sensitivities	none/mild/severe
Increased effect from alcohol and possible worse hangover	none/mild/severe

Part 4 – Diagnosis history

Section A- Other Diagnosis

Have you ever been diagnosed with any of the following illnesses? (Please check applicable)

- Multiple Sclerosis_____
- Chronic Fatigue Syndrome_____
- Fibromyalgia _____
- Rheumatoid Arthritis_____
- Amyotrophic Lateral Sclerosis (ALS)_____
- Systemic Lupus Erythematosus (Lupus) _____
- Mental illness_____
- Parkinson’s Disease_____
- Other disease or illness not mentioned above that has symptoms that could be attributed to Lyme Disease? _____(Please specify:_____)

Was diagnosis based on a blood test or a clinical diagnosis?

What date (month and year) was the diagnosis made?

By whom was the diagnosis made? (Please provide Doctor’s name if possible, as well as the hospital or location)

Section B - Diagnosis of Lyme disease:

Have you been diagnosed with Lyme disease? (By whom, and where?)

How was Lyme diagnosed? (Standard blood tests prescribed by a Canadian physician, including ELISA, or western blotting, testing sought out in the United States, etc.)

What were the results of the tests that were undergone?

Part 5 – Treatment history

Treatment of Lyme disease:

Were you treated for Lyme disease? *Yes/no*

By whom and where?

What was the prescribed treatment? (Type, duration, location of provision)

If you have been diagnosed with any of the other conditions listed in section A, what treatments have/are you receiving for those conditions?