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Symptom Presentation Frequency and Severity Associated with Adult Lyme Disease by ROSS Scale Review

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This is to certify that the doctoral dissertation by

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has been found to be complete and satisfactory in all respects,
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Abstract

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By

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MS, University of Scranton, 1996

BS, Marywood University, 1994

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

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Public Health - Epidemiology

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Abstract

Although Lyme disease is the most frequently reported vector-borne illness in the United States, recent evidence from the CDC suggests that Lyme disease incidence in the United States may be much higher than reported. Lyme disease symptoms can be mistaken for a wide variety of diseases, which can complicate the diagnosis. To date, no diagnostic criteria analysis has been conducted examining the association between sociodemographic variables (sex and age) and seasonality of infection with the severity and symptomology found in Lyme disease cases. Using the CDC's outbreak investigation model, a primary case/control study was conducted using the ROSS Scale to collect data. Comparisons were made between a Lyme disease-diagnosed group (n = 203) and a convenience sample of non-Lyme disease patients (n = 388). Novel symptom patterns were found to significantly predict a diagnosis of Lyme disease. Odds ratio results revealed a positive association between musculoskeletal (OR = 11; 95% CI), neurological (OR = 12; 95% CI), cognitive (OR = 10; 95% CI), and cutaneous (OR = 144; 95% CI) symptoms frequency and severity and the diagnosis of Lyme disease. In addition, overall symptom frequency and severity scores displayed significant differences between cases and controls, between males and females, and among certain age groups. No correlation was found between symptom frequency and severity with the seasonality of infection. Current diagnostic tools search for antibodies to the *Borrelia* bacteria, but antibody production takes a few weeks. The results of this study help identify at-risk patients based on the presentation and severity of Lyme disease symptoms when antibodies are not present in measureable quantities in the blood stream, allowing for earlier diagnosis.

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Dedication

This project is dedicated to my friend Dr. Joseph Falcone and his wonderful wife Donna Falcone, who opened my eyes to the daily difficulties associated with living with Lyme disease. Donna inspired me to try to help in some small way, and this research is the result of that desire.

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Chapter 1: Introduction to the Study

Problem Statement

Lyme disease is a tick-borne illness caused by the spirochete *Borrelia burgdorferi* (Borchers, Keen, Huntley, & Gershwin, 2015; Deluca, Eisendle, & Zelger, 2013; Henry et al., 2011; Mead, 2015). Currently, Lyme disease is reported more frequently than any other tick-borne illness (Binder, Telschow, & Meyer-Hermann, 2012; Mead, 2015). According to the most recent surveillance data provided by the Centers for Disease Control and Prevention (CDC), there were 33,461 cases of Lyme disease reported in the United States in 2014 (CDC, 2015). These cases represent a 38% increase over the previous year's total. In addition, reported and confirmed Lyme disease cases show a bimodal distribution with children between the ages 5-9 years and adults between 40-50 years showing the highest incidence rates. Males account for 54% of reported cases and, except in the 70-plus age groups, consistently have a higher incidence of confirmed Lyme disease (CDC, 2007). Based on preliminary data reported from three ongoing CDC research studies, Lyme disease may be underreported across all ages by a factor as high as 12-to-1 resulting in 300,000 actual cases yearly (Aucott & Seifter, 2011; Borchers et al., 2015; CDC, 2015; Johnson, Aylward, & Stricker, 2011; Mead, 2015). The cause of this underreporting remains unclear.

Sex differences in symptom presentation could be a possible reason why Lyme disease is underreported. In a study conducted in Slovenia (2013), sex differences existed with the presentation and appearance of the erythema migrans (EM) rash (Strle et al., 2013). The Slovenia study (2013) examined three specific symptoms commonly found in Lyme disease patients—EM rash, Lyme arthritis, and Lyme neuroborreliosis—for sex

differences. The results of the Slovenia study showed that of the 10,539 patients who displayed the EM rash, 58% were women, while males (42%) tended to present with other noncutaneous symptoms (Strle et al., 2013). In addition, the women who presented with the EM rash were 15 years younger than those women who presented with other cutaneous symptoms (Strle et al., 2013). In the same study, men were more likely to display Lyme arthritis and Lyme neuroborreliosis (later stage symptoms) than women (Strle et al., 2013).These results may be related to the specific type of *Borrelia* species found in Europe, which differs from the *Borrelia* species found in the United States. Because of these species variations, further study in the United States is warranted.

According to the Institute of Medicine's (IOM) report (2001), sex differences are based on factors related to being male or female. These differences result from biological differences at the genetic level, cellular level, and/or via hormonal variations between males and females (Wizeman & Pardue, 2001). Prior to puberty and the production of the sex hormones (estrogen, progesterone, and testosterone), male to female sex differences are related only to the anatomical differences present. This study will focus on adult male and female subjects.

Sex differences in symptom presentation have been reported in many chronic and autoimmune diseases. For example, coronary heart disease (CHD), stroke, Parkinson's disease, rheumatoid arthritis (RA), multiple sclerosis (MS), and fibromyalgia show distinct male to female differences in disease presentation (Casimir et al., 2010; Caracta, 2003; Hassan, Gordon, & Einstein, 2016; Hirsch, Jette, Frolkis, Steeves, & Pringsheim, 2016; Kure et al., 2016; Maselli et al., 2016; Ngo, Steyn, & McCombeet, 2014; Quintero, Amador-Patarroyo, Montoya-Ortiz, Rojas-Villarraga, & Anaya, 2012). Although Lyme

disease is caused by a bacterium, long term infection mimics a few autoimmune disorders such as RA, MS, and systemic lupus erythematosus (SLE) (Savely, 2010).

Sex differences may also be found in recreational or occupational exposure to ticks. Studies have been conducted examining both recreational and occupational risk of contracting Lyme disease, but none have examined this risk based on sex (Borchers et al., 2015; Finch et al., 2014; Piacentino & Schwartz, 2002). In addition, seasonality of infection may be important because exposure to ticks and risk for contraction of Lyme disease may increase based on outdoor exposure to the *Ixodes* tick during peak tick growth seasons (CDC, 2015; Borchers et al., 2015; Finch et al., 2014; Mead, 2015).

Age variations in Lyme disease presentation have also been reported. According to CDC data for the years 2000-2010, males in the age group 5-9 years contain the most reported confirmed cases of Lyme disease (Borchers et al., 2015; CDC, 2015). A second spike in the number of cases during the same time frame occurs in both males and females age 40-50 years old (Borchers et al., 2015; CDC, 2015). This bimodal distribution is also seen in Europe and was supported by a study in Germany (Borchers et al., 2015; Dehnert et al., 2012). Why do these age variations exist? Are these variations related to sex differences in symptom presentation?

Gender differences, although the term is often used interchangeably with sex differences, are related to an individual's interactions with and expectations from their social environment (Sieck, 2015; Wizeman & Pardue, 2001).According to the World Health Organization's report on sex and gender differences in epidemic-prone infectious diseases (2007), gender roles can determine exposure possibilities and treatment seeking behaviors. The potential for exposure to infectious agents such as the *Ixodes* tick can be

increased based on occupational and recreational exposures (Borchers et al., 2015; Finch et al., 2014; McKenna, Faustini, Nowakowski, & Wormser, 2004). Gender differences can be seen in many occupational and recreational choices. While occupational and recreational choices as part of the risk of exposure to the *Ixodes* tick were considered, the focus of this study remained on differences in symptom presentation based on biological sex and not on gender roles.

In addition, Lyme disease shows seasonality in incidence rates. According to the CDC (2015), the highest number of reported cases for Lyme disease occur between the months of May and August. This time frame corresponds directly with the life cycle of the *Ixodes scapularis* tick (Aucott & Seifter, 2011; Borchers et al., 2015; CDC, 2015; Mead, 2015). Although incidence data is collected and reported by the CDC, an analysis based on sociodemographic and clinical factors, including sex, age, time since exposure to the *Ixodes* tick, month of case confirmation, symptomology, and severity of symptoms, has not been performed. Factors associated with seasonality of infection, sex, and age could provide insight into the risk of exposure to Lyme disease and whether these factors play a role in the underreporting of Lyme disease.

Lastly, severity of reported symptoms must be considered. While the severity of the EM rash or Bell's palsy may not prevent someone from performing daily tasks, the changes in appearance may prevent a person from going to work, school, or out in public (Fu, Bundy, & Sadiq, 2011). In addition, some of the other symptoms of Lyme disease may significantly impact day-to-day activities.

Arthritic pain in the major joints can prevent sufferers from performing normal everyday tasks and is one of the most frequently (up to 60% of untreated cases) described

symptoms of Lyme disease in the United States (Borchers et al., 2015; Feder, Abeles, Bernstein, Whitaker-Worth, & Grant-Kels, 2006; Mead, 2015; Nadelman, & Schwartz, 2012; Wormser et al., 2006). According to the Arthritis Foundation (2014), arthritis is the leading cause of disability in the United States. Severity of arthritis pain in the joints due to *B. burgdorferi* infection is no different than that caused by RA or osteoarthritis and can lead to similar levels of disability (Borchers et al., 2015; Nadelman, & Schwartz, 2012; Wormser et al., 2006).

Symptom severity may lead to additional negative outcomes. These negative outcomes may include, but are not limited to, inability to work, loss of job, and loss of insurance coverage (Chandra, Keilp, & Fallon, 2013; Johnson et al., 2011). The more severe the symptom experienced, the more functional impact Lyme disease can have on the individual.

Significance

Lyme disease has been classified into three phases of infection: early localized Lyme disease, early disseminated Lyme disease, and late Lyme disease (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick, Morbach, & Tappe, 2009; Wormser et al., 2006). In addition to the three accepted phases of Lyme disease, posttreatment Lyme disease syndrome and chronic Lyme disease have also been reported (Aucott, Rebman, Crowder, & Kortte, 2012; Cairns & Goodwin, 2005; Cameron, 2010). In early or localized Lyme disease, symptoms include mild flu-like symptoms, malaise, fatigue, headache and the erythema migrans (EM) rash that begins around the site of the tick bite and slowly increases in size to a typical diameter of at least 5 cm (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick et al., 2009; Wormser et al., 2006). The EM

rash occurs more frequently with *B. burgdorferi* sensu stricto, which is the bacterial species found in the United States, than in Lyme disease cases in Europe caused by other species of the bacterium (Borchers et al., 2015; Mead, 2015; Girschick et al., 2009).

In 50 – 80% of all cases of Lyme disease, the erythema migrans (EM) rash is present and can be used as a primary method for diagnosis (Aucott, Crowder, Yedlin, & Kortte, 2012; CDC, 2015; Miraflor et al., 2016). The EM rash can serve as a strong clinical marker for diagnosis according to the case definition of the National Notifiable Diseases Surveillance System (CDC, 2008). Correct identification of this rash is vital to the diagnosis of early Lyme disease because current diagnostic tools result in negative tests 60% of the time in patients who show up for treatment during the earliest stage of infection (Aucott et al., 2009). Inaccurate identification of this rash can lead to delayed treatment or treatment with antibiotics that would have no effect on the organism leading to long term, serious effects on the patient (Aucott et al., 2009).

Early disseminated Lyme disease signals the spread of the bacteria from the initial site of infection. The hallmark of this stage of infection is nervous system symptoms such as meningitis and cranial nerve palsies (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick et al., 2009; Wormser et al., 2006). In some cases, secondary EM lesions may appear at sites not near the initial tick bite (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick et al., 2009; Wormser et al., 2006). Secondary EM lesions have been seen in approximately 40% of children who show up for treatment but are less common in adults (Girschick et al., 2009). In addition, mild musculoskeletal system symptoms have been reported during this phase, especially in the United States (Borchers et al., 2015; Girschick et al., 2009).

Late Lyme disease may occur many months after the initial tick bite and results from systemic spread of the bacteria (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick et al., 2009; Wormser et al., 2006). Typical manifestations of this stage include arthritis, especially in the large joints of the arms and legs, polyneuropathy, cranial neuropathy, cardiac complications, and encephalomyelitis (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick et al., 2009; Wormser et al., 2006).

If left untreated or inadequately treated, early Lyme disease can progress quickly to late phase infection and the serious complications that come with this phase of the disease. Most research suggests that earlier treatment provides the patient's best chance for a full recovery (Cameron, 2007; Donta, 2012; Johnson & Stricker, 2004; Miraflor et al., 2016). Once treatment is initiated, 80-90% of patients significantly improve (CDC, 2015). Delaying treatment can be costly, both in patient health costs and in reduced quality of life (Johnson et al., 2011). Zhang et al. (2006) determined that the annual health care costs associated with late Lyme disease treatment ($16,199 per person) can be 12 times higher than those costs associated with early Lyme disease treatment ($1,310 per person).

During the early stage of infection, treatment with beta-lactam antibiotics is highly successful at killing the bacteria and stopping the progression of the infection (Binder et al., 2012; Borchers et al., 2015; Cameron, 2007; Donta, 2012; Girschick et al., 2009; Wormser et al., 2006). Late phase infections may require longer term or intravenous treatments with antibiotics (Binder et al., 2012; Cameron, 2007; Donta, 2012; Girschick et al., 2009; Wormser et al., 2006). Unfortunately, antibiotic treatment for late phase infections does not always result in a complete recovery with no symptoms (Aucott

et al., 2013; Johnson et al., 2011). It is in these instances that post-treatment Lyme disease syndrome and/or chronic Lyme disease may be the diagnosis.

Because late phase manifestations can be incapacitating, adults could be burdened with "functional impacts" of Lyme disease (Aucott et al., 2013, p 2). Functional impacts occur when patients experience symptoms and/or increased severity of symptoms that prevent them from completing daily activities (Aucott et al., 2013). These functional impacts may include loss of job, loss of productivity, lapse of insurance coverage, and disability (Johnson et al., 2011). In fact, loss of productivity makes up more than half the costs of late Lyme disease infection (Johnson et al., 2011). Early, accurate diagnosis of Lyme disease can prevent all of these impacts by stopping the spread of the infection before the serious complications of late Lyme disease occur (Miraflor et al., 2016).

In cases of misdiagnosis in children and adults, the burden of Lyme disease may be measured in more than just the financial burden. In a study conducted by Tager et al. (2001), children diagnosed with Lyme disease showed many cognitive difficulties even after the completion of treatment. These cognitive difficulties included attention and organizational deficits, as well as memory and IQ issues (Tager et al., 2001). Similar cognitive difficulties have also been reported for adult patients with late Lyme disease (Borchers et al., 2015; Cairns & Godwin, 2005). Early, accurate diagnosis is vital to prevent these long term cognitive changes (Tager et al., 2001).

Identification of the comorbidities that alter the symptom presentation of Lyme disease would be helpful in ensuring the accurate diagnosis and appropriate treatment at the earliest stage of infection of Lyme disease. In chronic diseases such as cardiovascular disease and Parkinson's disease, sex and age differences in symptom presentation have

been identified (Fairweather, Petri, Coronado & Cooper, 2012; Hassan et al., 2016; Hirsch et al., 2016; Kure et al., 2016; Maselli et al., 2016; Ngo et al., 2014; Quintero et al., 2012). In autoimmune diseases such as RA and SLE, females are more likely to be affected than males based on the specific mechanisms of the female immune system (Fairweather et al., 2012; Ngo et al., 2014; Quintero et al., 2012). According to Dey et al. (2009), women with acute coronary syndromes were more likely to have a milder form of the disease and to report atypical symptoms, such as jaw pain and nausea, when seeking treatment. The study by Dey et al. supported the findings of a previous study on sex differences in symptom presentation for cardiovascular disease (Polk & Naqvi, 2005).

In Parkinson's disease, both age and sex differences play a role in the progression of the disease (Hirsch et al., 2016; Haaxma et al., 2007). Women develop symptoms 2.2 years later than men (Hirsch et al., 2016; Haaxma et al., 2007). After disease onset, women displayed different symptoms than men at initial diagnosis; women seemed to display the "tremor dominant form" of the disease (Haaxma et al., 2007, p. 822).

Understanding of these differences has led to targeted prevention and education programs for both patients and physicians, and timely administration of treatment for women who present with nonclassical symptoms of disease. These advances can be realized for Lyme disease as well if potential differences in symptom presentation can be identified.

Background

Lyme disease is caused by the spirochete *Borrelia burgdorferi* sensu lato (Borchers et al., 2015; Deluca et al., 2013; Mead., 2015; Miraflor et al., 2016). This classification of bacteria has been further delineated into more than 20 distinct

genospecies based on the bacterial genome, geographical location, tick vector, reservoir animal, and Lyme disease symptoms produced (Borchers et al., 2015; Deluca et al., 2013; Mead et al., 2015; Pritt et al., 2016). The five most commonly found pathogenic genospecies include *B. burgdorferi* sensu stricto found in North America and Europe; *B. garinii* and *B. afzelii* found in Europe; *B. japonica* found in Japan; and *B. andersonii* found in North America (Borchers et al., 2015; Mead, 2015; Pritt et al., 2016). A new genospecies of *Borrelia* was recently discovered in the Midwestern United States, specifically Minnesota, North Dakota, and Wisconsin (CDC, 2015; Pritt et al., 2016). This new genospecies, proposed name *Borrelia mayonii*, has similar symptoms to *Borrelia burgdorferi* (Pritt et al., 2016). Signs and symptoms of infection vary based on the *Borrelia* species causing the infection (Borchers et al., 2015; Deluca et al., 2013; Mead, 2015).

Lyme disease is transmitted through the bite of the *Ixodes* tick (Arsnoe, Hickling, Ginsburg, McElreath, & Tsao, 2015; Borchers et al., 2015; Deluca et al., 2013; Mead, 2015; Miraflor et al., 2016). The tick vector varies based on the geographic area where the tick is found. In the United States, the primary vectors of Lyme disease are *Ixodes scapularis* in the Eastern states and *Ixodes pacificus* on the West coast (Arsnoe et al., 2015; Borchers et al., 2015; Deluca et al., 2013; Mead, 2015). In order for Lyme disease to be transmitted to a human host, the tick must first be infected with *B. burgdorferi* while feeding from an infected animal host (Arsnoe et al., 2015; Deluca et al., 2013). Typical animal reservoirs for *B. burgdorferi* include small rodents like the white-footed mouse, hares, small birds, and white-tailed deer (CDC, 2015; Deluca et al., 2013). Infection of the tick is for life (Deluca et al., 2013). In addition, the tick must feed on the

human host for 24-48 hours to pass on the infection (Borchers et al., 2015; CDC, 2015; Hynote, Mervine, & Stricker, 2012).

Clinical manifestations of Lyme disease occur in three phases (Binder et al., 2012; Borchers et al., 2015; Deluca et al., 2013; Girschick et al., 2009; Johnson & Feder Jr., 2010). These phases include early or localized Lyme disease, early disseminated Lyme disease, and late Lyme disease (Binder et al., 2012; Borchers et al., 2015; Deluca et al., 2013; Girschick et al., 2009; Johnson & Feder Jr., 2010). In addition, posttreatment Lyme disease syndrome and chronic Lyme disease have been discussed in the literature. Each phase has typical signs and symptoms associated with movement of the bacteria from the initial tick bite to a systemic infection (Binder et al., 2012; Borchers et al., 2015; Deluca et al., 2013; Girschick et al., 2009; Johnson & Feder Jr., 2010).

Signs and symptoms include, but are not limited to, flu-like symptoms such as malaise, fatigue, fever, arthralgia, myalgia; arthritis; neuropathy including Bell's Palsy; meningitis and encephalitis; cardiac symptoms including atrial block; and erythema migrans (EM) rash (Binder et al., 2012; Borchers et al., 2015; Deluca et al., 2013; Girschick et al., 2009; Johnson & Feder Jr., 2010; Miraflor et al., 2016).

The classic bull's eye EM rash is the most widely known symptom associated with Lyme disease (Aucott et al., 2012a; Borchers et al., 2015; Miraflor et al., 2016). Key features of the EM rash include a shape that is round to oval; a red to bluish-red color; a clearly defined edge; occurs at the location of the tick bite; and increases in size over time from 5 cm to 16 cm (Moore, 2015; Muellegger, 2004). Typical locations for the EM rash appear to coincide with common tick bite locations. In adults, EM usually occurs on the calf, back of the knee, thigh, groin, buttocks, armpit, shoulder, waist, and occasionally,

the scalp/hairline (Muellegger, 2004). With the new genospecies *Borrelia mayonii*, the rash is more diffuse and distributed and does not take on the typical bulls-eye appearance (Pritt et al., 2016). In children, head and neck lesions are more common than in adults (Muellegger, 2004). Unfortunately, some of these sites are not conducive to finding the tick or viewing the EM rash, so patients may not seek treatment during the early phase of infection.

Because some form of the EM rash is present in 50 – 80% of reported cases, the EM rash alone can be used to definitely diagnose Lyme disease without any additional testing (Johnson & Stricker, 2004; Miraflor et al., 2016; Moore, 2015). The EM rash provides the best clinical marker for Lyme borreliosis (Moore, 2015; Muellegger, 2004). According to the National Notifiable Disease Surveillance System (NNDSS) case definition, a diagnosis can be made based on the presence of the EM rash or positive two-tiered serology testing with ELISA and Western blot (CDC, 2008). Unfortunately, the *classic* appearance of the bull's eye EM rash (concentric rings with a central clear area) is present only 20% of the time with *Borrelia burgdorferi* infections and not at all with *Borrelia mayonii* infections (Aucott et al., 2012a; Pritt et al., 2016).

Other manifestations of this rash can be present and can significantly confuse the accurate diagnosis of Lyme disease in the early stages. These manifestations include homogenous red lesions with no central clearing; secondary lesions; vesiculopustular lesions; lesions with bruising, which typically occur on the calves; and a "diffuse macular rash" occurring all over the body including the palms of the hands and soles of the feet (Aucott et al., 2012a ; Pritt et al., 2016, pg. 7;). In addition, other skin lesions may be confused with the EM rash lesion. This group of lesions includes small insect bites

(spider or mosquito); immediate allergic inflammatory responses to the initial tick bite which decreases in size over time; poison ivy rash; shingles rash; cellulitis; hand-foot-mouth disease; and *S. aureus* infection (Aucott et al., 2012a; Tibbles & Edlow, 2007). Lipsker, Lieber-Mbomeyo, and Hedelin (2004) found that almost 72% of physicians in Lyme endemic areas could not correctly diagnose the EM rash (Aucott et al., 2012a; Brett, Hickley, Zielinski-Gutierrez, & Mead, 2014). Early diagnosis is necessary to ensure full recovery from infection (Brett et al., 2014).

Lyme disease can be successfully treated with a 21 day course of oral antibiotics as long as neurological or cardiac symptoms are not present (Borchers et al., 2015; Gerstenblith & Stern, 2014; Johnson & Feder Jr., 2010). Neurological or cardiac symptoms may require up to 28 days of IV antibiotics (Borchers et al., 2015; Johnson & Feder Jr., 2010). The earlier this treatment begins, the more likely a full recovery will occur (Borchers et al., 2015; Brett et al., 2014; Johnson & Stricker, 2004; Mead, 2015). Delayed treatment may lead to more chronic symptoms and may require longer courses of antibiotics (Cameron, 2007; Johnson & Stricker, 2004). In addition, the direct and indirect medical costs may pose problems for patients over the long term. Zhang et al. (2006) found that the economic impact of Lyme disease nationwide was more than $200 million in direct and indirect medical costs, as well as nonmedical costs like loss of productivity. This cost can be avoided with accurate diagnosis in the early stages of infection so that appropriate treatment can be implemented.

Tager et al. (2001) discuss the cognitive deficits found in children with late phase Lyme disease. These deficits can lessen quality of life and increase the burden assumed by families where these cognitive deficits are present. Common issues found include

memory and IQ deficiencies, attention and concentration disorders, and withdrawal from social situations (Tager et al., 2001). Similar cognitive defects have also been found in adults in the later stages of the infection (Cairns & Godwin, 2005).

Posttreatment Lyme disease (PTLD) syndrome occurs when patients have been diagnosed with Lyme disease via the traditional methods of EM rash identification or enzyme immunosorbent assay (EIA) and Western blot, treated with a 21-day course of antibiotics, and still display symptoms like fatigue, arthralgia, myalgia, and memory and concentration issues (Lantos, 2011; Nichols & Windemuth, 2013). While rare, PTLD syndrome does occur more frequently in patients who are diagnosed in the later stages of infection or had severe symptoms at diagnosis (Lantos, 2011; Nichols & Windemuth, 2013).

Lastly, the controversy surrounding chronic Lyme disease must be presented. The controversy is centered on the existence of chronic Lyme disease. On one side of the controversy are the physicians and researchers who believe that chronic Lyme disease does not exist; that Lyme disease is rarely found in the general population, is not easily acquired, and is simple to cure with the standard course of antibiotics (Johnson et al., 2011; Stricker & Johnson, 2007). On the other side of the controversy are the physicians and researchers who believe that chronic Lyme disease exists and is extremely difficult to treat and cure (Johnson et al., 2011; Stricker & Johnson, 2007).

An important component of this controversy stems from the fact that the Infectious Diseases Society of America (IDSA) is influential in determining treatment regimens for Lyme and other infectious diseases in the United States (Johnson et al., 2011). The IDSA does not believe that chronic Lyme disease exists, so the

recommendations set forth do not cover treatment for long term infection, providing barriers to health care access and increased disease burden for those diagnosed with chronic Lyme disease (Johnson et al., 2011).

The International Lyme and Associated Diseases Society (ILADS) is on the opposite side of this controversy. ILADS was formed by a group of physicians and researchers to advance the options available for chronic Lyme disease sufferers (ILADS, 2009). This group has also offered a case definition of chronic Lyme disease with diagnostic criteria and treatment recommendations. The ILADS case definition is broader and covers many more presentations of symptoms (ILADS, 2009; Lantos, 2011). In addition, diagnostic criteria take into consideration the expertise of the physician and do not rely exclusively on the EM rash, EIA, and/or Western blot tests (ILADS, 2009; Lantos, 2011). Lastly, treatment options are expanded to allow for extended antibiotic treatment as needed (ILADS, 2009; Lantos, 2011). Although the existence of chronic Lyme disease remains controversial, identification of sex differences in symptom presentation may help inform this issue more fully.

Sex differences in symptom presentation have been seen in many other diseases. In coronary heart disease (CHD), women often do not present with chest pain as their primary symptom (Dey et al., 2008; Fairweather et al., 2012; Kure et al., 2016; Polk & Naqvi, 2005; Wizeman & Pardue, 2001). However, males experiencing CHD most often report chest pain as a primary symptom (Dey et al., 2008; Fairweather et al., 2012; Polk & Naqvi, 2005; Wizeman & Pardue, 2001). According to the American Heart Association (2015), a woman is likely to experience shortness of breath and/or pain and discomfort in the jaw or back instead of the classic chest pain symptom. The lack of chest

pain may prevent a woman from seeking help, since it is an accepted convention that heart attacks present with chest pain.

Parkinson's disease is another example where women and men present with different symptoms. Parkinson's disease is more common in men than women, where women tend to present with symptoms at an older age (Haaxma et al., 2006; Hirsch et al., 2016). In addition, women are more likely to present with the classic tremors associated with Parkinson's disease while men tend to present with rigidity and bradykinesia, which is slow, limited movement (Haaxma et al, 2006; Hirsch et al., 2016).

Observed advantages for women in both CHD and Parkinson's disease may be related to estrogen levels circulating in the bloodstream. Differences in symptoms of both CHD and Parkinson's disease between males and females seem to equal out after menopause (Haaxma et al., 2006; Hirsch et al., 2016; Kure et al., 2016; Wizeman & Pardue, 2001). While age of onset and estrogen levels may affect symptom presentation for CHD and Parkinson's disease, autoimmune diseases are another example where symptom presentation differs between males and females and estrogen levels may not be the cause. For many autoimmune diseases, the prevalence in women is 60 – 75% higher than it is in men (Maselli et al., 2016; Ngo et al., 2014; Quintero et al., 2012; Whitacre, 2001).

Late Lyme disease mimics a few autoimmune diseases and is often misdiagnosed as MS, SLE, and RA (Hassan et al., 2016; Ngo et al., 2014; Quintero et al., 2012; Savely, 2010). Some symptom similarities between MS and Lyme disease include confusion, weakness, peripheral nerve numbness, dizziness, and malaise (Savely, 2010). Joint and muscle pain is not found in MS, so patients presenting with musculoskeletal issues should

not receive a diagnosis of MS (Savely, 2010). Unfortunately, in SLE and RA, joint and muscle pain and malaise are primary symptoms (Hassan et al., 2016; Savely, 2010). Lastly, autoimmune diseases tend to present later in life, so misdiagnosis with autoimmune diseases versus Lyme disease may occur more frequently in older patients than in children (Savely, 2010; Whitacre, 2001).

Because Lyme disease displays a bimodal distribution when considering age as a variable, it becomes important to identify why this distribution occurs (Dehnert et al., 2012; Esposito, Bosis, Sabatini, Tagliaferri, & Principi, 2013). Are these variations due to sex differences in recreational behaviors and occupational exposures? While studies have been conducted on outdoor worker exposure and tick counts/types in recreational areas, no studies have looked at Lyme disease, occupational and/or recreational exposure, and sex (Belongia et al., 1999; Finch et al., 2014; Piacentino & Schwartz, 2002; Schwartz & Goldstein, 1990; Smith, Benach, White, Stroup, & Morse, 1988). Most of these studies collected sex (male/female) information, but none of the studies analyzed sex as a variable for study. This study will include a sex comparison in the collection and analysis of data.

In addition, none of the described studies examined seasonality of infection in relation to either sex or age. Seasonality of infection could be directly related to outdoor activities because exposure to the *Ixodes scapularis* tick occurs outdoors during certain stages of the tick's life cycle. Month of diagnosis and potential month of tick exposure will be collected from the medical records and ROSS scale surveys in order to make comparisons with sex and age.

Theoretical Framework

An appropriate theoretical framework for this study is the CDC's outbreak investigation model (Rohrer, 2013). Although Lyme disease is not considered a typical outbreak type of disease (like *Salmonella* or *E. coli*), the outbreak investigation model can still apply. This model allows for examining the relationship between a variable under consideration and a disease like Lyme disease (Reingold, 1998). This model also allows for the expansion of the variables studied as more information becomes available (Roher, 2013). In addition, the outbreak investigation model fits well with the case-control study design. Independent variables to be studied include socio-demographic characteristics like age and sex; seasonality of infection characteristics like month of infection and month of diagnosis; and time since tick exposure; dependent variables to be studied include symptoms present at diagnosis and severity of symptoms as reported by the patient (Table 1).

Table 1

Study Variables

Independent variables		**Dependent variables**		
Sociodemographic	Seasonality of infection	Symptoms*	Symptom severity	Symptom frequency
Age	Month of tick exposure	Musculoskeletal	Not affected	Never
Sex	Month of diagnosis	Neurological	Slight/ barely noticeable	1-2 days
		Cognitive	Minor but noticeable	3-4 days
State of Residence**		General	Moderate	5-6 days
		Cardiac	Major	7 days
		Cutaneous	disabling	

*See Appendix A for a complete list of symptoms.
**State of residence will be collected only for case/control matching.

The CDC outbreak investigation model has 10 components, but only seven were applied to this study (Reingold, 1998). The three components that were not applicable to the current study included environmental sampling and testing, controlling the spread of infection, and dissemination of information via the press to the public (Reingold, 1998). Dissemination of information on the finding of this study will occur in public forums at the completion of the study. The steps of the model that were applied include the following:

First, a case definition was established to ensure that all cases met specific, consistent criteria for inclusion in the study. Cases (n = 203) were defined as adult subjects (≥ 18 years of age) that met one of the following diagnostic criteria: (a) patient presented with a physician confirmed EM rash; (b) patient had a positive EIA and/or Western Blot laboratory result for IgG and IgM antibodies to *B. burgdorferi* surface proteins; or (c) patient had a score of 5 or higher on the Burrascano Diagnostic Criteria for Lyme Disease scale (Burrascano, 2005).

Controls (n = 388) were selected from adult subjects (≥ 18 years of age) from the primary care clinic who did not suffer from Lyme disease, family members of Lyme disease patients at the primary care clinic, and employees and students at a small, liberal arts college. Subjects who suffered from illnesses other than Lyme disease were not excluded from the study. All controls were Lyme disease free at the time of selections as determined by: (a) never having had a tick bite; (b) having no evidence of EM rash; or (c) no prior laboratory testing for or diagnosis of Lyme disease by a physician. Controls were selected at an approximate 1:2 case/control ratio to address the sampling bias introduced by non-random selection of participants.

Removal of participants who did not meet the established case criteria was performed. Cases were removed based on age (participant must be over 18 years old), missing data for date of birth, and state of residence. Because state of residence was used to match cases and controls, participants were required to live in a Lyme endemic state for this study to equalize the risk of exposure to the *Ixodes* tick vector. Additional information on case inclusion and exclusion criteria can be found in chapters 3 and 4.

Next, case confirmation was performed by a qualified physician who specialized in the treatment of Lyme disease. Completed forms were reviewed with the primary care physician to verify inclusion as a case or control for this study from a clinical standpoint. Because controls must NOT be Lyme disease positive, any indicators from the symptom checklist for a potential undiagnosed Lyme disease patient were reviewed carefully for appropriate case/control placement or exclusion from the study. No undiagnosed Lyme diseases cases were identified through this review.

Incidence rates for Lyme disease were established for all states of residence used in the study. This data was collected from the CDC, which reports data received via the National Notifiable Disease Surveillance System. Lyme disease is a vector-borne disease that, if caught early, can be cured with a 21-28 day course of antibiotics (Gerstenblith & Stern, 2014). Lyme disease is therefore reported in incidence rates because each case reported to the National Notifiable Disease Surveillance System (NNDSS) represents a newly diagnosed infection.

Cases and controls were enrolled from two sites – a primary care clinic that specializes in the treatment of Lyme disease and a small, liberal arts college campus. At both sites, cases and controls had to meet the inclusion criteria established for the study.

Because sex and age were two variables of interest in this study, matching between cases and controls based on sex or age could not occur. Matching based on state of residence was done to insure that exposure to the *Ixodes scapularis* tick was the same between cases and controls. In addition, both cases and controls had to be available from a particular state to be considered for inclusion in the study.

Descriptive data was collected and analyzed. Data collected included, but was not limited to: sex, date of birth, state of residence, education level, date of tick bite, and date of diagnosis. These demographic characteristics allowed for the establishment of person, place, and time variables. Cases consisted of both females (n = 130) and males (n = 73) ranging in age from 18 years to 75+ years. Controls consisted of both females (n = 268) and males (n = 120) also ranging in age from 18 years to 75+ years. States of residence for both cases and controls included Connecticut, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, and Vermont. All of these states are considered Lyme-endemic states by the CDC (2015).

Overall, 51% of the study population had at least a high school diploma and 44% of the population had completed at least a baccalaureate degree. Cases occurred in all twelve months; the majority of cases occurred in May (n = 34) and June (n = 29) and the fewest number of cases occurred in March (n = 3). Twenty one cases did not list a month of tick exposure or month of diagnosis, so they were excluded from the analysis for seasonality of infection only.

Research Questions

Five research questions were formulated, each with a null and alternate hypothesis for testing. The following research questions were generated following an extensive

review of the published literature. These research questions were an important guide for the inquiry into specific factors that ultimately affect the diagnosis of Lyme disease. Symptom presentation and severity were compared to defined sociodemographic variables, seasonality of infection variables, and time since tick exposure to identify areas to expand health providers' knowledge and awareness of Lyme disease which can lead to earlier diagnosis and treatment, reduced treatment costs and improved health outcomes for Lyme disease patients.

RQ1: Is the presentation of symptoms in Lyme disease-positive patients associated with the sociodemographic variables age and sex as assessed by patient medical record and ROSS Scale survey review?

$H_0$1: Lyme disease symptom presentation is not associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a1: Lyme disease symptom presentation is associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

RQ2: Is the severity of symptoms in Lyme disease positive patients associated with the sociodemographic variables age and sex as assessed by patient medical record and ROSS Scale survey review?

$H_0$2: Lyme disease symptom severity is not associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a2: Lyme disease symptom severity is associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

RQ3: Is the presentation of symptoms associated with the diagnosis of Lyme disease as assessed by patient medical record and ROSS Scale survey review?

H_03: Lyme disease symptom presentation is not associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a3: Lyme disease symptom presentation is associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

RQ4: Is the severity of symptoms associated with the diagnosis of Lyme disease as assessed by patient medical record and ROSS Scale survey review?

H_04: Lyme disease symptom severity is not associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a4: Lyme disease symptom severity is associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

RQ5: Is Lyme disease symptom presentation and severity associated with seasonality of infection variables as assessed by medical record and the ROSS Scale survey review?

$H_0$5: Lyme disease symptom presentation and severity are not associated with the seasonality of infection variables as assessed by patient medical record and the ROSS Scale survey review.

H_a5: Lyme disease symptom presentation and severity are associated with the seasonality of infection variables as assessed by patient medical record and the ROSS Scale survey review.

Nature of the Study

In this study, I followed a quantitative approach to data collection. Patient records and ROSS scales were examined for symptoms present at time of doctor's appointment for Lyme disease; severity of symptoms present as described by the patient; sex of patient; age at diagnosis; time elapsed between tick exposure and diagnosis; and month of possible tick exposure and month of initial diagnosis. Because sex is a nominal variable, age, month of exposure and month of diagnosis can be measured using an interval scale, and symptom severity is an ordinal variable, quantitative analysis of the data will be performed using SPSS (version 21).

Statistical tests performed include the Chi-square test (if the data are normally distributed) or the Kruskal-Wallis test (if the data are not normally distributed), which allowed for comparisons between the control group and the study population. The Chi-square test and the Kruskal-Wallis test can be performed because the sample size is well over 60 participants and there was more than five participants in each age category. Analysis of Variance (ANOVA) was performed on normally distributed data samples, both with and without the Tukey Post-hoc analysis as needed, to compare the various age groups. In addition, an odds ratio was calculated for each of the symptom categories

included in the study. Lastly, a receiver operating characteristic (ROC) curve was generated to analyze the usefulness of the symptom index score as a diagnostic tool.

In addition to the described univariate and bivariate statistical analyses, multiple linear regression analysis was conducted on sex and age variables in comparison to the symptom index score. The independent variables of age and sex were included in the analysis regardless of their association with the dependent variables after bivariate analysis based on evidence from previous literature (Katz, 2006).

Since the goal of this research was to identify additional potential factors to use for the earliest possible diagnosis of Lyme disease, an analysis based on the use of the information from this study for diagnosis was performed. Once associations between symptoms and/or symptom severity and the independent variables were determined, the sensitivity, specificity, positive predictive value, and the negative predictive values were calculated for the groups of symptoms. These values describe the ability of the test to identify correctly those individuals who have a disease. A ROC plot was also generated determine the diagnostic value of the symptom index score for Lyme disease.

Possible Types and Sources of Information or Data

1. ROSS Scale surveys from patient records from a primary care medical practice.
2. ROSS Scale surveys distributed and collected at a small, rural liberal-arts college.

Definition of Terms

The following section provides working definitions of the terms used in this research.

Age: A numerical value distinguishing the number of years an individual has been alive, beginning at birth and ending with death (Free Dictionary, 2013).

Chronic Lyme disease: Persistent symptoms of Lyme disease despite 30 days of treatment or a recurrence or relapse of symptoms without evidence of a new tick bite or incidence of EM rash (Cameron et al., 2004).

Early disseminated Lyme disease: The stage of Lyme disease infection associated with the spread of the *B. burgdorferi* bacteria from the initial site of infection as associated with neurological symptoms, like cranial nerve palsies and meningitis, and initial musculoskeletal complaints (Binder et al., 2012; Donta, 2012; Franz & Krause, 2003; Girschick et al., 2009; Wormser et al., 2006).

Early localized Lyme disease: The stage of Lyme disease infection signaled by symptoms that include mild flu-like symptoms, malaise, fatigue, headache and the erythema migrans (EM) rash that begins around the site of the tick bite and slowly *increases* in size to a typical diameter of at least 5 cm (Binder et al., 2012; Donta, 2012; Franz & Krause, 2003; Girschick et al., 2009; Wormser et al., 2006). Neurological symptoms, including Bell's palsy, may be present as well (Binder et al., 2012; Donta, 2012; Franz & Krause, 2003; Girschick et al., 2009; Wormser et al., 2006;).

Erythema migrans (EM) rash: A characteristic rash of early localized Lyme disease with the key features that include a shape that is round to oval; a red to bluish-red color; a clearly defined edge; occurs at the location of the tick bite; and increases in size over time from 5 cm to 16 cm (Muellegger, 2004).

Sex: For the purposes of this research, the term sex will be used to describe male or female biological sex, as well as the individual's interactions with and expectations from their social environment (Wizeman & Pardue, 2001).

Late Lyme disease: The stage of Lyme disease infection characterized by musculoskeletal complaints including arthritis, especially in the large joints of the arms and legs, polyneuropathy, cranial neuropathy, cardiac complications, and encephalomyelitis (Binder et al., 2012; Donta, 2012; Franz & Krause, 2003; Girschick et al., 2009; Wormser et al., 2006).

Posttreatment Lyme disease syndrome: The stage of Lyme disease characterized by the continuation or return of symptoms after a standard course of treatment for the disease (Aucott, Crowder, & Kortte, 2013).

Symptom presentation: The subjective signs of Lyme disease as reported by the patient (Free Dictionary, 2013).

Symptom severity: The intensity of the subjective signs of Lyme disease as reported by the patient (Free Dictionary, 2013).

Limitations

One of the main limitations for this study is related to sampling bias. The patient records examined all came from one clinic in New York. The patient records do not represent a random sampling of individuals because this study focuses on those individuals who have already been diagnosed with Lyme disease. In addition, all of these patients have access to health care and have health insurance, so results may not be typical of those individuals who do not have access to either.

Controls were selected by convenience sampling, which can lead to a reduction of external validity (Mann, 2003; McDermott, 2011; Pannucci & Wilkins, 2010). The convenience sample was chosen despite this reduction because the medical clinic treats patients from a broad sampling of states within the Lyme endemic region of the United States and the college also has students and staff from these same Lyme endemic regions. According to the CDC surveillance data, 97% of all reported Lyme disease cases come from only 14 states (CDC, 2015). These states include: Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, and Wisconsin (CDC, 2015). The medical clinic is centrally located and within driving distance from Connecticut, New York, Pennsylvania, and New Jersey. The college is located two hours west from the medical clinic. This central location provided the opportunity for both Lyme disease and non-Lyme disease participants to be chosen as both cases and controls. In addition, the age distribution of controls from the college was well matched to the age distribution of the medical clinic.

To address this non-random sampling bias, a ratio of 1:2 cases to controls was used. Frequency matching was used to insure that cases and controls had a similar percentage of participants that fell into the male/female and the selected age categories. Because age and sex are variables under investigation in this study, matching could not occur on these variables, but to make meaningful comparisons on these variables, there must be similar numbers of cases and controls for each sex and age category (McDermott, 2011; Pannucci & Wilkins, 2010; Zondervan, Cardon, & Kennedy, 2002). In addition, matching was performed between cases and controls on the basis of state of

residence. Because Lyme disease is endemic to only 14 states in the United States, matching of cases and controls on their state of residence insures that potential exposure to the *Ixodes* tick is equal in both groups.

Recall bias is a potential problem with case-control study designs (Mann, 2003; McDermot, 2011; Pannucci & Wilkins, 2010; Schulz & Grimes, 2002). Because this study collected data through medical record review and via the ROSS Scale survey, recall bias was minimized. Cases reported signs and symptoms experienced at the time of their medical office visit. Controls reported symptoms experienced within the previous week from the date the ROSS scale was filled out. The ROSS Scale survey collects data on symptoms for the week prior to filling out the survey. Recalling information from the past seven days helped to minimize recall bias considerably.

Lastly, confounding must be addressed. Because matching of cases with controls and stratification were difficult due to age and sex being variables under study, potential confounders were dealt with in the analysis portion of the study through multivariate regression analysis (McDermott, 2011; Schlesselman, 1982; Schulz & Grimes, 2002).

Statement of Positive Social Change Implications

The implications of this research for positive social change include increased knowledge of the sex differences found in Lyme disease; prevention of delays in diagnosis and treatment for patients with Lyme disease; decreased expenses associated with late Lyme disease due to increased diagnosis in the early stage of infection; and early access to needed health care services.

Summary

This chapter outlined the key components of this research project. Research questions and hypotheses were identified. Key definitions were provided. Significant background for and descriptions of Lyme disease were provided to show the importance of studying factors that could increase the accurate diagnosis and early treatment of Lyme disease. Further evidence to support the importance of studying Lyme disease is provided in chapter two via a thorough examination of the literature on Lyme disease to date.

Chapter 2: Literature Review

Introduction

This chapter provides a review of the literature in support of an examination of the factors that affect the presentation of Lyme disease. First, I provide a description of *Borrelia burgdorferi*, the causative agent of Lyme disease. Because Lyme disease is a tick-borne disease, *Ixodes scapularis* and *Ixodes pacificus*, the tick vectors found in the United States, must be described as well. In addition, I review the complex lifecycle of both *Borrelia burgdorferi* and *Ixodes scapularis*.

Next, I establish evidence for sex differences in other chronic and infectious diseases. These established differences, although not well studied in Lyme disease in the United States, can be utilized as support for identifying the same differences in Lyme disease. In addition, I review comparisons between the male and female immune response. I also discuss age differences in the incidence of Lyme disease. Lyme disease symptoms are clearly defined to provide the necessary basis for study measurements. Finally, I discuss the theoretical framework for the study.

I identified pertinent literature using Google and Google Scholar search engines, as well as Science Direct, PubMED, and ProQuest databases. Search terms included *Lyme disease, symptoms of Lyme disease, sex differences + Lyme disease, gender differences + Lyme disease, age + Lyme disease, sex differences + immune response, gender differences + immune response, sex differences + chronic disease, gender differences + chronic disease, sex differences + cardiovascular disease, gender differences + cardiovascular disease, sex differences + autoimmune disease, gender*

differences + autoimmune disease, sex differences + infectious disease, gender differences + infectious disease, Lyme disease + children, behavior risk factors + Lyme disease, occupational exposure + Lyme disease, recreational activity + Lyme disease, symptom severity + Lyme disease, and *environmental risk factors + Lyme disease*. Both sex differences and gender differences were searched separately because these terms are often used interchangeably.

The Causative Agent

The causative agent for Lyme disease, as it is known in the United States, or Lyme borreliosis, as it is known in Europe and Asia, was discovered in 1981 by Dr. Willy Burgdorfer, an entomologist who studied ticks and the organisms who lived inside them (Sternbach & Dibble, 1996). This bacterium, *Borrelia burgdorferi* sensu lato, has been separated into more than 20 distinct species to date, not all of which cause disease in humans (Borchers et al., 2015; Caimano, Hu, Radolf, & Stevenson, 2012; Mead, 2015). The primary disease causing agents for Lyme disease are *Borrelia burgdorferi* sensu stricto, found in the United States and Europe, *Borrelia garinii*, and *Borrelia afzelii*, found in Europe and Asia, and the recently discovered *Borrelia mayonii*, found only in the Midwestern United States (Borchers et al., 2015; Caimano et al., 2012; Mead et al., 2015; Pritt et al., 2016). Because the patient sample for this research will be in the eastern United States, the focus of this literature review will be on *Borrelia burgdorferi* sensu stricto.

Borrelia burgdorferi sensu stricto is a Gram negative, microaerophilic member of the family *Spirochaetaceae* (Johnson, Schmid, Hyde, Steigerwalt, & Brenner, 1984; Neelakanta et al., 2007; Rosa, Tilly, & Stewart, 2005). This organism displays the typical

flexible spiral shape of other members of the *Spirochaetaceae* family with both inner and outer protective membranes that enclose the periplasmic flagella which allow the organism to be motile by either rotational or translational movement (Johnson et al., 1984; Rosa et al., 2005). The cell wall of *B. burgdorferi* is missing lipopolysaccharides, a unique characteristic for a Gram negative organism, since most Gram negative cell walls contain a high concentration of lipopolysaccharides (Rosa et al., 2005). In addition, the peptidoglycan layer of the cell wall contains a high concentration of ornithine (Johnson et al., 1984). Peptidoglycan composition is important since peptidoglycan is a typical antimicrobial target for treatments.

The cell wall encloses a unique genome. First, the genome contains both a segmented linear chromosome and between 13 and 21 linear and circular plasmids (Brisson et al., 2012; Caimano et al., 2011; Rosa et al., 2005; Schutzer et al., 2011). While the linear chromosome contains much of the structural and metabolic information necessary for survival, this information is carried repeatedly on many different genes (Brisson et al., 2012; Caimano et al., 2011; Schutzer et al., 2011). In fact, many of the genes in the *Borrelia* genome are identical (Brisson et al., 2012; Schutzer et al., 2011). Bacterial plasmids generally carry information for virulence, exotoxins, endotoxins, or special enzymes. *Borrelia* plasmids carry information vital for survival, including the outer surface protein genes (Brisson et al., 2012; Caimano et al., 2011; Schutzer et al., 2011). In addition, no virulence factors have been discovered on any of the known plasmids (Brisson et al., 2012; Rosa et al., 2005).

Additional components of the bacterial cell wall and membrane with functional importance are the outer surface proteins (Osp). Six outer surface proteins (A through F)

have been identified, but only three have identified functions in the infection cycle (Kenedy, Lenhart, & Akins, 2012; Pal et al., 2000). Outer surface protein A (OspA) plays a role in replication in and infection of the *Ixodes* tick vector (Hartiala et al., 2008; Kenedy, Lenhart, & Akins, 2012; Pal et al., 2000). This protein is only produced when *Borrelia* is in the tick's gut and plays a role in binding to TROPSA receptors in the mid-gut region of the tick (de Silva, Fish, Burkot, Zhang, &Fikrig, 1997; de Silva, Telford III, Brunet, Barthold, &Fikrig, 1996; Hartiala et al., 2008; Kenedy, Lenhart, & Akins, 2012; Pal et al., 2004a; Schwan, Piesman, Golde, Dolan, & Rosa, 1995). Once the tick begins feeding on either the natural host or the human host, production of OspA stops and *Borrelia* no longer expresses this protein on the surface (de Silva et al., 1997; de Silva et al., 1996; Hartiala et al., 2008; Kenedy, Lenhart, & Akins, 2012; Schwan et al., 1995). OspA was a target for the Lyme vaccine because OspA is found only in the tick stage of infection, but the vaccine is no longer produced due to poor sales in the United States (de Silva et al., 1997; de Silva et al., 1996; Schwan et al., 1995).

Outer surface protein B (OspB) is found in conjunction with OspA on the bacterial cell surface when *Borrelia* is in the mid-gut of the *Ixodes* tick (de Silva et al., 1997; de Silva et al., 1996; Hartiala et al., 2008; Kenedy, Lenhart, & Akins, 2012; Neelakanta et al., 2007; Schwan et al., 1995). While not much is known about the function of OspB, a study by Neelakanta et al. (2007) confirmed the importance of OspB in binding of *Borrelia burgdorferi* to the epithelial lining of the *Ixodes* tick. In addition, Hartiala et al. (2008) suggested that OspB plays a role in immune system evasion by preventing phagocytosis by neutrophils at the site of initial infection. This role was

previously presented by Bundoc and Barbour (1989), but little evidence was provided to support this earlier claim.

While OspA and OspB are expressed by *Borrelia burgdorferi* within the *Ixodes* tick vector, down-regulation and reduced expression of these two proteins occurs once the blood meal commences and the bacterium enters the mammalian host (de Silva et al., 1997; de Silva et al., 1996; Kenedy, Lenhart, & Akins, 2012; Neelakanta et al., 2007; Schwan et al., 1995). Outer surface protein C (OspC) is expressed for a short while during *Borrelia* migration from the mid-gut to the salivary glands and upon first entering the mammalian host (Carrasco et al., 2015; Grimm et al., 2004; Kenedy, Lenhart, & Akins, 2012; Neelakanta et al., 2007; Pal et al., 2004a; Stewart et al., 2006; Tilly et al., 2006). The shift from OspA/OspB production to OspC production is related to the pH changes that occur in the mid-gut of the tick during the blood meal (Tilly et al., 2006).

Pal et al. (2004b) found that OspC is present in greater numbers than OspA or OspB during migration of the bacteria to the salivary glands of the tick, but Grimm et al. (2004), Tilly et al. (2006), and Stewart et al. (2006) all found that OspC was *not* required for *Borrelia* motility or adherence to the tick salivary glands. It seems that OspC plays a vital role during early infection of the mammalian host (because OspC expression down-regulates after 2 weeks post infection), but this role was unclear (Carrasco et al., 2015; Grimm et al., 2004; Stewart et al., 2006; Tilly et al., 2006). In a study conducted by Carrasco et al. (2015), OspC is important for evading phagocytosis by macrophages at the site of the infection. This phagocytic evasion allows the organism to colonize the mammalian host during the early stage of infection (Carrasco et al., 2015).

According to Samuels (2011), one role of OspC is binding to mammalian plasminogen (an important protein for dissolving blood clots). Onder et al. (2012) suggested that the binding between plasminogen and OspC on the surface of *B. burgdorferi* provided a few advantages. First, plasminogen binding helped the bacteria cross over multiple cell membranes: from tick mid-gut to salivary glands; from tick salivary glands into mammalian skin; from skin at bite site to mammalian blood stream (Onder et al., 2012). Second, plasminogen helps to break down antibodies and deactivates parts of the complement system (Onder et al., 2012). Each of these factors provides support for the importance of OspC for *B. burgdorferi* infection of mammalian cells.

OspC is not required for tick re-infection from the mammalian host (Tilly et al., 2006). In a study with mice infected with an OspC mutant form of *Borrelia burgdorferi*, naïve ticks were infected with the OspC mutant form after feeding on infected mice (Tilly et al., 2006). Both Tilly et al. (2006) and Stewart et al. (2006) suggest that OspC plays a role in either evading the mammalian host's innate immune system or recognition of the mammalian host tissue. Neither group provided adequate evidence to support either hypothesis, but Onder et al. (2012) provided evidence to support both.

Stewart et al. (2006) suggested that genetic variations in OspC may allow for evasion of the innate immune system and subsequent dissemination from the initial site of infection, but the research did not support this conclusion. OspC gene expression down-regulates within two weeks post-infection, right at the time dissemination from the initial tick bite normally occurs (Grimm et al., 2004). This early research suggested that if OspC is important for dissemination in the host, down-regulation of the gene for OspC and reduction in the expression of OspC would not occur at this point of the infection

cycle. Recent research by Onder et al. (2012) show that OspC helps the bacteria get into the bloodstream for dissemination, but is not necessary for the actual spread of the infection.

Samuels (2011) suggested that because OspC is considered a dominant immunogen, production shuts down to prevent antibody production. This shutdown would prevent adaptive and memory immune responses, but would not affect the innate immune system. Carrasco et al. (2015) suggest that OspC plays a role in evading innate immune system responses like macrophage phagocytosis. Onder et al. (2012) suggest that OspC serves as a plasminogen receptor that helps break down antibodies. The role of OspC in the immune process is still under investigation. While the functions of these outer surface proteins in the mammalian hosts are not completely understood, their interactions within the tick vector are important.

The Vector

The vector for Lyme disease is the *Ixodes* tick (Figure 1). Four primary species have been identified as a carrier for *Borrelia burgdorferi*: *Ixodes scapularis* in the eastern United States, *Ixodes pacificus* in the western United States, *Ixodes ricinus* in Europe, and *Ixodes persulcatus* in Europe and Asia (Borchers et al., 2015; Caimano et al., 2012; Mead, 2015; Rosa et al., 2005; Suss, Klaus, Gerstengarbe, & Werner, 2008). The tick becomes infected, usually during the larval stage of its life cycle, by feeding on an infected endemic host (Caimano et al., 2012; Mead, 2015; Subak, 2003). These hosts tend to be small mammals and birds (Caimano et al., 2012; Subak, 2003). In the United States, *Peromyscus leucopus* (white-footed mouse) is the primary reservoir (Caimano et al., 2012; Subak, 2003).

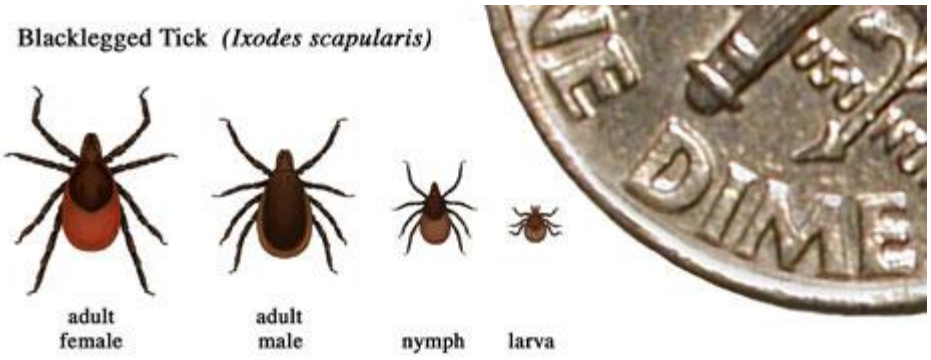

Figure 1. Ixodes scapularis tick sizes (CDC, 2015)

The Life Cycle

Ixodes ticks are born uninfected with *Borrelia burgdorferi* because passage of the bacteria does not occur through transovarial routes (Borchers et al., 2015; Rosa et al., 2005). As shown in Figure 2, *Ixodes* ticks lay eggs in May of the first year of their two year life cycle (CDC, 2015; Subak, 2003). Eggs hatch releasing larvae in the summer months (CDC, 2015; Subak, 2003). In order to continue to the next stage of development, larvae must feed and tend to feed on smaller mammals like *Peromyscus leucopus* (Caimano et al., 2012; Mead, 2015; Rosa et al., 2005; Subak, 2003). It is at this point that the tick becomes infected with *Borrelia burgdorferi* (Caimano et al., 2012; Mead, 2015; Rosa et al., 2005; Subak, 2003). The tick is infected with the *Borrelia* bacteria for life and can transmit the bacteria to any other organism it feeds on (Borchers et al., 2015; Caimano et al., 2012; Rosa et al., 2005; Subak, 2003).

The larvae become dormant over the winter and molt into the nymph stage in the spring (CDC, 2015; Caimano et al., 2012; Subak, 2003). A second blood meal must be taken at this time in order for the final stage of development to occur (Caimano et al., 2012; Subak, 2003). Humans and larger animals like deer and dogs are the prime target

for nymphal feeding (Caimano et al., 2012; Rosa et al., 2005; Subak, 2003). Nymphs develop into adults that are able to lay eggs and begin the cycle again (Caimano et al., 2012; Rosa et al., 2005; Subak, 2003).

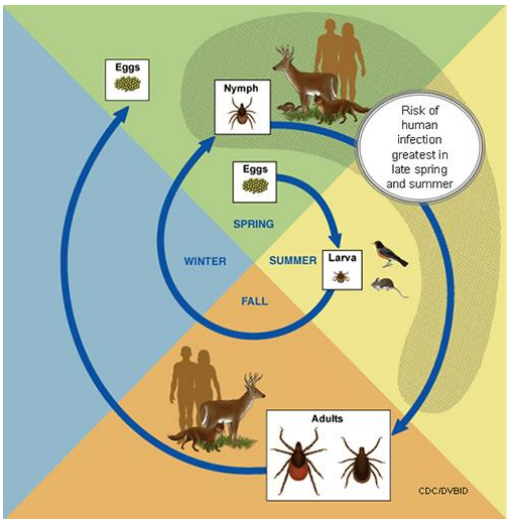

Figure 2. Tick two-year life cycle (CDC, 2015)

Larvae become infected with *Borrelia* during the blood meal and remain in the tick midgut until the tick enters the nymph stage (Rosa et al., 2005). At this point, *Borrelia* expresses both OspA and OspB (Rosa et al., 2005). The bacteria do not invade any other tissues within the tick until after becoming a nymph and the second blood meal occurs (Rosa et al., 2005). With the pH changes that occur during the second blood meal, OspA and OspB production is down-regulated and OspC production is up-regulated as the bacteria move from the midgut of the tick to the salivary glands (Caimano et al., 2012; Kenedy, Lenhart, & Akins, 2012; Rosa et al., 2005). Once in the salivary glands, *Borrelia* can be transferred to the next organism bitten (Borchers et al., 2015; Caimano et al., 2012; Rosa et al., 2005).

Borrelia is not transferred immediately to the new host (Caimano et al., 2012; Rosa et al., 2005). Ticks must be attached more than 24 hours for the transfer to occur because during the first 24 hours of attachment, little blood is actually taken in by the tick (Borchers et al., 2015; Caimano et al., 2012; Dai et al., 2010; Hynote, Mervine, & Stricker, 2012; Rosa et al., 2005). After 48 hours of attachment, blood meal intake by the tick increases rapidly and poses the largest chance for transfer of *Borrelia* to the new host (Dai et al., 2010). While the minimum number of spirochetes required to cause infections in humans in not currently known, the infective dose of *Borrelia* in mice models suggest as few as 18 bacteria can lead to infection (Borchers et al., 2015).

Incidence Rates

Because Lyme disease is a vector-borne disease, each new case reported to the CDC is considered a new infection. In addition, Lyme disease is endemic to only 14 states in the United States. These states are mostly located in the eastern United States and correspond to the habitat of the *Ixodes scapularis* tick. Incidence rates for Lyme disease for each state used in the study are shown in Table 2 (CDC, 2015).

Table 2

Incidence Rate by State of Residence of Study Participants for 2014 (CDC, 2015)

State of residence	Incidence rate (per 100,000 people)	Lyme disease cases (count)
Connecticut	47.8	2,360
Massachusetts	54.1	5,304
New Hampshire	46.9	724
New Jersey	29.0	3,286
New York	14.4	3,736
Pennsylvania	50.6	7,487
Vermont	70.5	599

The lower incidence rate in New York is related to the fact that New York has a state population of 19.75 million people, 8.5 million that live in New York City alone (US Census Bureau, 2015). Living in a large urban setting reduces your risk for exposure to the Lyme disease tick and lowers your risk for developing Lyme disease. According to CDC data, New York (*n* = 3,736) had more reported cases than Connecticut (*n* = 2,360) in 2014, but due to the population differences between the two states, Connecticut has a higher incidence rate (CDC, 2015). The other states with low numbers of confirmed cases but high incidence rates follow the same pattern – the overall population size is lower in states like Vermont and New Hampshire (US Census Bureau, 2015).

Risk Factors

Behavioral and environmental risk factors for tick-borne diseases have been studied since the early 90's. Risk factors associated with Lyme disease specifically have been studied in Pennsylvania, Maryland, New Jersey, California, Wisconsin, Rhode Island and Connecticut, all highly endemic states for Lyme disease and the normal habitat for the *Ixodes* tick that serves as the vector for Lyme disease. These studies examined the following risk factors: location of primary residence, activities associated with tick habitat contact, and pet or animal ownership. Occupational risk was also examined.

In the one of the earliest studies, Glass et al. (1995) performed a case-control study (*n* = 47/492) in Baltimore County, Maryland for incident cases in 1989 and 1990. This study focused on environmental factors associated with tick habitat and interaction venues for tick/human interactions. Study results suggested that living close to a forest increased the risk for contact with a tick and the subsequent development of Lyme disease by a factor of three for those individuals who lived at the forest edge (Glass et al.,

1995). As the distance between living space and forest increases, risk drops accordingly (Glass et al., 1995). In addition, living in a highly developed urban area provided a protective effect (Glass et al., 1995). Glass et al. (1995) were the only researchers to find this protective effect, although other studies did support distance from forested areas as a reduced risk for contracting Lyme disease.

Ley, Olshen, and Reingold (1995) examined common outdoor activities that could provide an opportunity for a tick-human interaction. The case-control study took place in California with 101 cases and 107 controls (Ley, Olshen, &Reingold, 1995). During the period 1992-1997, California had only 581 confirmed Lyme disease cases so the case sample size for the study period of June 1991-December 1992 provided a large case population (CDC, 2015).

Results of this study did not identify any activities that were significantly associated with contracting Lyme disease. Activities selected for examination included a variety of yard work activities, like gardening, clearing brush, and stacking wood, and leisure activities, like hiking, biking, camping, and fishing (Ley et al., 1995). The study did not examine distance of the home from a wooded area, but did examine whether a fenced in or natural yard was present (Ley et al., 1995).

These results are in direct contrast with the studies by Glass et al. (1995), Orloski et al. (1998), and Belongia et al. (1999) discussed in this review. Ley et al. (1996) suggested that this contrast may be due to the fact that California is a very populous state with 29 million people living there in 1990 (US Census, 2001). A sample size of 101 confirmed cases would not be representative of the entire state and significance levels would be difficult to reach (Ley et al., 1995). In addition, California covers a large

surface area with many different climates present across the state. Tick habitat availability would vary significantly across the many distinct climate areas in California, further diluting the potential for significant differences in Lyme disease risk (Ley et al., 1995).

In an earlier study (n = 83) in California conducted by Lane et al. (1992), outdoor risk factors for Lyme disease were also examined. Of the variables examined as a potential risk factor for Lyme disease, only woodcutting (OR = 4.8; 95% CI 1.01-23.10) showed statistical significance (Lane et al., 1992). In addition, living in a "natural" area (with a home close to a wooded area) was considered an increased risk but the statistical measures were not provided for this risk, so the level of risk can't be quantified from the reported data (Lane et al., 1992). Lane et al. (1992) were the only researchers to report a significant difference in risk between females and males (OR = 2.3; 95% CI 0.94-5.81) infected with Lyme disease. Sex data was collected in the studies conducted by Glass et al. (1995), Ley et al. (1996), Klein et al. (1996), Orloski et al. (1998), and Belongia et al. (1999) but were not analyzed as a potential risk factor for developing Lyme disease.

Klein, Epps, and Hunt (1996) specifically studied environmental factors and activities in children. In this case-control study (n = 44/44), twenty four environmental factors and 45 activities were examined for increased risk for Lyme disease in the northeastern endemic states of Maryland, New Jersey, and Pennsylvania (Klein et al., 1996). Based on the findings of this study, the only significant risk for Lyme disease in children is the presence of deer ticks in the home and yard (OR = 3.05; 95%CI 0.97-9.89) (Klein et al., 1996). Unfortunately, the authors did not provide what specific

environmental factors or activities were surveyed so no comparisons can be drawn with the other studies examined in this literature review.

Orloski et al. (1998) performed a case-control (n = 51) study that examined both behavioral and environmental factors for Lyme disease. Results showed the typical bimodal age distribution (<11 years and 40-60 years peaks), month of onset (May, June, or July), presence of erythema migrans (EM) rash (87%), and sex distribution (males in the younger age group; females in the older age group) as that reported by the Centers for Disease Control and Prevention (Orloski et al., 1998). In addition, living near wooded areas (OR = 15.0; 95CI) and clearing heavy brush (OR = 4.0; 95CI) on their properties produced the greatest risk for contracting Lyme disease. Lastly, rock walls present on the property also showed an increased risk for Lyme disease because rock walls provide a good habitat for small mammals, like the white-footed mouse, that serve as reservoir hosts for *Borrelia burgdorferi* (Orloski et al., 1998).

In contrast to the study by Glass et al. (1995), Orloski et al. found that living in an urban environment did not produce a protective effect (Orloski et al., 1998). Outdoor activities like walking, hiking, or jogging in grassy or wooded areas, gardening or lawn mowing, and hunting or fishing did not increase the risk for developing Lyme disease (Orloski et al., 1998). Cat ownership also did not increase the risk, but dog ownership was not tested and no reason for the exclusion was given (Orloski et al., 1998).

In a study by Belongia et al. (1999), dog ownership was identified as a risk factor for Lyme disease due to the fact that dog owners tend to actively check for and remove ticks from their pet dogs. Surprisingly, this study was one of the few conducted with dog ownership as a variable. Belongia et al. (1999) included cat ownership as a variable but

found no correlation between cat ownership and increased risk for Lyme disease. Other factors identified to increase the risk of contracting Lyme disease include: living in a rural area on a property with more than two acres of land, living near a farm, clearing heavy brush from property or land near property, hiking or jogging on forest paths, and camping (Belongia et al., 1999). Surprisingly, and in direct contrast to the studies by Glass et al. (1995) and Orloski et al. (1998), living near a wooded area and/or having a rock wall or wood pile on the property produced no significant increase in the risk for developing Lyme disease (Belongia et al., 1999). In addition, occupational exposures were not identified as a significant risk factor (Belongia et al., 1999).

Piacentino and Schwartz (2002) conducted a review of the extensive literature on occupational exposure risk of contracting Lyme disease. Workers identified as having a potential increased risk include: forestry workers and lumberjacks, farm workers, military personnel, veterinarians, and other workers who spend large amounts of time outdoors (Piacentino & Schwartz, 2002). Forty one articles were culled from the vast number of articles pertaining to occupational risk of Lyme disease. After careful examination of this literature, Piacentino and Schwartz concluded that there was no evidence to support an increased occupational risk of "symptomatic, clinically confirmed Lyme disease" in any of the categories of outdoor workers (Piacentino & Schwartz, 2002, p. 82).

These result directly supported the study by Smith, Wileyto, Hopkins, Cherry, and Maher (2001) where no increased occupational risk of Lyme disease for outdoor workers was found. The authors conducted the largest case-control study (n = 294/449) to date that examined occupational, behavioral, and environmental risk factors for Lyme disease. While this study showed the same bimodal distribution of cases by age as the data

reported by the CDC (2015), males did not make up more cases in the lower age range and females did not make up more cases in the 40-60 year age range (Smith, Wileyto, Hopkins, Cherry, & Maher, 2001).

Similar to the studies by Glass et al. (1995) and Orloski et al. (1998), living in a rural setting increased the risk of developing Lyme disease three times over the risk associated with living in an urban setting (Smith et al., 2001). In fact, living in a single family home increased the risk for developing Lyme disease 2 ½ times over living in a multi-family dwelling (Smith et al., 2001). In addition, living within 100 feet of a wooded area increased the risk of developing Lyme disease 4-5 fold (Smith et al., 2001). This result is in line with the findings of Glass et al. (1995) and Orloski et al. (1998) but is in direct contrast to the findings of Belongia et al. (1999).

Additional findings identified the following increased risk activities: gardening more than four hours per week (OR = 1.83; CI 1.21, 2.54); walking or jogging in the woods (OR = 1.48; CI 1.09, 2.00); and picnicking in non-traditional locations (OR = 1.47; CI 1.02, 2.12) (Smith et al., 2001). There was no increased risk associated with camping, which was surprising considering most of the other studies found an increased risk for camping (Belongia et al., 1999; Glass et al., 1995; Orloski et al., 1998; Smith et al., 2001).

A more recent study identified which examined risk factors for Lyme disease was conducted by Vazquez et al. (2008). The purpose of this case-control study (*n* = 709/1,128) was to examine personal protective measures utilized by the sample population, but a few occupational, environmental, and activity variables were measured as well. Hiking, camping, pet ownership, and proximity of the home to a wooded area

were not associated with increased Lyme disease risk (Vazquez et al., 2008). In addition, the only occupation with a positive association with increased risk for Lyme disease was farming (Vazquez et al., 2008).

The most recent study identified on risk factors for Lyme disease was conducted by Finch et al. (2014). This study (N = 486 participants/ 105 properties) focused mainly on peridomestic methods of Lyme disease transmission, including pet ownership and shrub cover at the edge of a property. Finch et al. (2014) focused mainly on shrub cover, but surveyed land owners about pet ownership, occupational and recreational activities, and prevention methods. Results suggested that the density of shrub cover and time spent outdoors was correlated to an increased risk of Lyme disease. As with the study conducted by Vazquez et al. (2008), pet ownership did not increase the risk for Lyme disease (Finch et al., 2014).

With the results from studies by Finch et al. (2014), Vazquez et al. (2008), Piacentino and Schwartz (2002), and Smith et al. (2001), occupational exposure to *Ixodes* ticks does not increase an individual's risk of developing Lyme disease. Environmental factors, like living in a wooded area, may or may not increase the risk of developing Lyme disease (Belongia et al., 1999; Glass et al., 1995; Klein et al., 1996; Lane et al., 1992; Ley et al., 1995; Orloski et al., 1998; Smith et al., 2001). Activities, like camping, hunting, hiking, or jogging in wooded areas, may or may not increase your risk of developing Lyme disease as well (Belongia et al., 1999; Glass et al., 1995; Klein et al., 1996; Lane et al., 1992; Ley et al., 1995; Orloski et al., 1998; Smith et al., 2001). With the conflicting data provided by these studies, additional information on risk factors for Lyme disease is needed.

Immune System Response

In order to understand the sex differences found in the immune response to Lyme disease, one must first examine the immune system response to the bacterial pathogen in general. *Borrelia burgdorferi* enters the host through the tick bite. The tick provides some protection to the bacteria upon injection into the host because the tick also injects certain molecules that insure the tick can feed undetected. These molecules prevent immune system activation by preventing the activation of immune cells including neutrophils, B-lymphocytes, T-lymphocytes and dendritic cells, as well as preventing the release of early cytokines and antimicrobial peptides (Radolf et al., 2012; Schuijt et al., 2008). One molecule used is Salp-15 (Schuijt et al., 2008). Salp-15 prevents $CD4^+$ T-lymphocyte (helper T-cell) activation by binding to its cellular receptor (Schuijt et al., 2008). In addition, using the Salp-15 protein helps *Borrelia* to prevent the activation of the complement system and, if activated, allows evasion of the complement system proteins (Schuijt et al., 2008).

Toll-like receptors (TLR2 and TLR1) on macrophages and dendritic cells circulating within the skin are activated by binding to lipoproteins (OspA) on the surface of *Borrelia*, causing the release of key cytokines to initiate the immune system response to the invading pathogen (Radolf et al., 2012; Shin et al., 2008). This response can be either a T_{H1}-cell or a T_{H2}-cell response. During a T_{H1}-cell response, cytokines gamma interferon (IFN-γ), transforming growth factor – beta (TGF-β) and interleukin-2 (IL-2) is released (Romagnani, 2000). During a T_{H2}-cell response, cytokines (IL-4, IL-5, IL-6, IL-9, IL-10, IL-13) may be released, depending on the stimulus (Romagnani, 2000). Specific cytokines released in response to *Borrelia* infection includes tumor necrosis factor alpha

(TNF-α), transforming growth factor beta (TGF-β_1), interleukins (IL-1, IL-6, IL-10, IL-17) and interferons (IFN-α, IFN-γ) (Glickstein et al., 2003; Radolf et al., 2012; Sehgal & Khurana, 2015; Widhe et al., 2002). The functions of the various cytokines are shown in Table 3.

Table 3

Cytokine Functions (Owen, Punt, & Stranford, 2013)

Cytokine	Secreted by	Effects
Tumor Necrosis Factor Alpha (TNF-α)	Macrophages Neutrophils T-lymphocytes	Inflammation
Transforming Growth Factor – beta (TGF-β_1)	Macrophages T-lymphocytes	Inhibits T-cell and B-cell proliferation; Inhibits macrophages
Interleukin -1 (IL-1)	Macrophages	Inflammation
Interleukin – 6 (IL-6)	Macrophages T_H2-lymphocytes	Proliferation of B-lymphocytes; Antibody production
Interleukin – 10 (IL-10)	B-lymphocytes T-lymphocytes Dendritic Cells	Regulatory cytokine
Interleukin – 17 (IL-17)	T_H-lymphocytes	Inflammation; neutrophil recruitment
Alpha – Interferon (IFN-α)	Macrophages Dendritic Cells	Increases MHCI expression
Gamma – Interferon (IFN-γ)	T_H1-lymphocytes T_C-lymphocytes	Activates macrophages; Increases MHCI and MCHII expression

Release of these cytokines initiate the inflammatory response, calls other immune cells to the site of infection, and activate B-lymphocyte differentiate and proliferation (Radolf et al., 2012; Widhe et al., 2002). While neutrophils are recruited early in the infection process, T- lymphocyte (both $CD4^+$ and $CD8^+$) activation leads to the

production of the EM rash in infected tissue (Glickstein et al., 2003; Radolf et al., 2012;). B-lymphocytes are not activated during the EM rash and the early localized portion of the infection cycle (Radolf et al., 2012).

Activation of B-lymphocytes occurs once *Borrelia* leaves the initial infection site and enters the bloodstream where it can come in contact with B-lymphocytes in the spleen during normal transport through the body (Radolf et al., 2012). Antibody production occurs in a two-fold process – IgM antibodies are produced first, but don't persist for very long (Radolf et al., 2012). IgG antibodies take longer to be produced, but last longer in tissues and circulation (Radolf et al., 2012). Antibodies are produced against many of the outer surface proteins (Osp), including OspA and OspC even though these proteins don't seem to play a role outside of the tick host (Liang et al., 2004; Radolf et al., 2012). In addition, the lipoprotein VlsE is found on the surface of the organism but can demonstrate significant variation in structure, leading to the need for multiple antibodies against this specific protein to offer protection (Kenedy, Lenhart, & Akins, 2012; Radolf et al., 2012).

One of the primary identified differences between the male and female sex is the presence of the steroid hormone estrogen. While estrogen's primary role in the body is during the reproductive cycle in women, estrogen does have an effect on the immune system (Baker et al., 2011; Bullard et al., 2012; Pennell et al., 2012). Several immune cells have membrane bound cell receptors that bind estrogen, which leads to the activation and amplification of a signal transduction cascade (Pennell et al., 2012). These immune cells include: B-lymphocytes, T-lymphocytes, dendritic cells, neutrophils, macrophages, and natural killer (NK) cells (Pennell et al., 2012).

In addition, binding of estrogen by immune cells can initiate a cell-specific response. Binding of estrogen to B-lymphocytes increases proliferation and antibody production (Pennell et al., 2012). Binding of estrogen by T_H-lymphocytes leads to increased production of IL-10, which also leads to increased proliferation of and antibody production in B-lymphocytes (Pennell et al., 2012). Estrogen inhibits IL-1 and IL-6, reducing the inflammatory response of effected tissue (Bullard et al., 2010).

TNF-α and IL-1, along with IFN-γ, initiate the inflammatory response in vascular tissue, allowing more fluid and immune cells to enter into the site of the tick bite (Baker et al., 2003). This response occurs in both sexes, although IL-1 production is inhibited by estrogen in women (Bullard et al., 2010; Pennell et al., 2012). Estrogen increases the amount of IgM and IgG antibodies produced by women and can induce IgM and IgG production if administered to men (Oertelt-Prigione, 2012). Unfortunately, testosterone inhibits IgM and IgG production by reducing the amount of IL-6 produced (Oertelt-Prigione, 2012). Lastly, estrogen tends to produce a T_H2-lymphocyte response, which includes increased B-lymphocyte activation and release of IL-4, IL-5, and IL-9 cytokines (Pennell et al., 2012). Androgens in males produce a T_H1-lymphocyte response, where IFN-γ is the primary cytokine released and $CD4^+$ and $CD8^+$ T-lymphocytes are activated (Pennell et al., 2012).

Infection with *Borrelia burgdorferi* causes the release of the following cytokines in both male and female cases: tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β_1), interleukins (IL-1, IL-6, IL-10, IL-17) and interferon (IFN-α, IFN-γ) (Glickstein et al., 2003; Radolf et al., 2012; Sehgal & Khurana, 2015; Widhe et al., 2002). Estrogen leads to inhibition of IL-1 and IL-6 secretion, providing a reduced

inflammatory effect and a reduction in the proliferation and antibody production by B-lymphocytes in females that would not be found in males (Pennell et al., 2012). In addition, estrogen causes an increase in the production of IL-10, a regulatory cytokine that helps to control inflammation, further reducing the inflammatory response (Pennell et al., 2012). IL-10 production is stimulated by testosterone in men (Giefing-Kroll et al., 2015).

Since the inflammatory response serves as an important factor to keep an infection localized and allow additional immune cells to enter the infection site, this reduction in the inflammatory response could lead to increased symptom frequency and severity in female cases. Reduced inflammation also allows the bacteria to spread from the site of infection to other locations, like the joints and nervous system. Lastly, androgens in males produce $CD8^+$ T-lymphocyte activation, which helps to clear the infection at the initial infection site (Giefing-Kroll et al., 2015). While the focus of the current study was not based on estrogen levels, the effect of estrogen on the immune system's response to an invading pathogen provides support for the reported sex differences.

Sex Differences

While sex differences in risk factors for Lyme disease was discussed by only Lane et al. (1992), sex differences for other diseases have been identified. Cardiovascular disease (CVD) sex differences have been documented and continue to be studied (DeVon, Ryan, Ochs, & Shapiro, 2008; Dey et al., 2009; Kure et al., 2016; Norris, Dasgupta, & Kirkland, 2007). CVD symptom presentation can be markedly different in males and females (DeVon et al., 2008; Dey et al., 2009; Norris et al., 2007). Males

suffering from CVD tend to present with the typical symptom of chest pain that radiates out to the left arm (DeVon et al., 2008; Dey et al., 2009; Norris et al., 2007). Women suffering from CVD tend to present with non-typical symptoms like pain in the back, neck, or jaw, shortness of breath, or indigestion (DeVon et al., 2008; Dey et al., 2009; Norris et al., 2007). This variation in symptom presentation often keeps women from seeking needed medical care early. In addition, non-typical symptom presentation increases the chances for misdiagnosis and administration of the wrong treatment when treatment delays can be life threatening (DeVon & Zerwic, 2002; Kure et al., 2016).

One hypothesis for the sex differences in CVD hinges on estrogen. Estrogen, a female sex hormone, is believed to have cardio-protective effects because women develop less CVD prior to menopause than men of the same age, but the risk of developing CVD becomes equal between men and women after menopause (Baker et al., 2003; Murphy et al., 2011). Estrogen not only controls the female menstrual cycle, but also plays a key role in regulating many other body mechanisms. One of those mechanisms is the inflammatory response (Murphy et al., 2011).

Estrogen regulates several key cytokines responsible for the inflammatory response (Bullard et al., 2010). First, estrogen inhibits production of interleukin-1 (IL-1) by direct action and through promoting the production of interleukin-4 (IL-4) and interleukin-10 (IL-10), which also inhibit IL-1 production (Bullard et al., 2010). IL-1 is the cytokine responsible for the initiation of the inflammatory response (Bullard et al., 2010). While estrogen levels remain in the normal physiological range, protection from inappropriate or excessive inflammation remains (Baker et al., 2003; Bullard et al.,

2010). Once menopause occurs, this protective effect is reduced with reduced estrogen concentrations (Bullard et al., 2010).

Parkinson's disease is another condition that displays sex differences in symptom presentation. In a cohort study (n = 253) by Haaxma et al. (2006), women developed symptoms of Parkinson's disease up to two years later than men. In addition, women suffered more frequently with dyskinesias at disease onset then men who tended to present with more bradykinesias/rigidity (Haaxma et al., 2006). Higher estrogen levels in women have been hypothesized to have protective value against Parkinson's disease development (Currie, Harrison, Trugman, Bennett, & Wooten, 2004; Haaxma et al., 2006; Liu et al., 2012). Unfortunately, not all research supports this theory (Lyons, Hubble, Troster, Pahwa, &Koller, 1998; Strijks, Kremer, &Horstink, 1999). In a study of 630 Parkinson's patients conducted by Lyons et al. (1998), the sex differences in symptom presentation was supported but estrogen as a protective factor against Parkinson's disease was not.

Autoimmune diseases affect only 8% of the entire population but almost 80% of those affected are women (Fairweather, Petri, Coronado, & Cooper, Jr., 2012; Ngo et al., 2014; Quintero et al., 2012). Diseases such as RA (2-3:1 female to male ratio), SLE (9:1 female to male ratio), MS (2-3:1 female to male ratio), scleroderma (up to 14:1 female to male ratio), and Sjogren's syndrome (9:1 female to male ratio) are especially prominent in women (Fairweather et al., 2012; Ngo et al., 2014; Quintero et al., 2012; Whitacre, 2001). All of these diseases display an inflammatory response of some type by the immune system that leads to the outward signs and symptoms of the disorder. These types of autoimmune diseases contradict what is known and accepted about the protective

anti-inflammatory effects of estrogen, but inflammation that remains for extended periods of time leads to tissue damage as well (Casimir et al., 2010). Unfortunately, it is not only the anti-inflammatory response that does the damage in autoimmune disease; self-antibodies cause long-term tissue destruction and damage (Whitacre, 2001).

Estrogen regulates B-lymphocyte production and differentiation through IL-4 and IL-10 (Bullard et al., 2010). IL-4 increases the production of new B-lymphocytes that will ultimately produce IgG and IgE antibodies (Bullard et al., 2010). In addition, IL-10 increase B-lymphocyte activation and stimulates antibody production (Bullard et al., 2010). Lastly, estrogen promotes helper ($CD4^+$) T-lymphocyte differentiation, increasing the body's ability to activate B-lymphocytes (Bullard et al., 2010). This activity ultimately leads to the ability to produce large amounts of self-antibodies and autoimmune disease. Women tend to have a higher $CD4^+$ T-lymphocytes numbers than men when in the healthy state, so increased activation and differentiation can lead to a disease state quickly (Whitacre, 2001).

So far, all of the diseases examined here are chronic diseases and Lyme disease is caused by an infectious agent. How are sex differences between these two vastly different types of conditions connected? First, Lyme disease is commonly misdiagnosed as an autoimmune disease (Savely, 2010). Frequently, a patient is bitten by a tick in an area where the tick and/or bite is not easily seen – back of the body, hairline, armpits, and groin (Bennet, Stjernberg, & Berglund, 2007; Savely, 2010). The American College of Rheumatology estimates the number of tick bites that goes unnoticed to be between 10-25% (Kalish, 2013). In addition, patients don't always remember being bitten by a tick at

all. In a study conducted in the Netherlands, the number of cases of Lyme disease where the participant didn't remember a tick bite was as high as 34% (Hofhuis et al., 2013).

This misinformation often leads the physician to look at other illnesses with similar symptoms (Savely, 2010). Lyme disease may be misdiagnosed as any of the following disease: autoimmune diseases including but not limited to RA, MS, and SLE, along with non-autoimmune diseases like Parkinson's disease and early onset Alzheimer disease (Savely, 2010). This misdiagnosis is based on symptoms that can mimic any or all of these conditions.

Sex and Age Differences

According to the CDC (2015), males in all age categories under age 70 suffer more frequently from confirmed Lyme disease than females of the corresponding age (Figure 3).

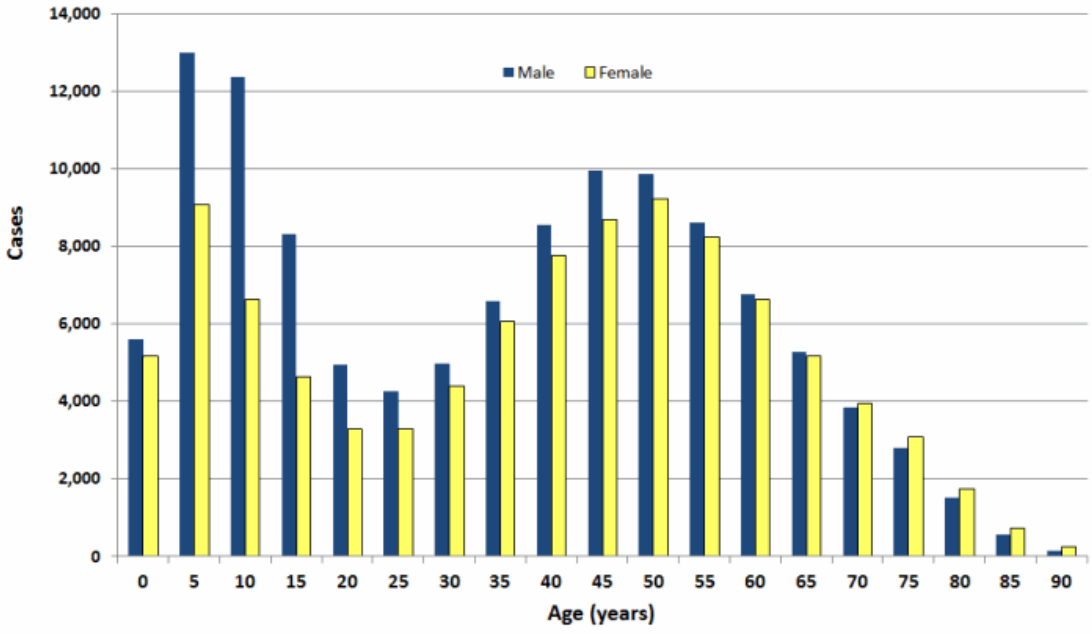

Figure 3. Confirmed cases of Lyme disease by sex and age, United States, 2001-2010 (CDC, 2015)

The immune system undergoes extensive changes as a person ages (Giefing-Kroll et al., 2015). Innate immune cells, like macrophages and dendritic cells, lose the ability to effectively present foreign antigens to T-lymphocytes for activation (Giefing-Kroll et al., 2015). In addition, thymus function declines with age so T-lymphocyte maturation is reduced leading to fewer T-lymphocytes available to fight infection (Giefing-Kroll et al., 2015). Lastly, effective antibody production also declines with age (Giefing-Kroll et al., 2015). Sex-differences of the immune system level off with declining production of estrogen by the ovaries as a woman ages (Giefing-Kroll et al., 2015).

Aging has also been shown to have an effect on the cytokines and immune cells of innate immunity. Castle (2000) discusses the effects of aging on specific cytokines, all of which are induced by infection with *Borrelia burgdorferi*. Production of IL-6 increases with age-related changes to the immune system (Castle, 2000). Increased production of IL-6 has two effects: 1) IL-6 inhibits macrophage activity and 2) increased B-lymphocyte antibody production (Castle, 2000). Production of IL-10 also increases with age-related changes to the immune system. Increased IL-10 production also has two effects: 1) increased anti-inflammatory effects and 2) inhibition of the T_H1-lymphocyte response (Castle, 2000). Lastly, TNF-α production increases with age leading to increased inflammatory responses in the individual (Castle, 2000). Even though immune system function changes with age and sex, the symptoms of Lyme disease produced by these changes may be an important tool in early diagnosis of the disease.

Symptoms of Lyme Disease

Lyme disease displays a wide array of symptoms based on the stage of the infection. Many of the symptoms can be mistaken easily for another disease or condition.

Stages of infection include: early localized Lyme disease, early disseminated Lyme disease, and late Lyme disease (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick et al., 2009). In addition to these three stages, two separate conditions have been discussed in the literature – post-treatment Lyme disease syndromes and chronic Lyme disease (Aucott et al., 2012; Cairns & Goodwin, 2005; Cameron, 2010; Wormser et al., 2006).

Early Localized Lyme Disease

In this stage of infection, symptoms include mild flu-like symptoms, headache, fatigue, and malaise (Binder et al.., 2012; Borchers, 2015; Donta, 2012; Girschick et al., 2009). During this stage, the characteristic "bull's eye" erythema migrans (EM) rash will also appear (Binder, 2012; Borchers, 2015; Donta, 2012; Girschick et al., 2009; Miraflor et al, 2016). The EM rash begins at the site of the tick bite as a raised red rash with a clear central area (Moore, 2015; Muellegger, 2004). To differentiate between a true EM rash and an allergic response to the tick bite, the rash should be monitored over time (Muellegger, 2004; Tibbles & Edlow, 2007). The classic EM rash will start out as a small round to oval lesion, but will *increase* in size to at least 5 cm in diameter (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick et al., 2009; Tibbles & Edlow, 2007). An allergic response to the tick bite will *decrease* in diameter over time (Muellegger, 2004).

The EM rash appears in 70-80% of Lyme disease patients but takes on the characteristic "bull's eye" appearance only 20% of the time (Aucott et al., 2012; Johnson & Stricker, 2004; Muellegger, 2004). Other, non-traditional appearances of the EM rash include: homogenous red lesions with no central clearing; secondary lesions;

vesiculopustular lesions; and lesions with bruising, which typically occur on the calves (Aucott et al., 2012).

One of the few reported sex differences with Lyme disease is related to the EM rash. In two separate studies, females were more likely to develop the EM rash than their male counterparts and of those females who developed the EM rash; the rash was more likely to appear in the non-typical form (Bennet et al., 2007; Strle et al., 2013). Strle et al. (2013) conducted a retrospective chart review of 15,539 patients over the age of 15 years diagnosed with an EM rash between 1990 and 2009. Results confirmed that 58% of the EM diagnoses were in women (Strle et al., 2013). This research was conducted in a single medical center in Slovenia, so confounding related to different diagnostic methods were minimized (Strle et al., 2013).

Bennet et al. (2007) conducted a retrospective study in Sweden of 123,495 patient records for the presence and appearance of the EM rash. Results showed that 20% more women were diagnosed with EM rash than men during the years 1997-2003 (Bennet et al., 2007). Of those women diagnosed with the EM rash, 40% of them displayed the non-typical presentation of the EM rash (Bennet et al., 2007).

Bennet et al. (2007) also reported that a few other European studies had similar results for the higher prevalence of EM rash in women but after reviewing these additional studies, the results were not as reported. In the Bennet et al. study, German cases of EM rash in females were reported at 55%, but the actual study by Mehnert and Krause (2005) stated that 55% of reported Lyme disease cases in Germany were female. Mehnert and Krause did not report the percentage of EM rash cases in terms of females or males. The same was true for the Stanek et al. (1987) study. Bennet et al. reported that

the number of cases of EM rash in females in Austria was 60%, but Stanek et al. reported that 60% of reported Lyme disease cases in Austria were females with no distinction in EM rash appearance (Stanek et al., 1987).

Although evidentiary support was weak for the Bennet et al. (2007) study, study data did display sex differences in EM reporting and appearance. Unfortunately, all reported studies were conducted in Europe where the *Borrelia* strain varies significantly from the *Borrelia* strain found in the United States. Studies within the United States where *Borrelia burgdorferi* sensu stricto is the predominant strain must be conducted.

An additional study was conducted by Schwarzwalder, Schneider, Lydecker, and Aucott (2010). This was a retrospective case series study of 125 patients from a Maryland clinic (Schwarzwalder et al., 2010). Early Lyme disease symptoms of EM rash and flu-like symptoms were measured for sex differences (Aucott et al., 2013). No significant sex differences were found within the study population, but the authors contribute these results to the small sample size and the difficulty with confirming true early cases of Lyme disease via serology (Aucott et al., 2013).

Early Disseminated Lyme Disease

This stage of infection occurs when the bacteria leave the initial site of infection and spread to other body tissues. Typical symptoms that coincide with the spread of the bacteria include neurological symptoms like meningitis and facial palsies (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Franz & Krause, 2003; Girschick et al., 2009; Muellegger, 2004; Wormser et al., 2006). Secondary EM rash lesions may appear at sites distant from the initial tick bite, but these lesions are more common in children than adults (Girschick et al., 2009). Cardiac complications can occur in untreated patients that

include various degrees of atrioventricular block (Alao & Decker, 2012). Heart complications tend to resolve with treatment (Alao & Decker, 2012). Finally, mild musculoskeletal symptoms may appear (Girschick et al., 2009).

The hallmark of this stage of infection is the development of neurological symptoms, but meningitis and facial palsies can have other causes besides the *Borrelia* bacteria. Unfortunately, treatment is different for *Borrelia burgdorferi* infection than treatment given for facial palsies of other etiology. Determining the cause of the neurologic symptoms is paramount in providing appropriate treatment.

Bremell and Hagberg (2011) conducted a study of 109 Swedish patients with some form of facial nerve palsy. The purpose was to identify clinical markers to increase the speed and accuracy for diagnosis of the causative agent of the palsy (Bremell & Hagberg, 2011). Bell's palsy is generally treated with corticosteroids, while Lyme neuroborreliosis is treated with antibiotics (Bremell & Hagberg, 2011). Unfortunately, an inaccurate diagnosis would, at best, provide treatment that would not help the patient at all, and in the case of Lyme neuroborreliosis, would actually hurt the patient more (Bremell & Hagberg, 2011). Results of the study demonstrated that in confirmed neuroborreliosis cases, patients displayed a significantly higher number of mononuclear cells in extracted cerebrospinal fluid than patients without neuroborreliosis (Bremell & Hagberg, 2011). Unfortunately, even with early diagnosis and proper treatment, Lyme neuroborreliosis may persist.

In a follow-up case control study conducted in Sweden five years after the initial study, recovery rate was only 73% in children (n = 84/84) diagnosed with and treated for Lyme neuroborreliosis (Skogman et al., 2012). Where facial palsy was the primary

symptom, partial facial palsy remained in 13% of subjects (Skogman et al., 2012). In addition, balance issues and persistent pain was found in 14% of subjects (Skogman et al., 2012). This impairment underscores the need for diagnosis in the earliest stage of infection to prevent these long term outcomes.

Late Lyme Disease.

If left untreated, Lyme disease can progress to late stage infection. This stage occurs months to years after the initial tick bite and is most commonly associated with arthritis in the large joints of the limbs (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick et al., 2009). Additionally, advanced neurological and cardiac symptoms can be found (Binder et al., 2012; Borchers et al., 2015; Donta, 2012; Girschick et al., 2009). In the United States, arthritis is the most common late stage affliction (Alao & Decker, 2012; Bennet et al., 2007; Borchers et al., 2015). In Europe, acrodermatitis chronica atrophicans (ACA) can also manifest in late stage infections due to chronic skin infection by *Borrelia burgdorferi* (Alao & Decker, 2012).

In a European study conducted by Strle et al. (2013), sex differences were present for arthritis symptoms. Within the patient sample (n = 60) diagnosed with Lyme arthritis, three quarters of the patients were male (Strle et al., 2013). This significant difference was supported even when the possibility of misdiagnosis was controlled for (Strle et al., 2013). In the same study, males diagnosed with Lyme neuroborreliosis made up 60% of the study population (Strle et al., 2013). These findings have not been supported by other published research in the United States or Europe to date.

Posttreatment Lyme Disease Syndrome

Controversy surrounds the existence of post-treatment Lyme disease syndrome. At its core, the controversy centers around the efficacy of current treatment guidelines for Lyme disease and whether longer courses of antibiotics are needed for complete eradication of the *Borrelia burgdorferi* from a patient. When diagnosed early and the treatment regimen is completed, most patients find relief from signs and symptoms of Lyme disease (Borchers et al., 2015; Deluca et al., 2013; Lantos, 2011; Moore, 2015). Unfortunately, in up to 10-15% of those patients who are not diagnosed early or complete a standard course of antibiotics, symptoms may persist after treatment is complete (Aucott, Crowder, & Kortte, 2013; Deluca et al., 2013). Persistent symptoms include musculoskeletal complaints including myalgia and arthralgia; headache; fatigue; and cognitive symptoms like difficulty concentrating and memory loss (Aucott, Crowder, & Kortte, 2013; Deluca et al., 2013; Lantos, 2011).

Based on the clinical practice guidelines for Lyme disease created by the Infectious Disease Society of America (IDSA), post-treatment Lyme disease does not exist (Lantos et al., 2010; Wormser et al., 2006). According to the IDSA, the standard course of 21-days of antibiotics will kill the *Borrelia* bacteria and any persistent symptoms may be related to co-infection with another organism or just the normal aches and pains of daily life (Wormser et al., 2006). Unfortunately, this explanation does not take into the account the large number of patients who experience persistent symptoms after treatment is complete (Deluca et al., 2013).

In order to address the IDSA's claim that there is no scientific evidence to support a diagnosis of post-treatment Lyme disease syndrome, Aucott, Crowder, and Kortte

(2013) designed a study to develop and support an operational definition for post-treatment Lyme disease syndrome so that further studies can be standardized and data can be collected. The study included 71 cases (14 matched controls) that entered the study with a diagnosis of Lyme disease via the presence of the EM rash (Aucott et al., 2013). Subjects were followed for a period of up to two years post diagnosis (Aucott et al., 2013). Based on results from this study, the following operational definition of post-treatment Lyme disease is proposed by Aucott et al.:

Inclusion criteria were as follows:

- Documented Lyme disease with evidence of systemic disease
- Treatment using doxycycline (FDA approved treatment regimen), resulting in the resolution of objective manifestation of disease
- Onset of any of the following subjective symptoms within 6 months of the diagnosis of Lyme disease and persistence of continuous or relapsing symptoms for at least a 6 month period after completion of antibiotic therapy:
    - Endorsement of fatigue at a level higher than pre-infection
    - At least 3 areas of the body affected by musculoskeletal pain
    - Complaints of difficulty finding words, difficulty focusing or concentrating, or memory impact
- A composite T-score < 45 (less than ½ standard deviation below normative mean) on SF-36

Exclusion criteria were as follows:

- Active co-infection
- Other underlying disease or condition that explains symptoms

- Previously diagnosed fibromyalgia or chronic fatigue syndrome
- Undiagnosed or unexplained complaints of musculoskeletal pain or fatigue before diagnosis of Lyme disease (pg. e3).

By establishing this operational definition, Aucott et al. (2013) looked to provide a framework for future research on posttreatment Lyme disease syndrome so that evidence either for or against this condition can be collected.

Each stage of Lyme disease has clearly defined symptoms experienced by patients. These symptoms, along with severity of symptoms, will be used to determine what other factors may contribute to symptom presentation in Lyme disease sufferers.

Theoretical Framework

The theoretical framework of the study follows the CDC outbreak investigation model. In this model, the relationship between a variable under study and a disease like Lyme disease can be examined (Reingold, 1998). The typical descriptive epidemiology factors corresponding to person, place, and time are identified and relationships between potential exposures or risk factors and the disease in question are explored (Reingold, 1998; Roher, 2013). While Lyme disease does not fall into the typical infectious disease category that would be examined as an outbreak, Lyme disease is a vector-borne disease and is well suited to the outbreak investigation model. In fact, the outbreak investigation model was initially used to identify the causative agent for Lyme disease after a significant number of cases of arthritis appeared in a group of children in the area of Lyme, Connecticut (Sternbach & Dibble, 1996).

Lyme disease is currently the most frequently reported vector-borne illness in the United States (Binder et al., 2012). Considering that Lyme disease may be underreported

by as high as 12 times, determining the factors that lead to this underreporting becomes paramount. Differences in symptom presentation and reporting based on sociodemographic factors like biological sex or age may hold the answers to identifying more cases earlier in the infection cycle, when treatment is most effective. This study will provide insights into the factors effecting symptom presentation and severity for Lyme disease.

Chapter 3: Research Method

Introduction

This chapter contains a review the methodology associated with studying symptom presentation and severity for Lyme disease. I explain the research design along with the rationale for the selection of a case-control research design. In addition, I clearly describe the study population and the clinic and college where data collection occurs. Recruitment methods, informed consent methods, and sampling methods will be discussed. I provide information on instrument selection, validity, and reliability as well. Variables will be discussed in terms of research questions generated. In addition, I review statistical analysis methods. Lastly, I discuss the protections in place for the study subjects w along with how the final report of findings to subjects will occur.

Research Design and Approach

In this study I employed a case-control study design because this study examined factors associated with symptom presentation and severity in Lyme disease patients compared to unaffected control subjects. ROSS scales from the primary care clinic and college were reviewed to provide insight into variations in symptom presentation and severity. This data is retrospective and aligns well with the case-control study design.

Case control study designs are often used to study rare diseases within the population because the number of subjects needed to reach statistical significance is smaller than the number of subjects needed for a cohort study design (Mann, 2003; Schulz & Grimes, 2002; Song & Chung, 2010). With 33,000 cases diagnosed in 2011 in the United States (population 310 million), Lyme disease would be considered a rare disease (CDC, 2015; US Census Bureau, 2014). Other rare diseases that have previously

been studied by a case-control study design include, but are not limited to: certain types of cancer (breast, ovarian, esophageal, prostate, colon, and pancreatic); psychiatric disorders (schizophrenia and bipolar disorder); infectious diseases (neonatal tetanus and Nipah virus); and autoimmune disorders such as SLE (Schulz & Grimes, 2002; Song & Chung, 2010).

A key advantage to the case control study design is the ability to examine multiple variables that may or may not be associated with a specific disease (Song & Chung, 2010). In the case of Lyme disease, there are a wide variety of potential exposures to the *Ixodes scapularis* tick, the key vector in the spread of Lyme disease. In addition, Lyme disease shows a bivariate bimodal age distribution, with the largest number of cases occurring in males under 13 years old and females over the age of 40 (CDC, 2015). Variables including sociodemographic variables (age and sex), seasonal variables (month of diagnosis, month of exposure), symptoms present, and severity of symptoms were examined during this case-control study.

Unfortunately, the case-control study design has a few disadvantages as well. The primary disadvantage for the case-control study design is the potential for bias (Pannucci et al., 2010; Song & Chung, 2010). The two main types of bias that must be considered when designing a case-control study are sampling bias and recall bias (Mann, 2003; Pannucci et al., 2010; Song & Chung, 2010). Sampling bias may be difficult to control for in a case-control study because the cases already represent a biased sample since they have the condition under study (Mann, 2003; Song & Chung, 2010). With a rare disease or condition, random sampling of a population is difficult since so few individuals may have the condition (Mann, 2003; Song & Chung, 2010). Careful selection of controls

must be performed to reduce the impact of sampling bias (Mann, 2003; Pannucci et al., 2010; Song & Chung, 2010).

The best way to reduce sampling bias is to make sure that the controls are matched as closely as possible to the case population (Mann, 2003; Pannucci et al., 2010; Song & Chung, 2010; Zondervan, Cardon, & Kennedy, 2002). Matching must be done carefully because any variable selected to match the controls with the cases cannot be assessed as a variable for the study (Song & Chung, 2010; Zondervan et al., 2002). Because sex and age were two variables under investigation in this study, controls could not be matched to cases based on these criteria. In order to ensure that comparisons could be made between the case and control groups, frequency matching was used to provide consistency within and between the two groups (Song & Chung, 2010). For example, frequency matching makes sure that the percentage of males in the case group is the same as the percentage of males in the control group (Song & Chung, 2010). Thus, frequency matching was used for sex and age variables in this study.

Matching was used among cases and controls on the basis of state of residence. Lyme disease is endemic to 14 states, primarily in the Northeastern United States (CDC, 2015). These states include Pennsylvania, New York, New Jersey, Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, Rhode Island, Vermont, Virginia, and Wisconsin (CDC, 2015). Because the primary care clinic treats patients from all over the country, cases and controls were matched on state of residence to ensure that the potential exposure to the *Ixodes* tick is equal between the two groups.

In addition, Lyme disease is a vector-borne disease that, if caught early, can be cured with a 21-28 day course of antibiotics (Gerstenblith & Stern, 2014). Lyme disease

is therefore reported in incidence rates because each case reported to the National Notifiable Disease Surveillance System (NNDSS) represents a newly diagnosed infection. The nature of disease reporting between the states and the CDC may also lead to underreporting of the disease, over reporting of the disease, and/or false surveillance numbers due to misdiagnosis of the infection (CDC, 2015). States used in this study were all Lyme endemic states. The current study's population (N = 591) came primarily from New York (39%) and Pennsylvania (42%), which is consistent with the high incidence rates reported for both states by the CDC (2015).

The second type of bias, recall bias, occurs when cases recall exposures more frequently than controls. This often occurs because the cases spend more time trying to determine what exposure may have led to their disease state (Pannucci et al., 2010; Schulz & Courtright, 2002). One way to overcome this type of bias is to use information gathered prior to the beginning of the study (Mann, 2003; Pannucci et al., 2010). With the current study, access to patient records for prior symptoms and complaints was possible, and they were examined as part of the data collection process.

In addition, the primary care site collects symptoms and severity of symptoms via the ROSS scale at every visit as part of the routine intake patient information. This symptom and severity information was part of the patients' medical records and was the primary source of data for the cases in the current study. All controls filled out a ROSS scale describing symptoms experienced within the previous week, which also helps to minimize recall bias.

Setting and Sample Population

Research was conducted at a primary care clinic in New York State. The clinician for this study is a primary care physician who also treats acute and chronic Lyme disease patients. According to clinic records, more than 500 new Lyme disease patients are treated each year, along with an equal number of recurrent Lyme disease patients (Clinical partner, personal communication, April 2014). In addition to primary care, the clinician has been a renowned and published Lyme disease researcher since the early part of the 1990s (Lyme Project, n.d.). Study participants came from the primary care clinic's patient population.

Cases were defined as adult subjects (≥ 18 years of age) who met one of the following diagnostic criteria: (a) patient presents with a physician confirmed EM rash; (b) patient has a positive EIA and/or Western Blot laboratory result for IgG and IgM antibodies; or (c) patient has a score of 5 or higher on the Burrascano Diagnostic Criteria for Lyme Disease scale (Burrascano, 2005). These criteria meet both the Infectious Disease Society of America (IDSA) and the International Lyme and Associated Diseases Society (ILADS) diagnostic guidelines (Cameron, Johnson, & Maloney, 2014; Wormser et al., 2006). Lyme disease cases were selected and confirmed by the clinical partner, a Lyme disease specialist. Cases were randomly selected by the physician, based on the weekly appointment schedule during the study period.

Because the primary care clinic could not provide enough controls for the study population, a secondary data collection site was added to the study. Controls were selected from adult patients (≥ 18 years of age) from the primary care clinic in New York State and the 4-year liberal arts college in Pennsylvania who do not suffer from Lyme

disease. Participants may suffer from other diseases or conditions. All controls were Lyme disease free as determined by: (a) never having had a tick bite; (b) having no evidence of EM rash; or (c) no prior laboratory testing for or diagnosis of Lyme disease by a physician. Study controls were as likely as cases to develop Lyme disease. Controls were selected at a 1:2 case/control ratio to address the sampling bias introduced by nonrandom selection of participants. Unfortunately, because sex and age were two variables of interest in this study, matching of cases and controls along these lines could not occur. Matching based on state of residence was performed to insure that exposure to the *Ixodes* tick was the same between cases and controls.

Sample size calculation is dependent on the types of statistical analyses planned to analyze the data. Each specific type of statistical analysis requires a slightly different number of participants in order to meet the minimal number for statistical significance (Cohen, 1992; Munro, 2005; Schlesselman, 1982). In order to satisfy all statistical analysis methods, the largest sample size was used. Based on the methods described in the statistical analysis section and tables provided by Cohen (1992) and a thorough power analysis performed using the Open Epi toolkit as described below, a minimum sample size of 120 cases and 240 controls was required (Dean & Sullivan, 2015). The actual sample size (N = 591) more than met this value with 203 cases and 388 controls included in the study. This value was above the minimum calculated value needed for statistical significance, met a 1:2 case to control ratio to address non-random sampling bias, and provided a larger sample size than had been examined previously by other researchers.

Power Analysis

For all power analyses, the sample size of 120 cases and 240 controls were used. For the EM rash symptom, the CDC (2015) and Aucott et al. (2012a) suggested that the EM rash is found in 70% of the individuals who are diagnosed with Lyme disease. This value (70%) was used to represent the percent of exposure among cases. Because the EM rash can be confused with many different types of rashes, determining what to use for the percent of exposure among controls was a little more difficult. After careful consideration, bacterial induced skin rashes were used as the comparison since Lyme disease is caused by a bacterial infection. According to Ki and Rotstein (2008), 10% of the population suffers from a bacterial induced rash. This value (10%) was used to represent the percent of exposure among controls.

Table 4

Power for Unmatched Case-Control Studies – EM Rash

	Input data
Two-sided confidence interval (%)	95
Number of cases	120
Percent of exposure among cases (%)	70
Number of controls	240
Percent of exposure among controls (%)	10
Odds Ratio	21
Power based on:	
Normal approximation	100%
Normal approximation with continuity correction	100%

For the arthritis symptom, the CDC (2015) reports that 31% of individuals who are diagnosed with Lyme disease report arthritis as a symptom. Arthritis associated with Lyme disease is often misdiagnosed as RA because of the similarity between the

symptoms of these two conditions (Savely, 2010). This value (31%) was used to represent the percent of exposures among cases. In addition, the CDC also reports that the annual incidence for RA is between 0.5-1.0%. One percent was used to represent the percent of exposure among controls for the power analysis.

Table 5

Power for Unmatched Case-Control Studies – Arthritis

	Input data
Two-sided confidence interval (%)	95
Number of cases	120
Percent of exposure among cases (%)	31
Number of controls	240
Percent of exposure among controls (%)	1
Odds Ratio	44
Power based on:	
Normal approximation	100%
Normal approximation with continuity correction	100%

For the Bell's palsy symptom, the CDC (2015) reports the percentage of diagnosed Lyme disease patients that suffer from Bell's palsy is 9%. This value (9%) was used to represent the percent of exposure in cases. According to Tiemstra and Khatkhate (2007), Bell's palsy is found in 0.023% of the population. This value (0.023%) was used to calculate the percent of exposure in controls.

Table 6

Power for Unmatched Case-Control Studies – Bell's Palsy

	Input data
Two-sided confidence interval (%)	95
Number of cases	120
Percent of exposure among cases (%)	9
Number of controls	240
Percent of exposure among controls (%)	0.023
Odds Ratio	430
Power based on:	
Normal approximation	97.73%
Normal approximation with continuity correction	96.02%

For cardiac symptoms, the most prevalent cardiac symptom found in diagnosed Lyme disease patients is AV block, which is found in only 1% of the diagnosed Lyme disease patients (CDC, 2015). This value (1%) was used to represent the percent of exposure among cases. AV block in the general population is rare, but the incidence does increase somewhat with age (Sandesara & Olshansky, 2012). At age 20 years, the incidence is only 0.5-2 %. This value increases up to 5% at age 60 years. A power analysis was done for each incidence representing the percent of exposures among controls.

Table 7

Power for Unmatched Case-Control Studies – AV Block Age 60 Years

	Input data
Two-sided confidence interval (%)	95
Number of cases	120
Percent of exposure among cases (%)	1
Number of controls	240
Percent of exposure among controls (%)	5
Odds Ratio	0.19
Power based on:	
Normal approximation	47.19%
Normal approximation with continuity correction	31.61%

Table 8

Power for Unmatched Case-Control Studies – AV Block Age 20 Years

	Input data
Two-sided confidence interval (%)	95
Number of cases	120
Percent of exposure among cases (%)	1
Number of controls	240
Percent of exposure among controls (%)	0.5
Odds Ratio	2
Power based on:	
Normal approximation	10.25%
Normal approximation with continuity correction	12.42%

According to this power analysis, the sample size was inadequate to find this rare condition. A sample size analysis was performed to identify the correct sample size to use. Based on this analysis, the potential to identify a significant difference between the cases and controls will be difficult. The sample size at 20 years is unrealistic based on the available population of cases at the primary care clinic where the study will take place (Table 9). At age 60 years, the sample size recommended is a little more realistic, but this

value is based on having this large number of patients above 60 years of age, which is also not possible at the primary care clinic (Table10).

Table 9

Sample Size for Unmatched Case-Control Study – AV Block Age 20 Years

		Input data	
Two-sided confidence level(1-alpha)		95	
Power(% chance of detecting)		80	
Ratio of Controls to Cases		2	
Hypothetical proportion of controls with exposure		2	
Hypothetical proportion of cases with exposure:		0.99	
Least extreme Odds Ratio to be detected:		0.49	
	Kelsey	Fleiss	Fleiss with CC
Sample Size - Cases	1889	1770	1916
Sample Size - Controls	3777	3540	3831
Total sample size:	5666	5310	5747

Table 10

Sample Size for Unmatched Case-Control Study – AV Block Age 60 Years

		Input data	
Two-sided confidence level(1-alpha)		95	
Power(% chance of detecting)		80	
Ratio of Controls to Cases		2	
Hypothetical proportion of controls with exposure		5	
Hypothetical proportion of cases with exposure:		0.99	
Least extreme Odds Ratio to be detected:		0.19	
	Kelsey	Fleiss	Fleiss with CC
Sample Size - Cases	259	228	264
Sample Size - Controls	517	456	528
Total sample size:	776	684	792

Lastly, mild cognitive impairment was considered. This group of symptoms is difficult to identify the percentage to use for cases because these symptoms are often only seen in those patients diagnosed with post-treatment Lyme disease disorder and/or chronic Lyme disease. Because the existence of these conditions is still controversial, finding a study that provided the incidence or prevalence of these symptoms was impossible. While the symptoms are listed as present, incidence rates were not calculated for the sample population or the sample populations were too small to generalize to the broader Lyme positive cases. In literature available from the International Lyme and Associated Diseases Society (ILADS), neurological symptoms, like memory or concentration issues, are found in 15-40% of Lyme patients (Caliendo et al., 1995). Unfortunately, this study was one of the most recently published articles on cognitive impairment in Lyme disease.

In order to determine a percentage to use for controls, mild cognitive impairment was used as the search criteria and included similar symptoms to those found in Lyme disease patients (memory issues, attention issues, confusion, etc.). Because Alzheimer's disease and dementia are accepted diagnoses, there were many more articles available with these symptoms. Unfortunately, these symptoms in the general population are often only found in older individuals (60 years +). In a study conducted by Iverson et al. (2011), mild cognitive impairment was found in 8.2% of the population within the 20-54 year age group. According to the CDC (as published in the MMWR for May 2013), mild cognitive impairment was found in 12% of the population between the ages of 60-74 years. Katz (2012) determined that mild cognitive impairment in the population over 70 years was 9.9%.

Multiple power analyses were performed to cover the range of ages discussed here. Because the clinician for this study, a respected Lyme disease specialist, treats many post-treatment Lyme disease and chronic Lyme disease cases at the primary care clinic, the odds of examining the records of a patient experiencing mild cognitive impairment was increased. Using the 40% value for the percentage of exposure in cases and the 8.2% value for the percentage of exposure in controls, a power analysis was conducted (Table 11). In addition, multiple power analyses were conducted to find the lowest percent of exposure in cases acceptable with the available sample size. Table 12 shows the minimum of 19% exposure in cases power analysis. Below 19% exposure in cases, additional patients must be added.

Table 11

Power for unmatched case-control studies – MCI symptoms 20-54 years age group

	Input data
Two-sided confidence interval (%)	95
Number of cases	120
Percent of exposure among cases (%)	40
Number of controls	240
Percent of exposure among controls (%)	8.2
Odds Ratio	7.5
Power based on:	
Normal approximation	100%
Normal approximation with continuity correction	100%

Table 12

Power for unmatched case-control studies – MCI symptoms 20-54 years age group

	Input data
Two-sided confidence interval (%)	95
Number of cases	120
Percent of exposure among cases (%)	19
Number of controls	240
Percent of exposure among controls (%)	8.2
Odds Ratio	2.6
Power based on:	
Normal approximation	82.48%
Normal approximation with continuity correction	78.01%

Because this study will include ages over 54, an additional power analysis was conducted that used the 40% for exposure in cases and 12% for exposure in controls (Table 13). In addition, another power analysis was done to test the lower end of required percentage for exposure in cases. As shown in Table 14, a minimum of 24% exposure in cases will be required at this sample size to reach the 80% power minimum. While this value is slightly higher than the 19% required for the lower age group, this value should be achievable within the primary care clinic patient population.

Table 13

Power for unmatched case-control studies – MCI symptoms 60+ years age group

	Input data
Two-sided confidence interval (%)	95
Number of cases	120
Percent of exposure among cases (%)	40
Number of controls	240
Percent of exposure among controls (%)	12
Odds Ratio	4.9
Power based on:	
Normal approximation	99.99%
Normal approximation with continuity correction	99.99%

Table 14

Power for unmatched case-control studies – MCI symptoms 60+ years age group

	Input data
Two-sided confidence interval (%)	95
Number of cases	120
Percent of exposure among cases (%)	24
Number of controls	240
Percent of exposure among controls (%)	12
Odds Ratio	2.3
Power based on:	
Normal approximation	81.49%
Normal approximation with continuity correction	77.37%

Based on this power analysis, the sample size was adequate to find a significant difference for the three major symptoms associated with Lyme disease in the United States (EM rash, arthritis, and Bell's palsy). For the rare symptom of AV cardiac block, the sample size selected for this study was inadequate to find a significant difference between cases and controls. Because AV cardiac block is rare, the expectation of finding cases with this symptom was small. Mild cognitive impairment (MCI) is found in the later stages of infection, post –treatment, and chronic Lyme disease, but is rare in the general population at ages below 60 years. Because the clinician treats patients in the later stages of infection, the potential for identifying these symptoms at the required percent exposure in cases was high. Significant differences between cases and controls may be found at the suggested sample size.

Level of significance (α) will be set at 0.05 following a review of the relevant literature and based on the statistical work of Cohen (1992). Probability (*p*) values will follow that a *p*-value < 0.05 resulted in rejection of the null (H_0) hypothesis and

acceptance of the alternate (H_A) hypothesis. With a *p*-value > 0.05, the null hypothesis was accepted. According to Cohen (1992), the effect size is the impact the independent variable has on the dependent variable. Based on the statistical method chosen for analysis, the effect size varied from 0.30 to 0.50 for a medium to large effect (Cohen, 1992). In addition, Cohen suggests setting the power of the study at 0.80 (Munro, 2005; Cohen, 1992).

Research Instrument

Data was collected using a modified Burrascano Symptom Checklist (ROSS Scale) that is based on the Wahler Physical Symptoms Inventory (PSI), which has been demonstrated previously to have a high level of internal consistency on test-retest scores over several different subgroups (Wahler, 1968). The PSI collects data on the frequency of general symptoms associated with most any illness, but does not address severity of symptoms. A modified version of the PSI was created by Burrascano (2008) to address the specific symptoms associated with Lyme disease and the severity of those symptoms. A modified version of the Burrascano Symptoms Checklist (ROSS Scale) was created to address the needs of this study. The modified Burrascano Symptoms Checklist (Ross Scale) that was used in this study can be obtained by request.

In addition to symptoms present, symptom severity, and symptom frequency, demographic data (age, sex, state of residence) and the seasonality of infection/time since tick exposure (month of diagnosis and/or month of tick bite were collected from the patients' medical records via the ROSS Scale and Chart review.

Data Collection and Analysis

Research questions one through five were answered through a thorough review of patient medical records and the ROSS Scale. Symptoms at the initial patient visit (related to Lyme disease diagnosis for cases and at first visit for controls) were collected and transcribed onto the modified Burrascano Symptom Checklist (ROSS Scale) (Burrascano, 2008).

All forms were numbered upon return (by the primary care clinic staff for cases) which ensured that each form was linked to a specific record in the Microsoft Access database where the ROSS scale results were stored and managed. The ROSS scales were NOT linked to a specific person by the numbering system, as there was no personal information on the ROSS scale that could identify an individual.

Analysis of data allowed for either the null hypothesis to be accepted or rejected so that the alternate hypothesis can be accepted. Independent variables included biological sex, age, time since tick exposure, month of diagnosis and/or tick exposure. Dependent variables included symptoms, symptom severity, and symptom frequency.

Computation of Variables

The independent variables for this study included age, sex, and time since exposure to the *Ixodes* tick. State of residence was collected to match cases and controls. Analysis was not performed using this variable because the primary care clinic was found within the Lyme disease endemic region identified by the CDC. Finding cases or controls from a non-endemic Lyme disease state for comparison was more difficult with the participant population available and required the addition of the secondary collection site (college).

The age variable was defined based on the CDC's surveillance groupings and included the following breakdown: < 20 years; 20-24 years; 25-29 years; 30-34 years; 35-39 years; 40-44 years; 45-49 years; 50-54 years; 55-59 years; 60-64 years; 65-69 years; 70-74 years; >75 years (CDC, 2015). Age categories may have been combined into 10 year intervals only if there are more than six categories with less than five subjects. Age category combination was not required. Biological sex included male and female categories.

Seasonality of infection variables included month of tick exposure and month of diagnosis. Based on CDC data (2015), most Lyme disease diagnoses occur during the months of June, July, and August. In addition, the winter months (December through March) contain the fewest reported Lyme disease diagnoses (CDC, 2015). Months with less than five diagnoses may have been combined into seasons (Spring, Summer, Fall, Winter) for statistical analysis. Combining months into seasons was not required at the time of analysis.

Independent variables were compared to the dependent variables of symptom presentation, symptom severity, and symptom frequency. Symptom presentation was examined for absence or presence of the described symptom using the modified Burrascano Symptom Checklist (ROSS Scale) instrument and calculated using the symptom index scoring system described below.

Symptom Index

Symptoms from the modified Burrascano Symptoms Checklist (ROSS Scale) were grouped together into six categories for analysis purposes. Grouping of symptoms into categories is common for diseases like fibromyalgia (Wolfe et al., 2010) and chronic

fatigue syndrome (Hickie et al., 2006), which are as difficult to diagnose as the later stages of Lyme disease. The six categories matched the major groups of reported symptoms in the NNDSS database and include: 1) musculoskeletal; 2) neurological; 3) cognitive; 4) cardiac; 5) general; and 6) cutaneous. The general category included the following symptoms: fever, sore throat, persistent swollen glands, unexplained weight loss or gain, nausea, diarrhea, and pain in the genital area. The musculoskeletal category included symptoms related to joint pain, stiffness, and swelling along with muscle stiffness, twitches, and aches/pain. The neurological category included facial paralysis (Bell's palsy) and other symptoms associated with cranial neuropathy, as well as meningitis not related to known bacterial or viral agents. The cognitive category included symptoms associated with memory, concentration, and speech difficulties. The cardiac category included chest pain, heart palpitations, or evidence of heart block. Lastly, the cutaneous category included the EM rash and other unexplained skin manifestations.

The frequency scale measured how often a patient experienced particular symptoms within the week prior to their appointment at the primary care clinic. The one week time frame was used to minimize recall bias on the part of the participant. Categories on the frequency scale included: never, 1-2 days, 3-4 days, 5-6 days, and everyday/7 days. The severity scale measured how the symptom affected the daily life of the participant. Categories on the severity scale included: not affected (0), slight/barely noticeable (1), minor problem but noticeable (2), moderate problem that interferes with some daily activities (3), major problem that interferes with most daily activities (4), and disabling problem (5) (Stricker et al., 2011).

An overall score (12 points) was calculated for the symptom index. Each category was worth 2 points of the total score – 1 point for severity and 1 point for frequency. Symptom scores in each category were averaged to reach this 2 point total.
This overall score was used to make comparisons between the Lyme positive case group and the Lyme negative control group, controlling for covariates like age, sex, and time since exposure to the *Ixodes* tick. Additional analysis made comparisons based on each symptom category that contributed to the total score. For example, if the symptom index score of the case group was based entirely on musculoskeletal and cutaneous symptoms, these category scores were compared between the case and controls by multivariate analysis methods.

Comparisons between cases and controls were conducted using the two way table and calculating the odds ratio for each symptom category reported. Since both cases' and controls' ROSS Scales provided information, these comparisons were easily made for each symptom category reported. This information provided insight into the symptoms that could be used for early diagnosis of Lyme disease.

In addition, univariate and bivariate statistical analyses were used. Univariate analysis allowed for descriptive statistics to be generated. Bivariate methods allowed comparisons to be made between two different variables to determine what, if any, relationship existed between these variables. The Chi-square test (if the data is normally distributed), the Kruskal-Wallis test, the odds ratio, and the odds ratio with the Mantel-Haenszel method (for age-adjusted comparison) were used to analyze the data and provided evidence to determine whether to accept or reject the null hypotheses (Munro, 2005).

In addition to the described univariate and bivariate statistical analyses, multivariate regression analysis was conducted on certain independent and dependent variable combinations. The independent variables age and sex were included in the analysis regardless of their association with the dependent variables after bivariate analysis based on evidence from previous literature (Katz, 2006). Independent or dependent variables were excluded from the multivariate regression analysis if there was a lot of missing data associated with that specific variable.

RQ1: Is the presentation of symptoms in Lyme disease-positive patients associated with the sociodemographic variables age and sex as assessed by patient medical record and ROSS Scale survey review?

$H_0$1 Lyme disease symptom presentation is not associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a1 Lyme disease symptom presentation is associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

RQ2: Is the severity of symptoms in Lyme disease positive patients associated with the sociodemographic variables age and sex as assessed by patient medical record and ROSS Scale survey review?

$H_0$2: Lyme disease symptom severity is not associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a2: Lyme disease symptom severity is associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

RQ3: Is the presentation of symptoms associated with the diagnosis of Lyme disease as assessed by patient medical record and ROSS Scale survey review?

H_03: Lyme disease symptom presentation is not associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a3: Lyme disease symptom presentation is associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

RQ4: Is the severity of symptoms associated with the diagnosis of Lyme disease as assessed by patient medical record and ROSS Scale survey review?

H_04: Lyme disease symptom severity is not associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a4: Lyme disease symptom severity is associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

RQ5: Is Lyme disease symptom presentation and severity associated with seasonality of infection variables as assessed by medical record and the ROSS Scale survey review?

$H_0$5: Lyme disease symptom presentation and severity are not associated with the seasonality of infection variables as assessed by patient medical record and the ROSS Scale survey review.

H_a5: Lyme disease symptom presentation and severity are associated with the seasonality of infection variables as assessed by patient medical record and the ROSS Scale survey review.

Statistical Analysis

Statistical analysis was performed with SPSS (version 21). Statistical tests included the Chi-square test (if the data are normally distributed) or the Kruskal-Wallis test (if the data are not normally distributed), which allowed for comparisons between the control group and the study population for specific symptoms and independent variable comparisons (Munro, 2005). A Chi-Square test was performed for age distribution categories and symptom presentation. This test was performed because the sample size is well over 60 participants and there were more than five participants in each age category (Munro, 2005). An ANOVA (with the Tukey Post Hoc test) was performed comparing age and symptom frequency and severity scores, because both of these categories had more than two groups (Munro, 2005). Lastly, an odds ratio was calculated both with and without the Mantel-Haenszel method (Munro, 2005).

In addition to the described univariate and bivariate statistical analyses, regression analysis was conducted on certain independent and dependent variable combinations. The independent variables age and sex were included in the analysis regardless of their association with the dependent variables after bivariate analysis based on evidence from previous literature (Katz, 2006). Independent or dependent variables were excluded from

the multivariate regression analysis if there was a lot of missing data associated with that specific variable.

Since the goal of this research was to identify additional potential factors to use for the earliest possible diagnosis of Lyme disease, an analysis based on the use of the information from this study for diagnosis was performed. Once associations between symptoms and/or symptom severity and the independent variables were determined, the sensitivity, specificity, positive predictive value, and the negative predictive values were calculated for the groups of symptoms. These values describe the ability of the test to identify correctly those individuals who have a disease. An ROC plot was generated to graphically show the data provided to help determine if the variables under investigation could be used to diagnose Lyme disease.

Protection of Human Participants

Participation in this study was completely voluntary. Study participants received a thorough explanation of the purpose of the study and the role the participant would play in the study. Each participant received an informed consent form prior to inclusion in the study. Participants were given an explanation of the study, a description of how to fill out the ROSS Scale, allowed to ask additional questions, and were told that returning the completed ROSS Scale was evidence of their consent to be included in the study.

Participant privacy has been maintained throughout the course of the study by coding each participant record to avoid the use of participant names or other identifying information. Coded records have been kept on a password protected computer and in a locked filing cabinet. In addition, the study was approved by the Walden University Institutional Review Board (approval number 10-30-14-00049220) and the Keystone

College Institutional Review Board (approval number 2015-000559) prior to data collection and again after the addition of the secondary data collection site.

Dissemination of Findings

Because the primary care clinic's participant population comes from all over the United States, study participants will receive information about the study results through a report mailed directly to their home. Researcher contact information will be provided to each study participant in case a participant has any questions about the study or the results. In addition, updates on publication or presentation of results will be provided to both the study clinician and study participants through post card updates.

In addition, a presentation of study results was given at the college that served as a secondary data collection site. The presentation was advertised through normal channels on the college campus, including the daily e-newsletter, announcements at meetings, and on the college website. Participants interested in the study results, as well as the general public, was encouraged to attend. Approximately, 120 individuals attended the presentation.

Summary

This study examined the factors associated with symptom presentation and severity in Lyme disease through a case-control methodology. Data was collected at a primary care clinic through an examination of patient records and ROSS Scale analysis. Data was collected at the secondary college site and analyzed through ROSS Scale analysis. The symptom index score was used to aid in the analysis portion of the study. Data collected provided information to either support or reject the null hypothesis for

each research question based on univariate, bivariate, and multivariate statistical analysis methods.

Chapter 4: Results

Introduction

Lyme disease is currently one of the most frequently reported vector-borne diseases (Binder, Telschow, & Meyer-Hermann, 2012; Borchers et al., 2015; Mead, 2015). According to the CDC (2015), Lyme disease is likely underreported by a factor of 12, leaving approximately 300,000 cases untreated. Successful treatment of Lyme disease requires early diagnosis and treatment. Delayed treatment may lead to long-term functional disability (Aucott et al., 2013; Johnson et al., 2011). The purpose of this study was to identify potential differences in the way Lyme disease symptoms are presented based on sex and/or age differences in order to help identify Lyme disease cases that might go undiscovered or misdiagnosed. In addition, a comparison was also made between symptom presentation and seasonality of infection to determine if symptoms vary based on month of tick exposure.

In this chapter, I will reiterate the research questions and hypotheses, describe the process of data collection and the addition of a secondary data collection site, discuss the modifications to the data collection procedures, describe the final make-up of the sample population, present the results obtained from data collection, and provide the statistical analysis methods used and results.

Data Collection Process

Data was collected at two collection sites, a primary care clinic that specializes in Lyme disease treatment and a small, 4-year liberal-arts college campus. The secondary collection site was added because the primary care clinic could not provide enough

controls for the study population. At both data collection sites, cases and controls were determined as follows.

Cases (n = 203) were defined as adult subjects (≥ 18 years of age) who met one of the following diagnostic criteria: (a) patient presents with a physician confirmed EM rash; (b) patient has a positive EIA and/or Western Blot laboratory result for IgG and IgM antibodies; or (c) a score of 5 or higher on the Burrascano Diagnostic Criteria for Lyme Disease scale (Burrascano, 2005). These criteria meet both the Infectious Disease Society of America (IDSA) and the International Lyme and Associated Diseases Society (ILADS) diagnostic guidelines (Cameron, Johnson, & Maloney, 2014; Wormser et al., 2006). Lyme disease cases were selected and confirmed by the clinical partner, a Lyme disease specialist. Cases were randomly selected by the physician, based on the weekly appointment schedule during the study period.

Controls (n = 388) were selected from adult subjects (≥ 18 years of age) who did not suffer from Lyme disease at the primary care clinic, family members of Lyme disease patients at the primary care clinic, and employees and students at a small, 4-year liberal arts college. Participants who suffered from illnesses other than Lyme disease were not excluded from the study. All controls were Lyme disease free at the time of selections as determined by: (a) never having had a tick bite; (b) having no evidence of EM rash; or (c) no prior laboratory testing for or diagnosis of Lyme disease by a physician. Controls were selected at an approximate 1:2 case/control ratio to address the sampling bias introduced by non-random selection of participants.

Because sex and age were two variables of interest in this study, matching between cases and controls based on sex or age could not occur. Matching based on state

of residence was done to insure that exposure to the *Ixodes scapularis* tick was the same between cases and controls. Participants were only included if they lived in one of the 14 states endemic for Lyme disease and the habitat for the *Ixodes scapularis* tick. These states included: Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, and Wisconsin. In addition, both cases and controls had to be available from a particular state to be considered for inclusion in the study.

At both data collection sites, the ROSS scale, an abbreviated and modified version of the Burrascano Symptom Scale, was used (Burrascano, 2008). The ROSS scale was administered to every participant at patient registration, as is standard procedure for intake at the primary care clinic. The ROSS scale became a permanent part of each patient's record and was used by the physician and staff to track symptom frequency and severity visit to visit. Each ROSS scale was scanned into the patient's record, de-identified, numbered and placed into a folder for entry as a participant into the study. These de-identified ROSS scales were collected once per week from the clinic over a period of four months.

At the secondary site, the ROSS scale was administered to several different populations including faculty, professional staff, hourly staff, and students. Information was distributed to the entire campus community about the study through daily newsletters, social media, and mass emails. This distribution of information was designed to increase interest in participation in the study. Data was then collected at large group meetings. The ROSS scale was passed out to all individuals in attendance. Informed consent forms were discussed and the study was explained.

Any questions from the group were answered as clearly as possible and directions were given on how to fill out the ROSS scale, highlighting key questions that required a response. Sex, date of birth, and state of residence questions were specifically discussed as important variables within the study. After completing this discussion, an envelope was left with the person in charge of the group for immediate return of the ROSS scale by participants. Instructions were also provided for on-campus mail return, scanning and email return, and/or drop box return of the ROSS scale, if the participants preferred to take the ROSS scale with them to fill out in private. Returning the completed form by any of the methods available was considered consent to participate in the study. The response rate at the college site was 84% and included primarily controls (Table 15). Response rate at the primary care clinic was 93%, and included both cases and controls (Table 15).

Table 15

Participant Response Rates by Collection Site and Return Method

Collection site	On-campus mail	Email	Drop box	Office staff	Response rate* (%)	Cases	Controls
Medical clinic (site 1)	0	0	0	357	94	232	125
College campus (site 2)	2	0	291	0	84	5	288

*Number of participant responses includes all participants collected prior to the application of exclusion criteria.

The college ROSS scales were numbered upon return, which ensured that each form was linked to a specific record in the Microsoft Access database, where the ROSS

scale results were stored and managed. The ROSS scales were NOT linked to a specific person by the numbering system, as there was no personal information on the ROSS scale that could identify a specific individual. Data collected from the primary care clinic used the same numbering system. The numbering system served a secondary purpose at the primary care clinic. In order to distribute information about the completed study to all participants, the primary care office staff used the numbers to identify which patients were included in the study. This identifying list was only available to the primary care office staff.

Exclusion Criteria

Completed forms were reviewed with the primary care physician to verify inclusion as a case or control for this study from a clinical standpoint. Because controls must NOT be Lyme disease positive, any indicators from the symptom checklist for a potential undiagnosed Lyme disease patient were reviewed carefully for appropriate case/control placement or exclusion from the study. No undiagnosed Lyme diseases cases were identified through this review. Forms containing ROSS scale data that was incomplete for the major study variables were excluded from the study. The ROSS scale does collect additional data that is not included in the current study, so if this additional data was incomplete, the ROSS Scale was not excluded.

Data collected was entered into Microsoft Access for storage and management. All records were double-checked for accuracy and completeness of required information. After review for accuracy in data entry, data records were reviewed to verify inclusion in the study. A total of 650 ROSS scales were completed and collected from participants. A

total of 591 participants were included in the final study analysis. Participants were removed for the following reasons.

Age under 18 years. Because the primary care site treats very few patients under the age of 18 years, the office staff did not pay special attention to the age requirement for the study and provided eleven ROSS scales for patients who were 17 years old. Participants must be 18 years of age for inclusion in the study, so these participants were excluded.

No date of birth listed. One of the major study variables is age. Without a birthdate listed, there was no way to determine how old the participant was so participants without a date of birth were excluded from the study. In addition, a few of the respondents listed the date the ROSS scale was completed as the date of birth. For the same reason, these respondents were excluded from the study.

State of Residence. State of residence was used to match cases and controls to address the non-random nature of participant selection. In addition, all participants needed to come from one of the 14 Lyme endemic states for inclusion in the study. The Lyme endemic states correspond to the habitat of the *Ixodes* tick, the vector for Lyme disease in the United States. Three participants were removed for not listing a state of residence at all. Seventeen additional participants were removed for not living in a Lyme endemic state because exposure to the *Ixodes* tick would not be possible thus eliminating the potential for developing Lyme disease. These participants lived in California, Georgia, Michigan, Tennessee, Texas, and Wyoming. Parts of northern California have a high incidence of Lyme diseases (even though the state as a whole has a low incidence of Lyme disease), but the tick vector is different in the western United States (Borchers et

al., 2015; Deluca et al., 2013). *Ixodes pacificus* is the tick vector in the western states and *Ixodes scapularis* is the tick vector in the eastern United States (Borchers et al., 2015; Deluca et al., 2013). While this variation in vector species may not have an effect on the study outcome, keeping the exposure possibilities as similar as possible within the study population was important.

An additional study participant was removed because the participant was from the United Kingdom. This participant was removed for two reasons: (a) there were no controls available from the same country for comparison; and (b) the causative agent and vector for Lyme disease is different in Europe than the causative agent in the United States (Borchers et al., 2015; Caimano et al., 2012). In Europe, the primary tick vector is *Ixodes ricinis*, which is different from the primary vector in the United States (Borchers et al., 2015; Caimano et al., 2012). In addition, the causative agent in Europe can be one of three bacteria, *Borrelia burgdorferi*, *Borrelia garinii*, and *Borrelia afzelii* (Borchers et al., 2015; Caimano et al., 2012). Each of these agents produces distinctly different symptom outcomes (Borchers et al., 2015; Deluca et al., 2013). European cases of Lyme disease tend to produce more neurological symptoms; American cases of Lyme disease tend to produce more musculoskeletal symptoms, which have been linked to the species of *Borrelia* that causes the infection (Borchers et al., 2015; Mead, 2015; Stanek et al., 2011).

Lastly, additional participants were excluded from the study that lived in Maryland, Rhode Island, and Wisconsin. Even though all three of these states are considered Lyme endemic states, all participants were controls. There were no reported Lyme cases from any of these states in the study population for comparison.

Study Population

The study population consisted of cases (n = 203) and controls (n = 388) that were similar in composition. Cases consisted of both females (n = 130) and males (n = 73) ranging in age from 18 years to 75+ years (Table 16). Controls consisted of both females (n = 268) and males (n = 120) also ranging in age from 18 years to 75+ years (Table 16). According to the CDC (2015), males in all age categories under age 70 suffer more frequently from confirmed Lyme disease (Figure 4). This age difference was not supported when recruiting case participants into this study (Figure 5). As shown in Figure 5 and Table 16, female cases outnumber male cases by almost 2:1. Most of the female cases were between the ages of 40-65 years. This discrepancy with the CDC's confirmed cases data may be due to the primary care clinic's much larger population of female cases over male cases to recruit.

Table 16:

Age Distribution of Study Lyme Disease Cases

Age Range	Female Cases (64%)	Male Cases (36%)	Female Controls (67%)	Male Controls (33%)
<20	6	6	133	38
25	2	6	37	16
30	6	8	10	6
35	9	2	4	10
40	12	5	7	6
45	17	11	10	14
50	18	5	10	9
55	24	7	11	8
60	10	6	12	4
65	11	9	10	6
70	7	2	10	7
75	5	4	5	2
80	2	1	4	2
85	0	1	3	1
90	1	0	2	0

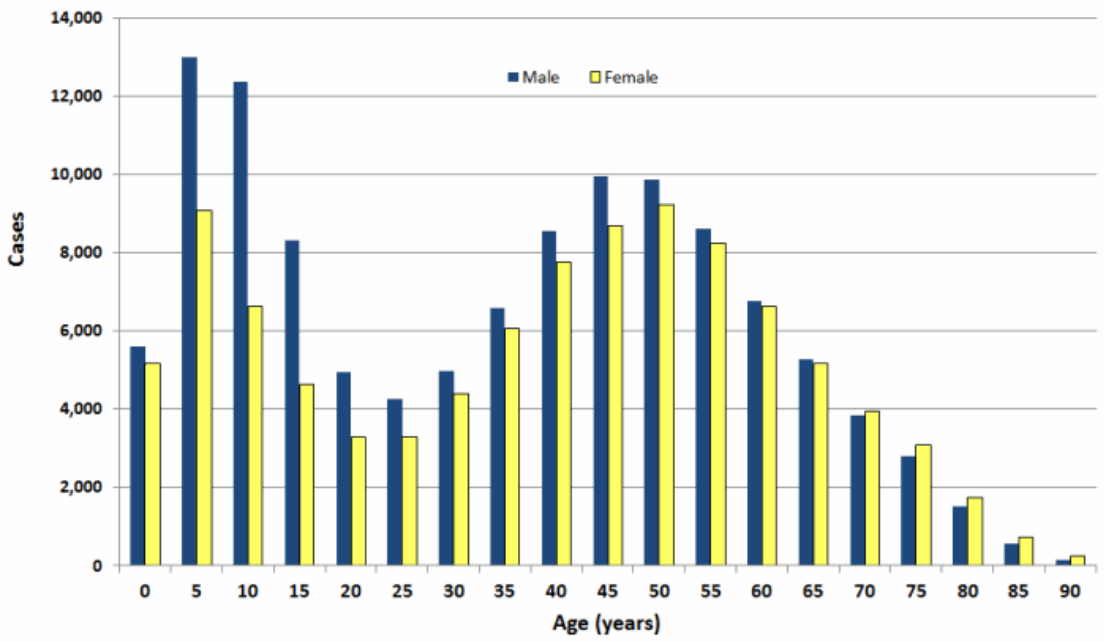

Figure 4. Frequency distribution of Lyme disease cases by gender – General population

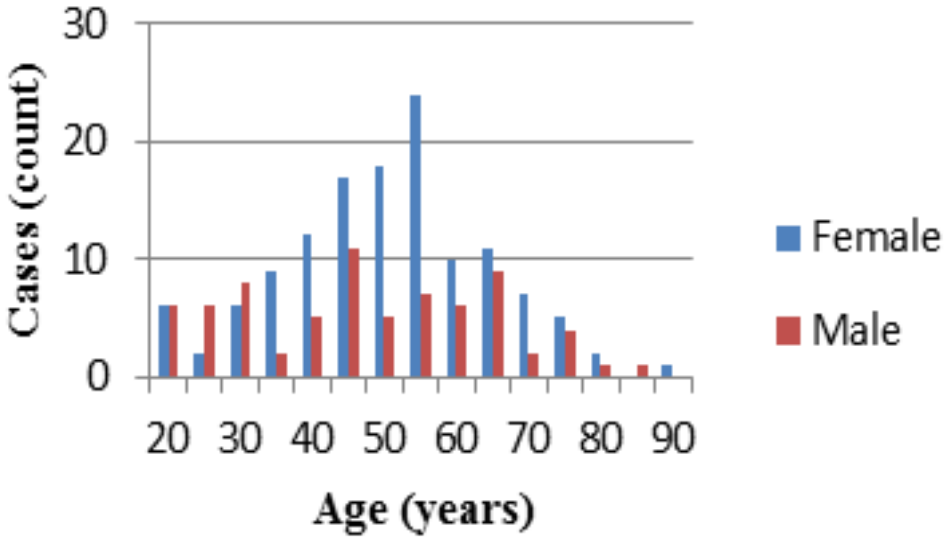

Figure 5. Frequency distribution of Lyme disease cases by gender – Study population

States of residence for both cases and controls included Connecticut, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, and Vermont (Table 17). All of these states are considered Lyme-endemic states by the CDC (2015).

Table 17

State of Residence of Study Participants by Case/Control

State of Residence	Female - Case	Female - Control	Male - Case	Male - Control	Total (%)
CT	16	9	17	6	48 (8)
MA	2	1	0	1	4 (0.7)
NH	2	2	0	0	4 (0.7)
NJ	9	19	6	12	46 (8)
NY	89	64	46	34	233 (39)
PA	8	168	3	66	247 (42)
VT	4	3	1	1	9 (2)
Total	130	268	73	120	591 (100)

In order to ensure that the sample population was as uniform as possible, matching based on state of residence and frequency matching was performed. State of residence matching (as shown in Table 17) required at least one case and one control from a state in order for the study participant to remain in the study. Frequency matching based on sex was performed so that the sample population did not contain a single sex in cases or controls. Females made up 64% of the case population and 69% of the control population. Males made up 36% of the case population and 31% of the control population. In addition, a case-control ration of 1:2 was used to reduce sampling bias.

Lastly, random sampling was utilized as much as possible given the fact that cases needed to be Lyme disease positive for inclusion. At the primary care site, cases were selected at random based on the daily appointment schedule. In addition, controls were

selected based on the daily appointment schedule (for non-Lyme disease patients) or because the control was at the primary care site with a patient. At the secondary site, ROSS scales were distributed at a variety of meetings on campus to anyone who was in attendance at the meeting. This method for selection of meetings and campus groups allowed for some random sampling of the population.

While educational level was not a variable under study, the secondary site was a college campus so matching based on educational level became an important consideration as advanced educational level can change socioeconomic status and effect, specifically, access to health care (Heck & Parker, 2002; Saydah, Imperatore, & Beckles, 2013). Because all but five of the cases came from the primary care site, a comparison of cases between collection sites was not necessary. Comparisons between controls collected at each site were performed.

Overall, 51% of the study population had at least a high school diploma and 44% of the population had completed at least a baccalaureate degree (Table 18). According to the United States Census Bureau (2014), 30% of the population of the United States over the age of 18 years old has at least a high school diploma. In addition, 29% of the population of the United States over the age of 18 years old has at least a baccalaureate degree. Site 1 females with a college degree made up 5% of the control population and site 2 females with a college degree made up 4% of the control population. Site 1 males with a college degree made up 5% of the control population and site 2 males with a college degree made up 0.5% of the control population. Site 1 females with a graduate degree made up 7% of the control population and site 2 females with a graduate degree

made up 3% of the control population. Lastly, males with a graduate degree at both sites made up 4% of the population.

Table 18

Education Level Comparison for Cases/Controls from Both Study Sites

Education Level	Female Case Site 1	Female Case Site 2	Female Control Site 1 (%)	Female Control Site 2 (%)	Male Case Site 1	Male Case Site 2	Male Control Site 1 (%)	Male Control Site 2 (%)	Total (%)
Unassigned	2	0	2	0	3	0	1	0	8 (2)
College Graduate	39	0	20 (5)	15 (4)	32	0	20 (5)	2 (0.5)	128 (22)
Graduate School	46	1	28 (7)	12 (3)	13	0	15 (4)	18 (4)	133 (22)
H.S. Graduate	28	3	23	163	19	2	4	58	300 (51)
Technical School	11	0	2	0	4	0	2	0	19 (3)
Total	126	4	75	190	71	2	42	78	588* (100)

*Three participants did not list educational level on the ROSS Scale.

The age variable was defined based on the CDC's surveillance groupings and included the following breakdown: < 20 years; 20-24 years; 25-29 years; 30-34 years; 35-39 years; 40-44 years; 45-49 years; 50-54 years; 55-59 years; 60-64 years; 65-69 years; 70-74 years; >75 years (CDC, 2015). Age categories were not combined into 10 year intervals because there were not more than six categories with less than five participants.

Table 19 shows an age range distribution for cases and controls collected from the primary care site and the secondary college site. Ages within the case and the control populations were well matched in all age categories with the exception of the <20 years and the 20-24 years age categories. Many more controls over cases are present in both of these age categories. This inconsistency between age categories should not disproportionally affect the results of the study because the controls should not be

experiencing symptoms of Lyme disease more frequently than cases or at a more severe level if the symptoms under study can truly be used as an indicator of the presence of *Borrelia burgdorferi*.

Table 19

Age Distribution of Sample Population

Age Range	Female Case Site 1	Female Case Site 2	Female Control Site 1	Female Control Site 2	Male Case Site 1	Male Case Site 2	Male Control Site 1	Male Control Site 2	Total (%)
<20	4	2	1	132	5	1	0	38	183 (33)
25	2	0	1	36	6	0	1	15	61 (10)
30	5	1	4	6	8	0	2	4	30 (5)
35	9	0	1	3	2	0	2	8	25 (4)
40	12	0	6	1	4	1	2	1	27 (5)
45	17	0	7	3	11	0	4	1	43 (7)
50	17	1	8	2	5	0	8	4	45 (8)
55	24	0	6	5	7	0	6	2	50 (9)
60	10	0	9	3	6	0	4	0	32 (5)
65	11	0	8	2	9	0	4	2	36 (6)
70	12	0	8	2	2	0	4	3	31 (5)
75	2	0	4	1	4	0	2	0	13 (2)
80	0	0	4	0	1	0	2	0	7 (1)
85	0	0	3	0	1	0	1	0	5 (1)
90	1	0	2	0	0	0	0	0	3 (1)
Grand Total	126	4	72	196	71	2	42	78	591 (100)

A power analysis was conducted to determine an adequate sample size to find statistically relevant results (see chapter 3 for the full analysis discussion). Based on this power analysis, the sample size of cases (n = 203) and controls (n = 388) was adequate to find a significant difference for the three major symptoms associated with Lyme disease in the United States (EM rash, arthritis, and Bell's palsy). For the rare symptom of AV cardiac block, the sample size selected for this study was inadequate to find a significant difference between cases and controls. Because AV cardiac block is rare, the expectation

of finding cases with this symptom was small. Mild cognitive impairment (MCI) is found in the later stages of infection, post–treatment, and chronic Lyme disease (Caliendo et al., 1995), but is rare in the general population at ages below 60 years (Iverson et al., 2011; Katz, 2012; CDC, 2015). Because the primary care clinic treats patients in the later stages of infection, the potential for identifying these symptoms at the required percent exposure in cases was high.

Level of significance (α) for the statistical analysis was set at 0.05 following a review of the relevant literature and based on the statistical work of Cohen (1992). Probability (*p*) values followed that a *p*-value ≤ 0.05 resulted in rejection of the null (H_0) hypothesis and acceptance of the alternate (H_a) hypothesis. With a *p*-value > 0.05, the null hypothesis was accepted, because a large *p*-value suggests that the null hypothesis is more likely to be true given the specific set of test parameters (Munro, 2005)

According to Cohen (1992), the effect size is the impact the independent variable has on the dependent variable. Based on the statistical method chosen for analysis, the effect size varied from 0.30 to 0.50 for a medium to large effect (Cohen, 1992). In addition, Cohen suggests setting the power of the study at 0.80 (Munro, 2005).

Symptom Index

Symptoms were grouped together into six categories for analysis purposes. The six categories matched the major groups of reported symptoms to the NNDSS database and included: 1) musculoskeletal; 2) neurological; 3) cognitive; 4) cardiac; 5) general; and 6) cutaneous. The general category included the following symptoms: fatigue, fever, chills, headaches, sore throat, persistent swollen glands, dizziness, lightheadedness, nausea, diarrhea, and night sweats. The musculoskeletal category included the following

symptoms: painful joints, stiff neck, back pain, stiff joints, and sore muscles. The neurological category included facial paralysis (Bell's palsy), blurred vision, eye pain, ear ringing, jaw pain, testicular/pelvic pain, and tingling/burning/numbness. The cognitive category included the following symptoms: disturbed sleep, poor concentration, memory loss, irritability, crying, and sadness/depression. The cardiac category included chest pain and heart palpitations. Lastly, the cutaneous category included the presence of the EM rash.

The frequency scale measured how often a patient experienced the listed symptoms within the week prior to filling out the ROSS scale. The one week time frame was used to minimize recall bias on the part of the participant. Categories on the frequency scale included: never, 1-2 days, 3-4 days, 5-6 days, and everyday/7 days (Table 20). The severity scale measured how each specific symptom affected the daily life of the participant (Table 20). Categories on the severity scale included: not affected (0), slight/barely noticeable (1), minor problem but noticeable (2), moderate problem that interferes with some daily activities (3), major problem that interferes with most daily activities (4), and disabling problem (5) (Stricker et al., 2011).

Table 20

Frequency and Severity Symptom Score Breakdown

Frequency	Frequency Score	Severity	Severity Score
Never	0.2	Not Affected (0)	0.17
1-2 Days	0.4	Slight/Barely Noticeable (1)	0.34
3-4 Days	0.6	Minor Problem but Noticeable (2)	0.51
4-5 Days	0.8	Moderate Problem that Interferes with Some Daily Activities (3)	0.68
Every Day/7 Days	1.0	Major Problem that Interferes with Most Daily Activities (4)	0.85
		Disabling Problem (5)	1.00

An overall score (12 points) was calculated for the symptom index. Each of the six symptom categories was worth 2 points of the total score – 1 point for severity and 1 point for frequency. Symptom scores in each category were assigned a value (which is a fraction of 1 point based on the level of frequency and severity shown in Table 20), totaled, and averaged to reach this 2 point total.

To illustrate a symptom index calculation in Table 21, a female case reported a list of symptoms experienced the week prior to filling out the ROSS scale. For frequency scores, there were five potential responses (never, 1-2 days, 3-4 days, 5-6 days, and everyday/7 days). Each of these responses was assigned a numerical value in 0.2 increments as shown in Table 20. For severity scores, there were six potential responses ranging from zero (no effect on daily life) to five (severely disrupts daily activities). Each of these responses was also assigned a numerical value that was $1/6^{th}$ of the 1 point allowed for severity (Table 21). Scores were recorded, grouped into the six categories of

symptoms, and then averaged to determine the frequency and severity score for that set of symptoms.

Table 21

Female Case Reported Symptoms and Scores

Symptoms Category	Reported Symptom	Frequency	Frequency Score	Severity	Severity Score
Musculoskeletal	Back Pain	Everyday	1	4	0.85
Neurological	Blurred Vision	1-2 Days	0.4	0	0.17
General	Chills	3-4 Days	0.6	1	0.34
Cognitive	Disturbed Sleep	Everyday	1	4	0.85
General	Dizziness	1-2 Days	0.4	1	0.34
General	Fatigue/Tiredness	3-4 Days	0.6	4	0.85
General	Headaches	Everyday	1	4	0.85
Cognitive	Irritability	Everyday	1	4	0.85
General	Lightheadedness	1-2 Days	0.4	1	0.34
Cognitive	Memory Loss	5-6 Days	0.8	4	0.85
General	Nausea	1-2 Days	0.4	1	0.34
General	Night Sweats	3-4 Days	0.6	1	0.34
Musculoskeletal	Painful Joints	Everyday	1	5	1
Cognitive	Poor Concentration	5-6 Days	0.8	4	0.85
Cognitive	Sadness/Depression	3-4 Days	0.6	0	0.17
Musculoskeletal	Sore Muscles	Everyday	1	5	1

Using the data displayed in Table 21, the female case reported the following five musculoskeletal symptoms: back pain, painful joints, stiff joints, stiff neck, and sore muscles. Based on the scale in Table 20, a frequency of everyday is awarded a 1.0 score. Since all of the symptoms were experienced every day, the average score for the five symptoms is 1.0 (the third column in Figure 6).

To calculate the severity score for the musculoskeletal symptoms, the severity score shown in Table 20 was used. Three of the symptoms were considered (by the participant) to be disabling (score of 5 on the severity scale) and were assigned a severity

score of 1.0. Two of the symptoms were considered major problems that interfere with most daily activities (score of 4 on the severity scale) and were assigned a severity score of 0.85 for each. To calculate the final severity score, the average of the five scores ([1.0 + 1.0 + 1.0 + 0.85 + 0.85]/5) resulted in a severity score of 0.94 (the fourth column in Figure 6).

Lastly, each symptom category score was added together to compose the final symptom index score (the last column in Figure 6). An example symptom index score is shown in Figure 6 below.

General		Musculoskeletal		Cognitive		Neurological		Cardiac		Cutaneous		Symptom Index
Freq	Sev	Freq	Sev	Freq	Sev	Freq	Sev	Freq	Sev	Freq	Sev	
0.44	0.34	1.00	0.94	0.40	0.34	0.47	0.34	0.00	0.00	0.50	0.50	5.27

Figure 6. Symptom index score card showing total symptom index score and frequency and severity scores for each symptom category.

Results

Symptom Frequency

Overall symptom frequency scores were calculated by adding the calculated frequency scores for each of the six symptom categories together. From Figure 6 above, frequency scores for general (0.44), musculoskeletal (1.00), cognitive (0.40), neurological (0.47), cardiac (0.00), and cutaneous (0.50) symptoms were combined to represent the overall symptom frequency score. A comparison was made of the overall symptom frequency score based on sex of the participant. Results are shown in Table 22 below. Because individual scores displayed a broad range of scores, individual scores

were combined into the categories listed in Table 22 for analysis to increase the number of participants in each category.

Table 22

Frequency Score for Participants

Frequency Score	Female Case	Female Control	Male Case	Male Control
0.00 – 0.99	5	120	13	49
1.00 – 1.99	5	79	47	44
2.00 – 2.99	55	55	13	23
3.00 – 3.99	44	10	0	4
4.00 – 4.99	20	4	0	0
5.00 – 5.99	1	0	0	0
6.00	0	0	0	0

Both male and female controls displayed an overall frequency score on the lower end of the scale (less than 3.00) with only 14 female controls scoring above 3.00. Noteworthy is the fact that male cases also had frequency scores below 3.00, while female cases were clustered primarily between 2.00 and 5.00. Figure 7 shows the distribution of score by cases and controls.

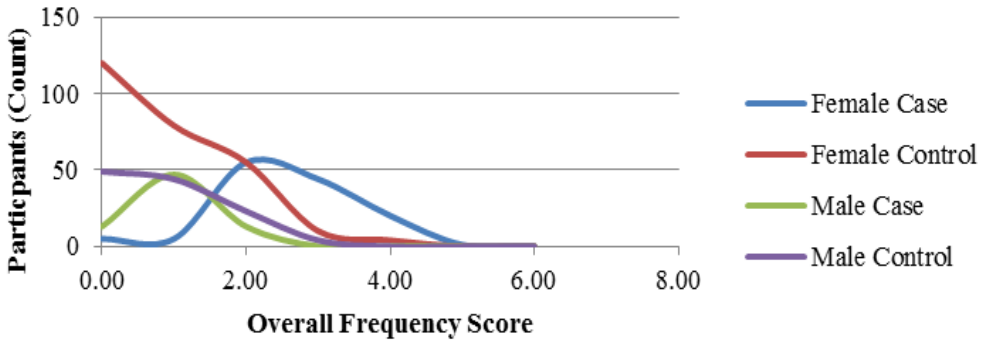

Figure 7. An overall frequency comparison between cases and controls based on sex of participant.

Univariate statistical analysis of frequency scores between cases and controls (Table 24), as well as between male and female cases (Table 23), support this arrangement as statistically significant [χ^2 (15, N = 588) = 290.42, $p < 0.05$; χ^2 (5, n = 203) = 122.04, $p < 0.05$].

Table 23

Frequency Scores in Cases by Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	122.036[a]	5	.000
Likelihood Ratio	144.646	5	.000
N of Valid Cases	203		

Table 24

Frequency Scores by Cases/Controls

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	290.418[a]	15	.000
Likelihood Ratio	289.682	15	.000
N of Valid Cases	588		

Raw data is shown below for breakdown by age of the overall frequency scores for cases (Table 25) and controls (Table 26). Most of the overall frequency scores for cases were between 1.00 and 5.00, while most of the overall frequency scores for controls were below 3.00.

In order to compare case and control overall frequency scores and the age category variable, the Kruskal-Wallis test was conducted. Initially, an ANOVA was conducted because both the age category and the frequency scores had multiple levels

within the groups. Unfortunately, the Levene's Test of Equality of Error Variances was 0.011, which is not larger than the *p*-value of 0.05. This test looks for homogeneity within the samples (Munro, 2005). This low score suggested that the test sample is not normally distributed. The Kruskal-Wallis test is a non-parametric test that does not require the sample population to be normally distributed across the age groupings.

Based on the Kruskal Wallis test comparison (Table 27), whether a participant was a case or a control was correlated to both the age group category, [χ^2 (1, N = 591) = 84.80, p = 0.00] and the symptom frequency score, [χ^2 (1, N = 591) = 118.43, p = 0.00] as shown in Table 28.

Table 25

Overall Frequency Scores of CASES by Age Groups

Freq Score	<20	>75	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74
0.00-0.99	3	0	2	2	1	1	4	2	1	1	1	1	0
1.00-1.99	4	2	4	4	4	3	6	4	5	3	8	2	3
2.00-2.99	1	1	1	7	3	8	6	11	8	7	5	4	6
3.00-3.99	1	3	1	0	2	5	6	3	14	1	5	3	0
4.00-4.99	0	0	0	1	2	0	3	4	4	1	3	1	0
5.00-5.99	0	0	0	0	0	0	0	0	1	0	0	0	0
6.00	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 26

Overall Frequency Scores of CONTROLS by Age Group

Freq Score	<20	>75	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74
0.00 - 0.99	64	5	43	6	7	1	8	6	7	6	5	6	3
1.00 - 1.99	41	3	28	5	7	4	3	8	8	3	3	9	1
2.00 - 2.99	24	4	16	4	1	3	2	7	4	4	5	3	1
3.00 - 3.99	0	0	0	0	0	1	3	0	1	2	2	2	3
4.00 - 4.99	1	0	2	0	1	0	1	0	0	0	0	0	0
5.00 - 5.99	0	0	0	0	0	0	0	0	0	0	1	0	0
6.00	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 27

Kruskal-Wallis Comparison

	Case or Control	N	Mean Rank
Frequency Score	Case	203	397.73
	Control	388	242.77
	Total	591	
Age Category	Case	203	384.53
	Control	388	249.68
	Total	591	

Table 28

Overall Frequency Score by Case/Control and Age Categories

	Frequency Score	Age Category
Chi-Square	118.429	84.800
df	1	1
Asymp. Sig.	.000	.000

RQ1: Is the presentation of symptoms in Lyme disease-positive patients associated with the sociodemographic variables age and sex as assessed by patient medical record and ROSS Scale survey review?

$H_0$1: Lyme disease symptom presentation is not associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a1: Lyme disease symptom presentation is associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

Based on the statistical evidence presented, the null hypothesis can be rejected and the alternate hypothesis for research question one can be accepted – Lyme disease symptom presentation is associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records.

Symptom Severity

A comparison was made between the overall severity score and the sex of the participants. Results are presented in Table 29 and Figure 8. As with the frequency scores, overall severity scores for male cases and controls and female controls are less than 3.00. Female cases' overall severity scores ranged from 1.00 – 5.00.

Table 29

Overall Severity Scores by Participant

Severity Score	Female Case	Female Control	Male Case	Male Control
0.00 – 0.99	12	141	29	63
1.00 – 1.99	34	95	32	42
2.00 – 2.99	46	24	11	12
3.00 – 3.99	28	6	1	3
4.00 – 4.99	10	2	0	0
5.00 – 5.99	0	0	0	0
6.00	0	0	0	0

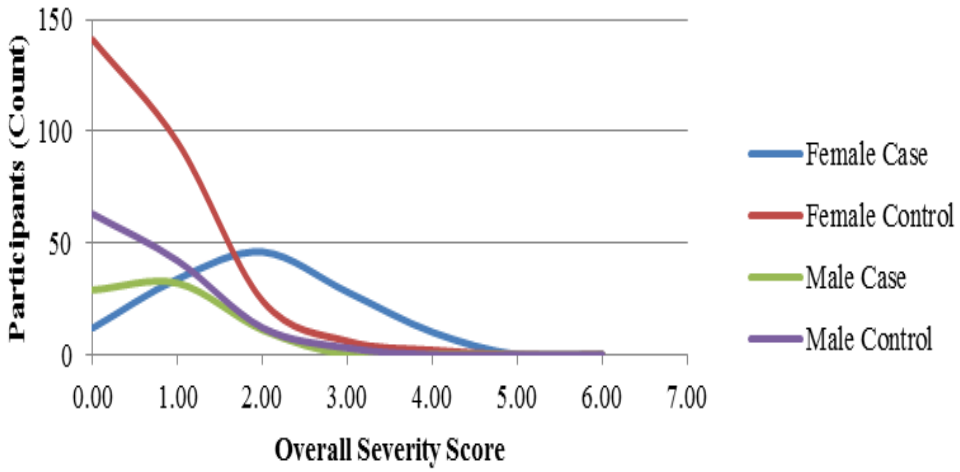

Figure 8. A comparison of the overall severity score between cases/controls based on sex.

After Chi-Square analysis, results (Table 30) indicate that there is a correlation between sex of the participant and the overall symptom severity score [χ^2 (4, N = 591) = 20.94, p = 0.00]. In addition, statistical analysis (Table 31) suggests that there is a significant difference between cases and controls based on overall symptom severity score [χ^2 (4, N = 591) = 106.39, p = 0.00]. Lastly, the comparison (Table 32) between overall symptom severity scores and participant age also suggests a significant correlation [χ^2 (48, N = 591) = 106.81, p = 0.00].

Table 30

Overall Severity Scores by Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	20.937[a]	4	.000
Likelihood Ratio	26.455	4	.000
Linear-by-Linear Association	17.523	1	.000
N of Valid Cases	591		

Table 31

Overall Severity Scores Between Cases and Controls

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	106.394[a]	4	.000
Likelihood Ratio	106.483	4	.000
Linear-by-Linear Association	103.092	1	.000
N of Valid Cases	591		

Table 32

Overall Severity Scores by Age Categories

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	106.807[a]	48	.000
Likelihood Ratio	111.154	48	.000
Linear-by-Linear Association	32.938	1	.000
N of Valid Cases	591		

RQ2: Is the severity of symptoms in Lyme disease positive patients associated with the sociodemographic variables age and sex as assessed by patient medical record and ROSS Scale survey review?

$H_0$2: Lyme disease symptom severity is not associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a2: Lyme disease symptom severity is associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

Based on the statistical evidence presented, the null hypothesis can be rejected and the alternate hypothesis for research question two can be accepted – Lyme disease symptom severity is associated with the sociodemographic variables age and sex as assessed by patient reported symptoms recorded in patient medical records.

In order to discuss how the presentation and severity of symptoms are associated with the diagnosis of Lyme disease (RQ3 and RQ4), the specific categories of symptoms were examined individually first for an effect on accurate diagnosis. The overall symptom score was then examined for an effect on accurate diagnosis of Lyme disease. Symptom categories of Musculoskeletal, Neurological, Cognitive and Cutaneous were examined because the study population size was adequate in power to identify a statistically relevant correlation if one was present. General symptoms were reviewed because these symptoms are common indicators of many other diseases and conditions, as well as indicators for Lyme disease. Cardiac symptoms, due to their rare occurrence in both the general and Lyme disease populations, required a much larger sample population than was feasible for this study (CDC, 2015; Sandesara & Olshansky, 2012). Analysis based on the cardiac symptom category was not performed individually, but the cardiac category symptom scores were included in the total symptom index score calculations and the overall effect calculations.

General Symptoms

The general symptoms category contains a variety of symptoms that can be an indicator of many different diseases, including Lyme disease. General symptoms examined included fatigue, fever, chills, headaches, sore throat, persistent swollen glands, dizziness, lightheadedness, nausea, diarrhea, and night sweats. Early localized Lyme

disease presents as fever, chills, fatigue, headache, and the erythema migrans rash (discussed separately next).

Based on Chi-square analysis of the general symptom category (Tables 35 & 36), significant differences were found between cases and controls [χ^2_{freq} (4, N = 591) = 105.468, p = 0.00; χ^2_{sev} (4, N = 591) = 71.243, p = 0.00] (Tables 33 & 34), but not based on sex [χ^2_{freq} (4, N = 591) = 5.403, p > 0.05; χ^2_{sev} (4, N = 591) = 6.496, p > 0.05]. In addition, analysis was performed comparing frequency and severity with the age variable. ANOVA analysis (Table 37) showed a significance based on both frequency score (p = 0.000) and severity score (p = 0.019) when compared to age. The Tukey post-hoc analysis showed the significance was between the < 20 years category and the 40-44 years, 45-49 years, and the 60-64 years categories for frequency, but showed no real significance between age groups based on general symptom severity scores (Tables 38 & 39).

Table 33

General Symptom Frequency vs Case/Control

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	105.468[a]	4	.000
Likelihood Ratio	115.894	4	.000
Linear-by-Linear Association	96.333	1	.000
N of Valid Cases	591		

Table 34

General Symptom Severity vs Case/Control

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	71.243[a]	4	.000
Likelihood Ratio	76.269	4	.000
Linear-by-Linear Association	64.690	1	.000
N of Valid Cases	591		

Table 35

General Symptom Frequency by Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.403[a]	4	.248
Likelihood Ratio	5.412	4	.248
Linear-by-Linear Association	1.629	1	.202
N of Valid Cases	591		

Table 36

General Symptom Severity by Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.496[a]	4	.165
Likelihood Ratio	6.496	4	.165
Linear-by-Linear Association	2.083	1	.149
N of Valid Cases	591		

Table 37

General Symptom Frequency Comparison via ANOVA by Age

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	66.327	12	5.527	4.142	.000
Within Groups	771.337	578	1.334		
Total	837.665	590			

Table 38

General Symptom Tukey Post-Hoc Analysis by Age

(I) Age Category	(J) Age Category	Mean Difference (I-J)	Std. Error	Sig.
<20 Years	20-24 Years	-.06910	.15264	1.000
	25-29 Years	-.58249	.23887	.418
	30-34 Years	-.36821	.23887	.946
	35-39 Years	-.76381	.24642	.097
	40-45 Years	-.77297*	.20291	.010
	45-49 Years	-.83646*	.19762	.002
	50-54 Years	-.57441	.18595	.099
	55-59 Years	-.55294	.23540	.482
	60-64 Years	-.89202*	.21556	.003
	65-69 Years	-.24148	.22901	.998
	70-74 Years	-.78418	.29648	.286
	>75 Years	-.44757	.28903	.944

Table 39

General Symptom Severity Comparison via ANOVA by Age

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.533	12	2.711	2.044	.019
Within Groups	766.566	578	1.326		
Total	799.100	590			

Erythema Migrans Rash

According to the CDC (2015), the erythema migrans (EM) rash "occurs in approximately 60 to 80 percent of infected persons" (para. 1). This ratio was also supported by other studies (Aucott, Seifert, & Rebman, 2012a). Based on this percentage, the EM rash is currently used as a diagnostic tool for Lyme disease. The presence of the EM rash with a known tick exposure can be used to diagnose Lyme disease without any further laboratory testing (Aucott, Crowder, Yedlin, &Kortte, 2012; CDC, 2015).

This 60-80% of cases ratio was not supported by the current study. According to the data collected (Table 40), only 28% of participants experienced the EM rash. Of the 28% of participants who experience the EM rash, female cases constituted 61% of the cases that experienced the rash.

Table 40

EM Rash Presence by Case Sex

EM Rash Present	Male	Female	Total	% of Total Case Population	% of Cases with EM Rash Male	% of Cases with EM Rash Female
Yes	22	34	56	28	38	61
No	50	94	144	72	34	67
Total	72	128	200	100	72	128

Three participants were unsure whether they experienced the EM rash and were excluded from the analysis.

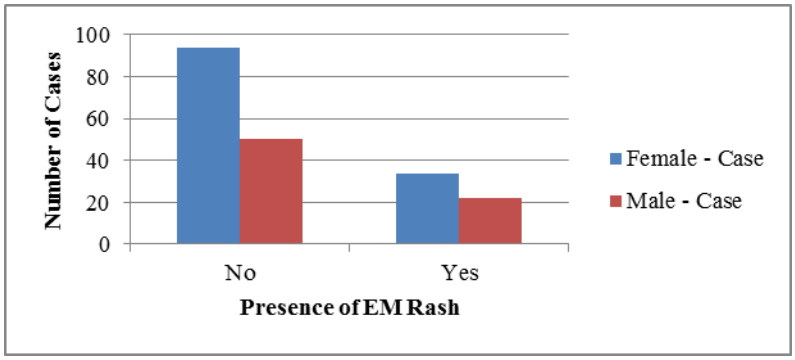

Figure 9. Comparison of cases and controls who displayed the EM rash by sex of participant.

Based on Chi-Square analysis of EM rash in males and females (Table 41), the relationship was not significant [χ^2 (1, n = 200) = 0.546, $p > 0.05$]. When comparing the presence of EM rash between cases and controls (Table 42), the relationship was significant [χ^2 (1, N = 588) = 120.076, $p < 0.05$]. This relationship supports using the EM rash as a diagnostic tool, but does not show a statistically relevant difference based on sex of the patient.

Table 41

EM Rash by Sex

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.364[a]	1	.546		
Continuity Correction[b]	.193	1	.660		
Likelihood Ratio	.362	1	.548		
Fisher's Exact Test				.623	.328
N of Valid Cases	200				

Table 42

EM Rash in Cases vs. Controls

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	120.076[a]	1	.000		
Continuity Correction[b]	116.848	1	.000		
Likelihood Ratio	132.662	1	.000		
Fisher's Exact Test				.000	.000
N of Valid Cases	588				

Analysis was also conducted based on the age of the participants. As shown in Table 43, presence of the EM rash in women was present primarily in cases over the age of 35, with the largest number of female cases with EM rash falling in the 50-54 year age category.

Table 43

EM Rash Comparison by Sex and Age Categories

Age Categories	Female - Case		Male - Case		Total
	No	Yes	No	Yes	
<20	4	1	3	1	9
>75	3	1	1	1	6
20-24	3	0	5	0	8
25-29	5	0	6	2	13
30-34	9	0	2	2	13
35-39	8	4	3	1	16
40-44	11	4	9	1	25
45-49	15	2	5	2	24
50-54	17	9	6	0	32
55-59	7	2	2	2	13
60-64	8	4	5	5	22
65-69	3	4	1	2	10
70-74	1	3	2	3	9
Grand Total	94	34	50	22	200

A comparison was made between the age categories (as listed in the Table 43 above) and the presence of the EM rash. The results (Table 44) suggest a statistical significance between age and presence of the EM rash [χ^2 (12, = 200) = 21.43, $p < 0.05$], although the level of significance is relatively small ($p = 0.044$). Kruskal Wallis testing (because the data was not normally distributed) was unable to support this level of significance ($p = 0.05$), suggesting age and presence of the EM rash were not related.

Table 44

Age vs Presence of EM Rash

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	21.426[a]	12	.044
Likelihood Ratio	22.405	12	.033
N of Valid Cases	200		

Musculoskeletal Symptoms

Because Lyme disease caused by *Borrelia burgerferi* tends to produce musculoskeletal symptoms in the United States, these symptoms were examined next for diagnostic use (Stanek et al., 2011). The musculoskeletal symptoms category included painful joints, stiff joints, stiff neck, back pain, and sore muscles. Analysis was conducted using sex, age, and case/control comparisons. Population sample size ($N = 591$) for the musculoskeletal category was slightly different than that used for the EM rash analysis. Three participants were unsure about the presence of the EM rash, so these participants were excluded from the EM rash analysis. Data was available for these three participants

for the other symptom categories, so these participants were included in the rest of the symptom analyses.

Breakdown of musculoskeletal symptoms frequency scores are shown in Table 45 and Figure 10. Of note, male (Mcase) cases experienced musculoskeletal symptoms across the entire range of frequency categories with the score evenly distributed across the scores. Female cases (Fcase) were also distributed across all frequency levels, but the largest concentration of scores was located in the highest musculoskeletal symptom frequency score. Additionally, both male (Mcontrol) and female (Fcontrol) controls experienced musculoskeletal symptoms across all score categories with the highest concentration of scores at the lowest end of the scale.

Table 45

Musculoskeletal Symptom Frequency Score by Sex and Case/Control

Frequency Score	Female Case	Female Control	Male Case	Male Control
0.00-0.20	7	140	10	53
0.21-0.40	12	53	17	23
0.41-0.60	24	33	17	28
0.61-0.80	27	15	16	9
0.81-1.00	60	27	13	7

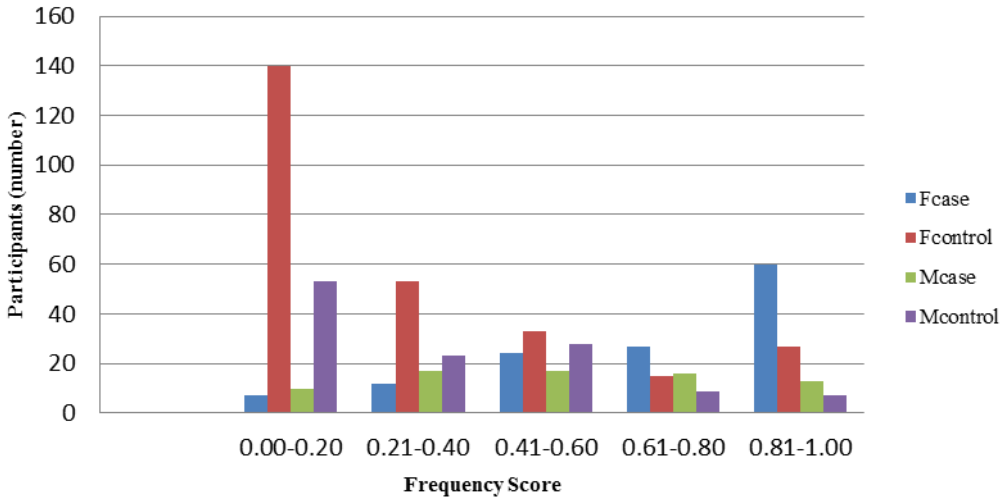

Figure 10. Comparison of musculoskeletal frequency scores by case/control and sex of participant.

Initial descriptive statistics demonstrated that all participants (N = 591) could be included in the analysis. Upon Chi-Square analysis, relationships between the frequency score for musculoskeletal symptoms compared to sex (Table 46), age (Table 47), and case or control (Table 48) displayed a significant correlation [χ^2 (4, N = 591) = 19.85, p = 0.01; χ^2 (48, N 591) = 120.34, p = 0.00; χ^2 (4, N = 591) = 148.74, p = 0.00].

Table 46

Musculoskeletal Frequency Score vs Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	19.852[a]	4	.001
Likelihood Ratio	20.645	4	.000
Linear-by-Linear Association	1.671	1	.196
N of Valid Cases	591		

Table 47

Musculoskeletal Frequency Scores vs. Age

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	120.344[a]	48	.000
Likelihood Ratio	123.063	48	.000
Linear-by-Linear Association	49.507	1	.000
N of Valid Cases	591		

Table 48

Musculoskeletal Frequency Score vs Case/Control

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	148.736[a]	4	.000
Likelihood Ratio	159.936	4	.000
Linear-by-Linear Association	145.614	1	.000
N of Valid Cases	591		

After performing Levene's Test of Equality of Error Variances, the significance value was 0.059, which is higher than the alpha value of 0.05. Because the test statistic was higher than the accepted level of significance, the values being tested were normally distributed and a one way analysis of variance (ANOVA) was performed (Munro, 2005). When comparing the musculoskeletal frequency score with case/controls or male/female, differences found were significant, F (1, 1) = 74.51, p = 0.00; F(1,1) = 8.33, p = 0.00 respectively. In addition, comparison of the musculoskeletal frequency scores with cases/controls and male/female variables together produced a significant result, F (1,1) =

8.03, $p = 0.01$. Further post hoc analysis was not performed on these variables because there were only two categories for each variable.

When examining the musculoskeletal frequency score with the age variable by ANOVA, initial results implied a lack of significance between age as a whole and the musculoskeletal frequency score. Post hoc analysis, via the Tukey post hoc test, comparing frequency scores of each age group with each other age group did show some areas of significance (highlighted in yellow). The <20 years age group was significantly different from most of the other age groups with the exception of the 20-24 years, 25-29 years and the >75 years age groups (Table 49). The 20-24 years age group was only significantly different from the 40-44 years and the 50-54 years age groups. The other age groups were not significantly different from each other, with the exception of those groups that were significantly different from the <20 years age group and mentioned previously.

Table 49

Tukey Post Hoc Test Comparing Musculoskeletal Frequency Score with Age Categories (Partial Table)

Dependent Variable: Frequency Score							
	(I) Age Category	(J) Age Category	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	<20 Years	20-24 Years	-.3041	.17107	.860	-.8734	.2652
		25-29 Years	-.6836	.26771	.342	-1.5745	.2073
		30-34 Years	-1.0407*	.26771	.007	-1.9316	-.1499
		35-39 Years	-1.1479*	.27618	.003	-2.0670	-.2288
		40-45 Years	-1.3384*	.22741	.000	-2.0951	-.5816
		45-49 Years	-1.0590*	.22148	.000	-1.7960	-.3220
		50-54 Years	-1.6196*	.20840	.000	-2.3131	-.9261
		55-59 Years	-1.1824*	.26383	.001	-2.0603	-.3044
		60-64 Years	-1.0646*	.24159	.001	-1.8685	-.2606
		65-69 Years	-.8898*	.25667	.032	-1.7439	-.0357
		70-74 Years	-1.2655*	.33228	.010	-2.3713	-.1598
		>75 Years	-1.0368	.32393	.073	-2.1147	.0412

Musculoskeletal symptom severity scores were also calculated. Scores were broken down by sex and case or control status (Table 50). Female (Fcase) cases had a more even distribution of scores across all severity levels with the highest scores in the top severity level. Male (Mcase) cases had lower severity scores overall. Female (Fcontrol) and male (Mcontrol) controls had the highest number of individuals experience little to no discomfort associated with their musculoskeletal symptoms (as expected), but also had participants experience symptoms across the entire range of scores in higher numbers than expected.

Table 50

Musculoskeletal Severity Score for Male/Female Cases and Controls

Severity Score	Female Case	Female Control	Male Case	Male Control
0.00-0.20	15	158	15	63
0.21-0.40	29	48	24	27
0.41-0.60	24	32	20	18
0.61-0.80	23	16	5	11
0.81-1.00	39	14	9	1

Musculoskeletal symptoms severity scores were also statistically analyzed. Comparisons were made between these severity scores for cases/controls (Table 51), males/females (Table 52), and with the various age categories (Table 53). Pearson Chi-Square analysis suggested a significant relationship between musculoskeletal severity and being a case or control [χ^2 (4, N = 591) = 116.54, p = 0.00]; being a male or female [χ^2 (4, N = 591) = 14.55, p = 0.01]; and with age [χ^2 (48, N = 591) = 128.36, p = 0.00].

Table 51

Musculoskeletal Severity Score vs Case/Control

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	116.537[a]	4	.000
Likelihood Ratio	122.993	4	.000
Linear-by-Linear Association	107.236	1	.000
N of Valid Cases	591		

Table 52

Musculoskeletal Severity Score vs Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	14.553[a]	4	.006
Likelihood Ratio	15.454	4	.004
Linear-by-Linear Association	2.452	1	.117
N of Valid Cases	591		

Table 53

Musculoskeletal Severity Score vs Age Category

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	128.359[a]	48	.000
Likelihood Ratio	129.435	48	.000
Linear-by-Linear Association	24.788	1	.000
N of Valid Cases	591		

Neurological Symptoms

Neurological symptoms are the hallmark of the second stage of Lyme disease infection (early disseminated Lyme disease) where the *B. burgdorferi* bacteria leave the initial site of infection and spreads to other areas of the body, including the nervous system. Neurological symptom data collected included: facial numbness (Bell's palsy); blurred vision, eye pain, ear ringing, and jaw pain (cranial nerve involvement), testicular/pelvic pain, and tingling/burning/numbness. Variables compared with symptom frequency and severity scores included case/control, sex, and age. All participants (N = 591) were included in the analysis.

Neurological symptom frequency scores (Table 54 and Figure 11) vary in their distribution between cases and controls. Both male (Mcase) and female (Fcase) cases display a more even distribution across the scale of scores available. Conversely, male (Mcontrol) and female (Fcontrol) controls' scores are heavily skewed to one side of the scale of available scores with a sharp decrease in the number of controls that experience these symptoms more than once per week.

Table 54

Neurological Symptom Frequency Scores by Sex and Case/Control

Frequency Score	Female Case	Female Control	Male Case	Male Control
0.00-0.20	14	182	14	76
0.21-0.40	26	45	14	23
0.41-0.60	30	19	16	12
0.61-0.80	29	14	16	6
0.81-1.00	31	8	13	3

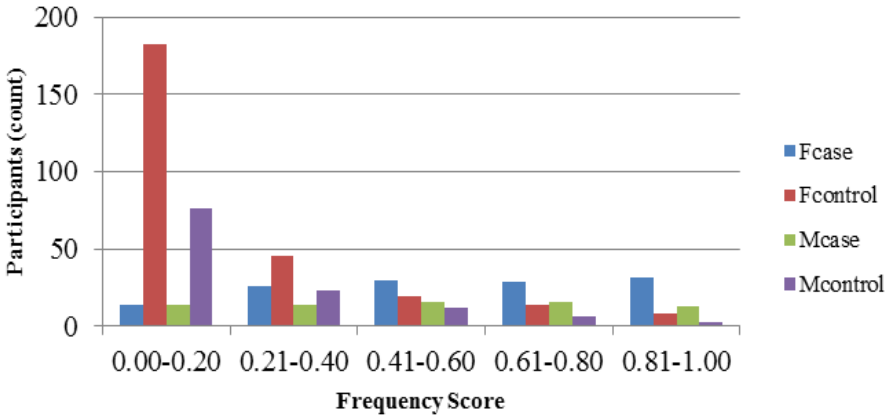

Figure 11. Comparison of the neurological symptom frequency score between cases/controls and by sex of participant.

Comparison was first performed based on the neurological symptom category frequency score. As shown in Table 55, Chi-Square analysis comparing frequency scores of cases and controls supported a significant difference between cases and controls based on neurological symptom frequency scores [χ^2 (4, N = 591) = 184.76, p = 0.00]. In addition, Chi-Square analysis based on age (Table 56) supported a significant difference based on age of participant [χ^2 (48, N = 591) = 155.81, p = 0.00].

Table 55

Neurological Symptom Frequency Score by Case/Control

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	184.755[a]	4	.000
Likelihood Ratio	195.642	4	.000
Linear-by-Linear Association	177.378	1	.000
N of Valid Cases	591		

Table 56

Neurological Symptom Frequency Score by Age Category

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	155.810[a]	48	.000
Likelihood Ratio	158.491	48	.000
Linear-by-Linear Association	72.536	1	.000
N of Valid Cases	591		

Not surprisingly, Chi-Square analysis based on sex (Table 57) did not support a significant difference between the frequency a neurological symptom was experienced and the sex of the participant [χ^2 (4, N = 591) = 1.15, p = 0.89]. Both male and female

cases displayed a broad distribution of frequency scores, so the lack of significance between the sexes based on the data collected was not unexpected.

Table 57

Neurological Symptom Frequency Score by Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.151[a]	4	.886
Likelihood Ratio	1.148	4	.887
Linear-by-Linear Association	.015	1	.902
N of Valid Cases	591		

Next, neurological symptom severity scores were examined. Comparisons were made between neurological symptom severity scores and case/control, sex, and age categories. Aggregate data can be found in Table 58 and Figure 12 below. As with the neurological symptom frequency scores, the neurological symptom severity scores displayed a similar distribution. Male (Mcase) and female (Fcase) cases experienced neurological symptoms with varying degrees of severity across all possible score categories. In comparison, male (Mcontrol) and female (Fcontrols) controls experienced neurological symptoms mainly on the less severe end of the severity scale, if at all.

Table 58

Neurological Symptom Severity Scores by Sex and Case/Control

Severity Score	Female Case	Female Control	Male Case	Male Control
0.00-0.20	22	201	20	88
0.21-0.40	36	34	21	16
0.41-0.60	33	21	16	8
0.61-0.80	25	9	10	6
0.81-1.00	14	3	6	2

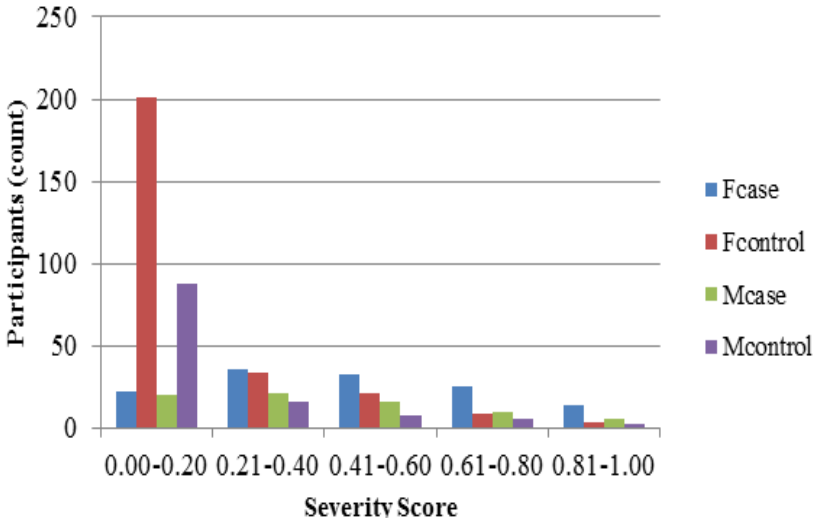

Figure 12. Comparison of neurological symptom severity score between cases and controls by sex of participant.

Statistical analysis was performed. Chi-Square analysis supported a significant difference between the neurological symptom severity scores of cases and controls [χ^2 (4, N = 591) = 165.51, p = 0.00], as well as neurological symptom severity scores found between age categories [χ^2 (48, N = 591) = 163.85, p = 0.00]. Results for these analyses are found in Tables 59 and 60 below.

Table 59

Neurological Symptom Severity Score by Case/Control

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	165.551[a]	4	.000
Likelihood Ratio	171.980	4	.000
Linear-by-Linear Association	145.122	1	.000
N of Valid Cases	591		

Table 60

Neurological Symptom Severity Score by Age Category

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	163.854[a]	48	.000
Likelihood Ratio	166.690	48	.000
Linear-by-Linear Association	44.193	1	.000
N of Valid Cases	591		

As with the neurological symptom frequency score, the neurological symptom severity score comparison based on sex of the participant (Table 61) did not support a significant difference between the severity levels of neurological symptoms experienced by males and females [χ^2 (4, N = 591) = 0.445, p = 0.98]. This finding was not surprising based on the distribution of neurological symptom severity scores reported.

Table 61

Neurological Symptom Severity Score by Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.445[a]	4	.979
Likelihood Ratio	.445	4	.979
Linear-by-Linear Association	.045	1	.832
N of Valid Cases	591		

Cognitive Symptoms

The last area for individual examination was cognitive symptoms frequency and severity scores. These scores were compared with case/control status, male/female sex, and the various age categories. All participants (N = 591) were included in this analysis.

Cognitive symptoms included in this category were: disturbed sleep, poor concentration, memory loss, irritability, crying, and sadness/depression.

Cognitive symptom frequency scores were examined first for significant differences across all variable categories. Aggregate data for cognitive symptom frequency scores based on sex and case/control status are shown in Table 62 and Figure 13. Both male (Mcase) and female (Fcase) cases experienced cognitive symptoms across a broad range of frequencies in an almost even distribution across categories. Male (Mcontrol) and female (Fcontrol) controls experienced cognitive symptoms infrequently (Table 62 & Figure 13).

Table 62

Cognitive Symptom Frequency Scores by Sex and Case/Control

Frequency Score	Female Case	Female Control	Male Case	Male Control
0.00-0.20	14	182	14	76
0.21-0.40	26	45	14	23
0.41-0.60	30	19	16	12
0.61-0.80	29	14	16	6
0.81-1.00	31	8	13	3

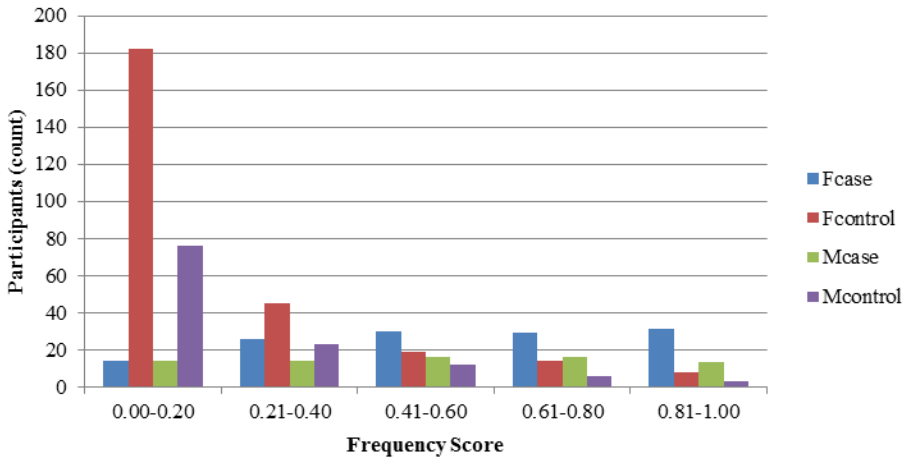

Figure 13. Comparison of cognitive frequency scores between cases and controls based on sex of the participants.

Chi-Square analysis of cognitive symptom frequency scores in cases and controls (Table 63) confirmed a significant difference [χ^2 (4, N = 591) = 141.71, p = 0.00] between how often an individual with Lyme disease experiences cognitive issues and how frequently an individual who does not have Lyme disease experiences these same symptoms. In addition, a significant difference was found between the different age categories (Table 64) based on cognitive symptom frequency score [χ^2 (48, N = 591) = 148.61, p = 0.00].

Table 63

Cognitive Symptom Frequency Score by Case/Control

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	141.713[a]	4	.000
Likelihood Ratio	151.486	4	.000
Linear-by-Linear Association	140.018	1	.000
N of Valid Cases	591		

Table 64

Cognitive Symptom Frequency Score by Age Category

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	148.616[a]	48	.000
Likelihood Ratio	151.897	48	.000
Linear-by-Linear Association	43.250	1	.000
N of Valid Cases	591		

As with neurological symptom frequency, cognitive symptoms (Table 65) occurred no more frequently in male or female participants [χ^2 (4, N = 591) = 5.22, p = 0.27]. This outcome was slightly less expected than the outcome observed for the neurological symptom frequency because the cognitive frequency raw data shows an upward trend in scores for females while the male cognitive frequency scores remain fairly steady across all frequency categories (Figure 13).

Table 65

Cognitive Symptom Frequency by Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.216[a]	4	.266
Likelihood Ratio	5.301	4	.258
Linear-by-Linear Association	.730	1	.393
N of Valid Cases	591		

Severity scores for the cognitive symptom category followed a similar pattern to the cognitive symptom frequency scores with a significant difference shown in the severity of cognitive symptoms experienced between cases and controls [χ^2 (4, N = 591)

= 105.23, p = 0.00] (Table 66) and between the various age categories [χ^2 (48, N = 591) = 83.61, p = 0.01] (Table 67). Significant differences in the severity of cognitive symptoms between males and females were absent [χ^2 (4, N = 591) = 5.66, p = 0.23] (Table 68).

Table 66

Cognitive Symptom Severity by Case/Control

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	105.230[a]	4	.000
Likelihood Ratio	112.883	4	.000
Linear-by-Linear Association	89.192	1	.000
N of Valid Cases	591		

Table 67

Cognitive Symptom Severity by Age Category

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	83.610[a]	48	.001
Likelihood Ratio	83.519	48	.001
Linear-by-Linear Association	12.701	1	.000
N of Valid Cases	591		

Table 68

Cognitive Symptom Severity by Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.656[a]	4	.226
Likelihood Ratio	5.853	4	.210
Linear-by-Linear Association	1.908	1	.167
N of Valid Cases	591		

For each symptom category analyzed, differences between cases and controls and between age categories were supported. Because these categories were developed to help diagnose Lyme disease, variations between cases and controls would be important to identify. Interestingly, there were no significant differences between male and female symptom presentation in these four categories, but from a diagnostic stand point, determining that there is no difference between male and female symptom presentation is also an important finding.

RQ3: Is the presentation of symptoms associated with the diagnosis of Lyme disease as assessed by patient medical record and ROSS Scale survey review?

$H_0$3: Lyme disease symptom presentation is not associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

H_a3: Lyme disease symptom presentation is associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

RQ4: Is the severity of symptoms associated with the diagnosis of Lyme disease as assessed by patient medical record and ROSS Scale survey review?

> $H_0$4: Lyme disease symptom severity is not associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.
>
> H_a4: Lyme disease symptom severity is associated with the diagnosis of Lyme disease as assessed by patient reported symptoms recorded in patient medical records or on the ROSS Scale survey.

Based on the reported statistical analyses and the resultant significant differences between cases and controls for musculoskeletal, neurological, cognitive, and cutaneous symptoms in both the frequency of experienced symptoms and the severity of those symptoms, the null hypothesis can be rejected and the alternative hypothesis can be accepted for both RQ3 and RQ4. Musculoskeletal, neurological, cognitive, and cutaneous symptom frequency and severity can be used to help diagnose Lyme disease.

Odds Ratio

Odds ratios were calculated (using the OpenEpi calculator) for the musculoskeletal, neurological, cognitive and cutaneous symptom categories (Dean, Sullivan, & Soe, 2015). These odds ratios were based on the symptom frequency score only for the specific symptom category, since this score represents the presence of the symptom. All participants were included in the calculations with the exception of the odds ratio calculation for the cutaneous symptom category. This group had three participants who were not sure whether the EM rash was present or not, so these three

participants were excluded from the odds ratio calculation. The odds ratios were calculated as shown in Figure 14.

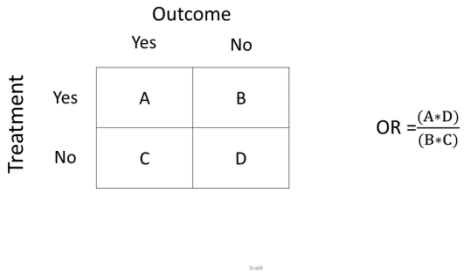

Figure 14. Odds ratio calculation chart.

According to the odds ratio calculation, the likelihood of an individual experiencing musculoskeletal symptoms with Lyme disease is 11 (95% CI: 6.34, 18.49) times higher than experiencing those symptoms in the absence of Lyme disease. Because the confidence interval is greater than and does not contain 1.0, there is a significant difference between the cases and controls based on the presence of musculoskeletal symptoms (Table 69). This result supports the statistical analysis discussed previously.

Table 69

Musculoskeletal Symptoms Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	Value	Lower, Upper	Type
CMLE Odds Ratio*	10.79	6.434, 18.93[1]	Mid-P Exact
		6.258, 19.67[1]	Fisher Exact
Odds Ratio	10.83	6.342, 18.49[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	83.16%	75.32, 91.01	
Etiologic fraction in exposed(EFe|OR)	90.77%	84.23, 94.59	

Next, the odds ratio was calculated for the presence of neurological symptoms in study participants. Based on this calculation, the likelihood of an individual experiencing

neurological symptoms with Lyme disease is 12 (95% CI: 7.90, 19.48) times more likely than experiencing neurological symptoms in the absence of Lyme disease. Because the 95% confidence interval is larger than and does not include 1.0, the results for neurological symptom odds ratio analysis are significant between cases and controls in this study. The odds ratio results (Table 70) support the statistical relevance determined by the statistical analysis discussed previously.

Table 70

Neurological Symptoms Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	12.34	7.937, 19.66[1]	Mid-P Exact
		7.77, 20.18[1]	Fisher Exact
Odds Ratio	12.4	7.9, 19.48[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	79.26%	71.97, 86.54	
Etiologic fraction in exposed(EFe|OR)	91.94%	87.34, 94.87	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.
° [1] 95% confidence limits testing exclusion of 0 or 1, as indicated
P-values < 0.05 and confidence limits excluding null values (0,1, or [n]) are highlighted.

The odds ratio was then calculated for the presence of cognitive symptoms in study participants. Based on this calculation, the likelihood of an individual experiencing cognitive symptoms with Lyme disease is 10 (95% CI: 5.24, 17.30) times more likely than experiencing cognitive symptoms in the absence of Lyme disease. Because the 95% confidence interval is larger than and does not include 1.0, the results for cognitive symptom odds ratio analysis are significant between cases and controls in this study. The

odds ratio results (Table 71) support the statistical relevance determined by the statistical analysis discussed previously.

Table 71

Cognitive Symptoms Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	9.486	5.347, 17.92[1]	Mid-P Exact
		5.175, 18.81[1]	Fisher Exact
Odds Ratio	9.516	5.235, 17.3[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	83.76%	74.99, 92.53	
Etiologic fraction in exposed(EFe|OR)	89.49%	80.9, 94.22	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.
° [1] 95% confidence limits testing exclusion of 0 or 1, as indicated
P-values < 0.05 and confidence limits excluding null values (0,1, or [n]) are highlighted.

Lastly, the odds ratio was calculated for the presence of cutaneous symptoms in study participants. Based on this calculation, the likelihood of an individual experiencing cutaneous symptoms with Lyme disease is 144 (95% CI: 19.72, 1048.73) times more likely than experiencing cutaneous symptoms in the absence of Lyme disease. Because the 95% confidence interval is larger than and does not include 1.0, the results for cutaneous symptom odds ratio analysis are significant between cases and controls in this study. The odds ratio results (Table 72) support the statistical relevance determined by the statistical analysis discussed previously. This result also supports the common use of the presence of the EM rash as a diagnostic tool for Lyme disease.

Table 72

Cutaneous Symptoms Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	Value	Lower, Upper	Type
CMLE Odds Ratio*	142.9	27.55, 2931[1]	Mid-P Exact
		24.12, 5797[1]	Fisher Exact
Odds Ratio	143.8	19.73, 1049[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	26.91%	20.76, 33.05	
Etiologic fraction in exposed(EFe|OR)	99.3%	94.93, 99.9	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.
° [1] 95% confidence limits testing exclusion of 0 or 1, as indicated
P-values < 0.05 and confidence limits excluding null values (0,1, or [n]) are highlighted.

Symptom Index Score

The symptom index score was evaluated as a diagnostic tool for Lyme disease. As stated previously, an overall symptom index score can be achieved based on a maximum score of two for each of the six symptom categories. The symptom index score was first evaluated by multiple linear regression based on the age and sex variables. All participants (N = 591) were included in this analysis. Symptom index scores were calculated as shown in Figure 6.

The multiple regression analysis was performed to determine if the sociodemographic factors sex and age could be used to predict the presence of Lyme disease based on the symptom index score. Based on the linear model, sociodemographic factors are significantly related to the symptom index score [F (2, 588) = 34.98, p = 0.00] and account for approximately 10% of the variance (R^2 = 0.106) between the symptom index scores and the age groups tested. This correlation is considered a weak, positive

correlation; for each incremental increase in age group, there is a small increase in symptom index score.

When looking at each individual factor's effect, age is the only factor that displayed any significant results ($p = 0.00$) when holding the sex variable constant (Table 73). When holding the age variable constant, sex ($p > 0.05$) is not a significant contributor to the symptom index score. Based on the multiple regression model, for each increase in age category, the symptom index score increase by a factor of 0.2. In addition, the symptom index scores for females are 0.18 points higher than those found in male participants but these variations do not reach the level of significance.

Table 73

Multiple Regression Analysis Results

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	2.379	.291		8.177	.000	1.808	2.951
	Sex	-.178	.193	-.036	-.922	.357	-.557	.201
	Age Category	.200	.024	.327	8.356	.000	.153	.247

a. Dependent Variable: Symptom Index

Next, the symptom index score was evaluated by construction a receiver operating characteristic (ROC) curve to determine whether the symptom index score can be a valid method for diagnosing Lyme disease. The resultant ROC curve for symptom index scores in cases vs. controls is shown in Figure 15.

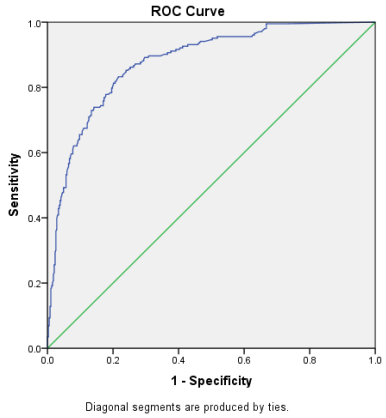

Figure 15. ROC curve comparing the symptom index scores of participants with case vs control status.

The area under the curve (AUC) can be used to show the validity of a diagnostic test (Kumar & Indrayan, 2011). With an AUC value equal to 1 showing a perfect diagnostic test, AUC values should be as close to 1 as possible. The AUC for the ROC curve shown in Figure 15 is 0.88, as shown in Table 74. This result supports using the symptom index score as a diagnostic tool for Lyme disease.

Table 74

Area Under the Curve

Test Result Variable(s): Symptom Index

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.878	.015	.000	.850	.907

The test result variable(s): Symptom Index has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

From the ROC curve, the optimal diagnostic cut-off value can be determined based on the sensitivity and specificity needs for the accurate diagnosis of the disease.

Because these two values are inversely proportional to each other, increasing sensitivity leads to a decrease in specificity (Kumar & Indrayan, 2011). If you increase sensitivity to correctly identify all cases of Lyme disease (true positives), you lose the ability to correctly identify all those individuals who don't have Lyme disease (specificity) at the same time (true negatives). Using any symptom index score above zero for diagnosis provides 99.5% sensitivity but 28% specificity (Table 75). Anyone who has Lyme disease will be diagnosed with Lyme disease, but there will be a large number of Lyme disease negative individuals who are also diagnosed as positive for Lyme disease. Using the ROC curve, a symptom index score of 3.7 points as a cut-off point will provide an 82% sensitivity and a 78% specificity, providing some balance to the diagnostic test and a more informed approach to disease diagnosis (Table 76).

Table 75

High Sensitivity/Low Specificity Using Symptom Index Score > 0.00

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	99.51%	(97.26, 99.91[1])
Specificity	27.58%	(23.37, 32.23[1])
Positive Predictive Value	41.82%	(37.5, 46.27[1])
Negative Predictive Value	99.07%	(94.94, 99.84[1])
Diagnostic Accuracy	52.28%	(48.26, 56.28[1])

Table 76

Moderate Sensitivity/Specificity Using Symptom Index Score > 3.69

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	81.77%	(75.89, 86.48[1])
Specificity	77.84%	(73.44, 81.68[1])
Positive Predictive Value	65.87%	(59.82, 71.45[1])
Negative Predictive Value	89.09%	(85.32, 91.98[1])
Diagnostic Accuracy	79.19%	(75.73, 82.27[1])

Based on the ROC analysis and the multiple linear regression analysis, the symptom index score can be used as another tool in the arsenal for diagnosing Lyme disease.

Seasonality of Infection

Seasonality of infection variables included month of tick exposure and month of diagnosis. Based on CDC data (2015), most Lyme disease diagnoses occur during the months of June, July, and August. In addition, the winter months (December through March) contain the fewest reported Lyme disease diagnoses (CDC, 2015).

As shown in Figure 16, the distribution of reported month of exposure to the *Ixodes scapularis* tick or the month of symptom onset (for those cases that did not recall a tick bite) differs from the distribution of cases by month reported to the Centers for Disease Control and Prevention shown in Figure 17 (CDC, 2015). The greatest number of cases in this study occurred in May (n = 34) and June (n = 29) with May showing the most cases in the study. According to CDC data, July is the month with the greatest number of cases overall (CDC, 2015). In addition, study participants reported January with a large number of cases (n = 21) where CDC data suggests that January and

February have the lowest number of reported cases (CDC, 2015). The study distribution directly contradicts CDC data showing the summer months as the most likely months for disease onset (CDC, 2015).

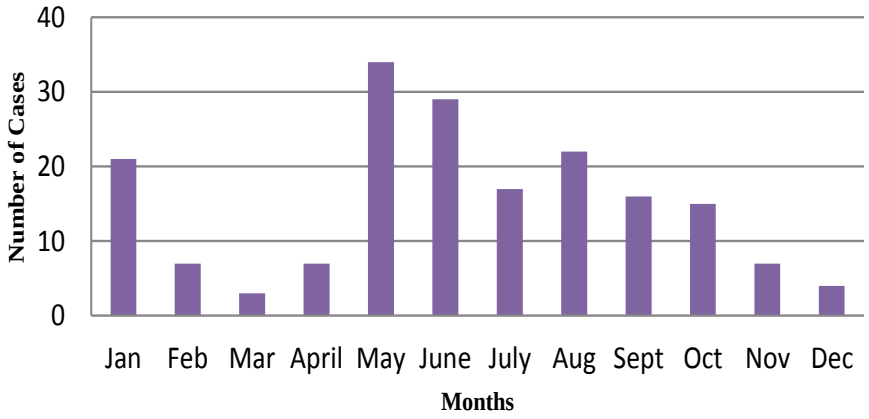

Figure 16. Total number of Lyme disease cases per month within the study population.

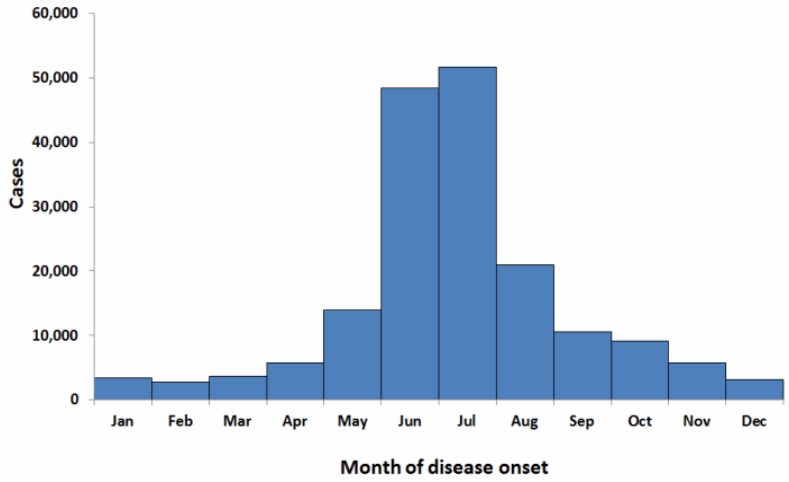

Figure 17. Confirmed cases of Lyme disease by month of disease onset – United States, 2001-2010 (CDC, 2015).

A few cases (n = 21) were removed from this analysis due to missing data for either tick bite exposure or month of initial symptom appearance. Either of these dates could be used in the analysis because initial symptoms occur 3-10 days after infection

with the *Borrelia burgdorferi* bacteria (CDC, 2015). If bitten by an *Ixodes scapularis* tick that is in the nymphal stage, a person may not necessarily notice the bite, but would experience the same symptoms at disease onset. When both dates were present, date of tick bite exposure was used in the analysis.

Statistical analysis was conducted using SPSS (ver. 21). Descriptive statistics for the symptom index scores used for analysis are shown in Table 77. A comparison was made between the symptom index score for all cases and the month of diagnosis of Lyme disease. The Chi-square results are shown in Table 78. Results show a *p*-value of 0.371, which is greater than the accepted *p*-value of 0.05. Month of diagnosis is not related to the symptom index score.

Table 77

Symptom Index Score

	N	Minimum	Maximum	Mean	Std. Deviation
Symptom Index Score	174	.84	9.20	4.4171	1.93778
Valid N (listwise)	174				

Table 78

Month of Diagnosis vs Symptom Index Score

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1643.505[a]	1617	.317
Likelihood Ratio	759.037	1617	1.000
Linear-by-Linear Association	.003	1	.958
N of Valid Cases	174		

RQ5: Is Lyme disease symptom presentation and severity associated with seasonality of infection variables as assessed by medical record and the ROSS Scale survey review?

> $H_0$5: Lyme disease symptom presentation and severity are not associated with the seasonality of infection variables as assessed by patient medical record and the ROSS Scale survey review.
>
> H_a5: Lyme disease symptom presentation and severity are associated with the seasonality of infection variables as assessed by patient medical record and the ROSS Scale survey review.

Because the symptom index score encompasses both symptom presentation and severity of symptoms, neither of these characteristics are associated with the seasonality of infection of Lyme disease. The null hypothesis is accepted.

Summary

Based on the analysis and results presented, Lyme disease symptom presentation and severity was affected by the sociodemographic variables age and sex. Assessing frequency and severity scores independently provided significant differences between cases and controls based on age and sex. Unfortunately, these differences between the sexes were not maintained when examining most symptom categories individually.

In addition, Lyme disease symptom presentation and severity can be used as a diagnostic tool (in the form of the total symptom index score), although differences between the sexes cannot be used reliably. Musculoskeletal symptoms appeared to show a distinct difference based on both age and sex, but the other symptom categories did not maintain this difference.

Lastly, seasonality of Lyme disease infection was not associated with symptom presentation or severity of reported symptoms. There was no correlation between the month of tick exposure and the severity and frequency of Lyme disease symptoms.

Overall, the study proposed that the differences in symptom presentation based on age and sex could lead to a decrease in the rate of misdiagnosis and under-diagnosis of Lyme disease in the general population. The finding that there were few differences between males and females in their experience of Lyme disease symptoms is important. Many hard to diagnose autoimmune diseases can be mistaken for Lyme disease and are found more prominently in one sex over the other. Removing sex from the diagnosis can help narrow the diagnostic search for root cause of disease. The following chapter will extrapolate on these ideas more fully.

Chapter 5: Discussion, Conclusions and Recommendations

Introduction

Although Lyme disease is currently the most frequently reported vector-borne illness in the United States, recent evidence from the CDC suggests that the estimates of Lyme disease prevalence from the National Notifiable Disease Surveillance System (NNDSS) may be inaccurate and actual prevalence levels may be much higher (Binder et al., 2012; Borchers et al., 2015; CDC, 2015; Mead., 2015). Lyme disease typically presents with many symptoms that can be mistaken for a wide variety of other diseases, which can complicate the diagnosis. Unfortunately, the only way to ensure complete recovery after treatment with no long term functional disabilities is to receive treatment while still in the early localized stage of infection (Aucott et al., 2013; Miraflor et al., 2016).

The purpose of this study was to determine the predictive value of symptom frequency and severity to diagnose Lyme disease at an earlier stage of infection, and the possible confounding effects of sex, age, and seasonality of infection. The results of this study suggest that novel diagnostic symptom patterns can significantly predict a diagnosis of Lyme disease at an earlier stage of infection, which may lead to dramatic improvements in health outcomes for afflicted patients.

Females over 30 years of age experienced musculoskeletal symptoms more frequently and with greater severity than their male counterparts. In addition, the presence of the musculoskeletal, neurological, cognitive, and cutaneous symptoms observed in this study served as a useful diagnostic tool because musculoskeletal symptoms appear 11 times more frequently in Lyme disease cases than in controls;

neurological symptoms appear 12 times more frequently in Lyme disease cases than in controls; cognitive symptoms appear 10 times more frequently in Lyme disease cases than in controls; and cutaneous symptoms appear 144 times more frequently in Lyme disease cases than in controls. Based on the receiver operator characteristic (ROC) analysis, the symptom index score, generated to measure symptom frequency and severity, can be used as a diagnostic tool. Unfortunately, no significant relationship was found between seasonality of infection and the symptom index score measured in this study, so the month of diagnosis was not related to the frequency or severity of participant symptoms. The implications of the study findings in terms of advancing knowledge in Lyme disease research will be discussed further in this chapter.

Interpretation of the Findings

Symptom Frequency and Severity

Symptom frequency and severity scores were compared between cases and controls, males and females, and between different age groups. Significant differences were found between cases and controls, based on sex of participants, and between certain age groups based on symptom frequency and severity scores. Lyme disease cases of both sexes experienced selected symptoms more frequently and with greater severity than controls. In addition, significant differences were seen based on the age of the participant. The specific symptom categories that contribute most to the identified difference will be discussed in this chapter.

Sex differences. The identified differences between male and female symptom frequency and severity scores provided some interesting results. One of the primary differences between the male and female sex is the presence of the steroid hormone

estrogen. While estrogen's primary role in the body is during the reproductive cycle in women, estrogen does have an effect on the immune system (Baker et al., 2011; Bullard et al., 2012; Pennell et al., 2012). Molecular research suggests a possible sex basis with estrogen regulation of the immune system response to *Borrelia burgdorferi*, and is an area for future research. The focus of the current study was not based on estrogen levels, but the effect of estrogen on the immune system's response to an invading pathogen provides support for the reported sex differences found in this study.

Few studies have been conducted in the United States that discuss sex and age differences in relation to Lyme disease infection. Wormser and Shapiro (2009) found that females were more likely to suffer from the chronic stages of infection than other stages, although the authors do not feel this difference is related to an inherent difference between males and females. Wormser and Shapiro attributed this increase in females with chronic Lyme disease to misdiagnosis of Lyme disease in the earlier stages of infection. According to the most recent available surveillance data analyzed by the CDC, 53.1% of reported cases of Lyme disease were male (Bacon et al., 2008). Based on the 2001-2010 surveillance data, more males suffered from confirmed Lyme disease than females across all age groups (up to age 70 years) in the United States (CDC, 2015). Data collected in the current study does not support this trend.

European studies have found sex and age differences in Lyme disease incidence. In a German study conducted by Wilkings and Stark (2014), 55.3% of the Lyme disease cases in the study were female (N = 18,894). In addition, women aged 25 – 69 years old had an increased rate of diagnosed Lyme disease (Wilkings & Stark, 2014). The data collected by Wilking and Stark supports the data collected by Bochnickova, Szilagyiova,

and Celec (2014) in Slovakia, Strle et al. (2013) in Slovenia, and Bennet, Stjernberg, and Berglund (2007) in Sweden that showed a higher incidence of Lyme disease in females over males. The current study supports the European data, but contradicts the few studies conducted in the United States. The lack of current studies on sex differences in Lyme disease in the United States shows an important area for continued research in the future.

Erythema migrans rash. The hallmark sign of early stage Lyme disease is the presence of the EM rash (Binder et al, 2012; Donta, 2012; Miraflor et al., 2016). According to Aucott et al. (2012), the EM rash appears in 70 – 80% of Lyme disease cases, but takes on the classic bull's eye appearance only 20% of the time. In a study conducted by Stonehouse, Studdiford, and Henry (2010), the classic bull's eye appearance may actually occur in as few as 9% of cases sampled. The CDC (2015) reported that 60 – 80% of Lyme disease cases report the EM rash. Miraflor et al. (2016) reported that 50 – 80% of Lyme disease cases display the EM rash.

One of the few reported sex differences with Lyme disease is related to the EM rash. In two separate studies, females were more likely to develop the EM rash than their male counterparts and of those females who developed the EM rash; the rash was more likely to appear in the nontypical form (Bennet et al., 2007; Strle et al., 2013). The studies conducted by Aucott et al. (2012), Stonehouse, Studdiford, and Henry (2010) and the CDC (2015) did not report sex differences in EM rash presentation.

The EM rash percentage (50-80%) reported by the CDC (2015), Miraflor et al. (2016), and Aucott et al. (2012) was not supported by the current study. According to the data collected, only 28% of case study participants experienced the EM rash, as opposed to 60 – 80% of cases reported by Aucott et al. (2012). Of the 28% of participants who

experienced the EM rash, female cases constituted 61% of the cases that experienced the rash. The EM rash percentage found in the current study supported recent research conducted by Strle et al. (2013), which determined that the EM rash was found more frequently in female cases (58%) than male cases (42%).

In the same study by Strle et al. (2013), women who displayed the EM rash were 15 years younger than those who did not display the rash. The results from the current study may or may not support the results presented by Strle et al. (2013). As shown in Table 35, presence of the EM rash in women was present primarily in cases over the age of 35, with the largest number of female cases with EM rash falling in the 50-54 year age category. This result closely matched the data reported by Strle et al. (2013). Unfortunately, other cutaneous symptoms were not measured in the current study so additional comparison to Strle et al. (2013) cannot be made.

In a study conducted by Dandache and Nadelman (2008), the EM rash presence shows a bimodal distribution. Caucasian males in age ranges 514 years and 45-54 years reported the most cases of EM rash (Dandache & Nadelman, 2008). In addition, Dandache and Nadelman (2008) were the first to discuss race in data reporting. The current study did not support the reported sex and bimodal age distribution reported by Dandache and Nadelman (2008). In the current study, females were more likely to have the EM rash. Of the males who had the EM rash in the current study, most were above age 55 years. In addition, the current study did not collect data on race or on participants under 18 years of age.

Musculoskeletal symptoms. Musculoskeletal symptoms tested in the current study include: painful joints, stiff neck, back pain, stiff joints, and sore muscles.

Musculoskeletal symptoms are more common during the late stages of *B. burgdorferi* infection, especially in the United States and with the species of *Borrelia* endemic to this area of the world (Feder et al., 2006). In the current study, musculoskeletal symptoms occurred more frequently and with greater severity in females over males; in cases more often than controls; and between the ages of 30 and 75 years than in other age groups below 30 years and over 75 years. Unfortunately, few other studies have been conducted examining the variables of age and sex with regard to symptom frequency and severity.

In a European study conducted by Strle et al. (2013), sex differences were present for arthritis symptoms. Within the patient sample (n = 60) diagnosed with Lyme arthritis, three quarters of the patients were male (Strle et al., 2013). This significant difference was supported even when the possibility of misdiagnosis was controlled for (Strle et al., 2013). In a study conducted in Germany, male participants made up 52% of the subjects positive for Lyme arthritis (Wilking & Stark, 2014). These findings have not been supported by other published research in the United States or Europe to date and have not been supported by the current study either.

In the current study, 46% of female cases experienced musculoskeletal symptoms every day, while only 18% of males experienced musculoskeletal symptoms every day. In a study conducted in Slovakia, 56% of women with Lyme disease had musculoskeletal symptoms (Bochnickova, Szilagyiova, & Celec, 2014). In addition, 30% of female cases from the current study classified the musculoskeletal symptoms as being completely disabling, as opposed to only 12% of the male cases ranking the musculoskeletal symptoms with the same severity. These results are directly contradictory to most of the European studies conducted to date, but this difference may be directly related to the

species of bacteria causing the infection. In the United States, *Borrelia burgdorferi* is the primary causative agent of Lyme disease and typically presents with musculoskeletal symptoms (O'Connell, 2014). In Europe, *Borrelia garinii* and *Borrelia afzelii* tend to present with neurological symptoms (O'Connell, 2014).

Neurological symptoms. Neurological symptoms included in the current study included: facial paralysis (Bell's palsy), blurred vision, eye pain, ear ringing, jaw pain, testicular/pelvic pain, and tingling/burning/numbness. Neurological symptoms occur during the early disseminated stage of Lyme disease (Binder et al., 2012; Donta, 2012). Neurological presentation of Lyme disease is often described as neuroborreliosis (Koedel, Fingerie, & Pfister, 2015).

Strle et al. (2013) found that males diagnosed with Lyme neuroborreliosis made up 60% of the study population. Unfortunately, Strle et al. only provided the age range of participants (15-79 years) but did not provide specific data on age distribution by sex for neuroborreliosis cases. Bochnickova, Szilagyiova, and Celec (2014) found that males with Lyme neuroborreliosis made up 44% of the study population and morbidity occurred between the ages 35-54 years. This morbidity was not differentiated based on sex, so direct correlation to the current study cannot be completed. In addition, Wilking and Stark (2014) discovered that 57% of the Lyme neuroborreliosis cases were male and showed a bimodal distribution. The first mode occurred in males between ages 5-9 years; the second mode occurred in males between ages 50-69 years. The current study did not support these results.

While the current study found a significant difference in neurological symptom frequency and severity between cases and controls and based on the age of the

participant, there was no significant difference between neurological symptoms experienced by the males and females in the study. The age range for cases experiencing neurological symptoms from the current study were primarily in the 35-65 years age range, which is consistent with previously published findings (Bochnickova, Szilagyiova, & Celec, 2014; Bremell & Hagberg, 2011; Strle et al. 2013; Wilking & Stark, 2014).

Unfortunately, the neurological symptom results of the current study do not support the reported results from these four European studies. All studies reported male cases experiencing neurological symptoms more frequently than female cases (Bochnickova, Szilagyiova, & Celec, 2014; Bremell & Hagberg, 2011; Strle et al. 2013; Wilking & Stark, 2014). The current study shows male and female cases experiencing neurological symptoms equally across the range of frequency and severity scores.

This discrepancy may be related to the fact that all four studies were conducted in Europe where the causative agent for Lyme disease is different than the causative agent present in the United States. There is strong evidence that the different species of *Borrelia* produce distinctly different symptoms (O'Connell, 2014; Rizzoli et al., 2011). *Borrelia* burgdorferi sensu stricto causes musculoskeletal symptoms; *Borrelia afzeli* causes cutaneous symptoms; and *Borrelia garinii* causes neurological symptoms (Bochnickova, Szilagyiova, & Celec, 2014). The current study did not identify the bacterial species that caused Lyme disease in the cases. Because *B. afzeli* and *B. garinii* are found only in Europe, the assumption was made that all cases were caused by *Borrelia burgdorferi*, the causative agent for Lyme disease in the United States.

Cognitive Symptoms. Cognitive symptoms included in this category were: disturbed sleep, poor concentration, memory loss, irritability, crying, and

sadness/depression. Aucott et al. (2013) conducted a study that examined Post-treatment Lyme disease syndrome (PTLDS) and the functional disability related to long term Lyme disease symptoms. This study by Aucott et al. was the only study that examined cognitive symptoms as outlined above. While the Aucott et al. study provided demographic information on the study participants, the study did not make comparisons based on age or sex. The study had 63 participants (35 males/28 females) whose ages ranged from 20-75 years (Aucott et al., 2013). In addition, 43 out of the 63 study participants were college graduates, which is similar to the education level found within the current study case population. Lastly, Aucott et al. found that one-third of the study participants experienced cognitive symptoms at the end of the study.

In the current study, cognitive symptoms were found more frequently and with greater severity in cases over controls and upon comparison between age groups. No significant difference was found based on sex. Cognitive symptoms associated with Lyme disease have not been studied extensively, partially due to the controversy over whether PTLDS and chronic Lyme disease exist. Cognitive symptoms are not typically found in the early stages of infection, but do show up in the later stages (Aucott, Seifter, & Rebman, 2012). In the current study, comparisons were not made based on length of time from diagnosis, so participants may have been in the later stages of infection where cognitive symptoms would occur.

General symptoms. Analysis of general symptom frequency and severity results are complicated. All of the symptoms are not cause specific and can occur in relation to many different infections. General symptoms included in the current study were fatigue, fever, chills, headaches, sore throat, persistent swollen glands, dizziness, lightheadedness,

nausea, diarrhea, and night sweats. For example, fever and chills are generated in response to a bacterial or viral infection, so the presence of these two symptoms in both the case and control groups was not surprising (Mayo Clinic, 2015). Dizziness, lightheadedness and headache also have a wide variety of causes including infection, inner ear problems, eye problems, and blood pressure issues (Mayo Clinic, 2015).

Each symptom could have multiple causes. Cases and controls were only selected based on Lyme disease status. No one was excluded based on the presence of another illness, so the presence of the general category symptoms in both groups was expected. Comparisons of frequency and severity scores between cases and controls and males and females based on the whole group of symptoms did reach the level of statistical significance. Because many of the symptoms found in the general symptom category are also present during the early localized stage of Lyme disease, further exploration of this group of symptoms in diagnosis is warranted.

Age differences. The immune system undergoes extensive changes as a person ages (Giefing-Kroll et al., 2015). Innate immune cells, like macrophages and dendritic cells, lose the ability to effectively present foreign antigens to T-lymphocytes for activation (Giefing-Kroll et al., 2015). In addition, thymus function declines with age so T-lymphocyte maturation is reduced leading to fewer T-lymphocytes available to fight infection (Giefing-Kroll et al., 2015). Lastly, effective antibody production also declines with age (Giefing-Kroll et al., 2015). Sex-differences of the immune system level off with declining production of estrogen by the ovaries as a woman ages (Giefing-Kroll et al., 2015).

Lyme cases displayed a significant difference from controls with regards to both symptom frequency and severity. Symptom frequency and severity scores for cases increased progressively as the case ages increased, with the highest frequency and severity scores falling in the 35-60 age range. Females made up 2/3 of both the case and control populations and most were under 60 years of age. Sixty-four percent of female cases and 69% of female controls had not completed menopause yet. Estrogen effects were equal between both groups, so differences in symptom frequency and severity related to age were based on Lyme disease differences.

In a study conducted by Nelson et al. (2015), Lyme disease incidence supported the CDC bimodal distribution of cases based on age. Males aged 5-9 years and males/females aged 60-64 had the highest incidence rate of Lyme disease (Nelson et al., 2015). In addition, physician diagnosed Lyme disease showed an increased incidence in females aged 15-44 years (Nelson et al., 2015). The study by Nelson et al. did not examine specific symptoms associated with Lyme disease, but the study does support the increased incidence of Lyme disease found in the current study population. Unfortunately, few studies have been conducted on symptom frequency and severity of Lyme disease symptoms based on age and sex. Reported differences in specific symptom presentation have been discussed previously in this chapter.

Seasonality of infection. In the current study, no differences in symptom presentation based on sex and age were found related to month of tick exposure or month of diagnosis. Lyme disease transmission to a susceptible host is dependent on the tick life cycle and availability of host-tick interactions (Moore et al., 2014). Cases in the current study were exposed to the *Ixodes* tick during either late spring or early fall, which is

typical for tick questing and feeding behaviors (Moore et al., 2014). Month of diagnosis varied among cases dependent mainly on whether the EM rash was present or the tick was discovered. Based on the current study data, the largest number of cases occurred in May (n = 34) and June (n = 29).

In addition, a large number of cases were diagnosed in January (n = 21). This winter diagnosis may be because the EM rash was not present and/or no tick was discovered. According to Moore et al. (2014), temperature and moisture levels can affect the length of time tick questing and feeding behaviors continue. With a warm or moist fall, ticks are able to seek blood meals later in the season leading to later cases of Lyme disease (Moore et al., 2014). In addition, warmer weather in fall or spring encourages humans to spend more time outdoors, allowing for the opportunity for the human-tick interaction to occur (Moore et al., 2014). No matter what month tick exposure or diagnosis occurred, there was no significant difference in the way symptoms presented in cases.

Theoretical Framework

The CDC outbreak investigation model was used as a basis for the current study. Ultimately, this model is designed to determine the causative agent involved in a disease outbreak and prevent the continued spread of the disease. Lyme disease surveillance has been limited, resulting in the under-reporting of Lyme disease cases (Hinkley et al., 2014). According to Hinkley et al (2014), the number of actual cases of Lyme disease in the United States in 2008 was between 288,000 and 440,000 people based on clinical laboratory tests performed. The number of cases reported to the CDC was only 38,000.

Results from the current study can be used to reduce the number of under-reported (and untreated) cases of Lyme disease in the United States.

Lyme disease symptoms frequency and severity are associated with both sex and age as supported by the statistically significant differences found between cases and controls, males and females (for certain symptoms categories), and various age groups. The differences in symptom frequency and severity can be used to aid in the early diagnosis of Lyme disease. In addition, the Lyme disease symptom frequency and severity symptom index score can be used as a diagnostic tool. Odds ratio calculations were performed based on the symptom index score. The musculoskeletal (OR = 11), neurological (OR = 12), cognitive (OR = 10) and cutaneous (OR = 144) symptoms all displayed significant variation between cases and controls. Clinical symptom observation would fall under the "physician-based surveillance" method (Ertel, Nelson, & Carter, 2012, pg. 246).

Ertel, Nelson, and Carter (2012) examined the importance of physician-based surveillance methods compared to laboratory-based surveillance methods alone or in combination with physician-based surveillance. A combination method of laboratory diagnosis with physician surveillance provided the most complete and accurate surveillance information for case reporting in the earliest stages of infection (Ertel, Nelson, & Carter, 2012). The calculated odds ratios and the observed differences between Lyme disease cases and controls in symptom frequency and severity found in the current study support physician-based surveillance in the diagnosis and reporting of Lyme disease cases.

Limitations of the Study

One of the main limitations for this study was sampling bias. Cases needed to be Lyme disease positive. This fact reduces the ability to have a true random sample population. The sampling bias was addressed in several ways. At the primary care site, cases were selected at random based on the daily appointment schedule. During the study period, cases who came in for an appointment (whether the appointment was Lyme disease related or not) were enrolled in the study. In addition, controls were selected based on the daily appointment schedule (for non-Lyme disease patients) or because the control was at the primary care site with a patient. At the secondary site, ROSS scales were distributed at a variety of meetings on campus to anyone who was in attendance at the meeting. This method for selection of meetings and campus groups allowed for some random sampling of the population.

Matching was also utilized to reduce sampling bias (Mann, 2003; Zondervan, Cardon, & Kennedy, 2002). First, matching was performed based on state of residence for participants. All participants came from one of the 14 Lyme endemic states for inclusion in the study. The endemic states include Pennsylvania, New York, New Jersey, Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, Rhode Island, Vermont, Virginia, and Wisconsin (CDC, 2015). The Lyme endemic states correspond to the habitat of the *Ixodes* tick, the vector for Lyme disease in the United States. States of residence for both cases and controls included Connecticut, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, and Vermont. State of residence matching required at least one case and one control from a state in order for the study participant to remain in the study.

In addition, no cases or controls were enrolled in the study from Wisconsin or Minnesota. During data collection, a new species of bacteria was linked to Lyme disease – *Borrelia mayonii*. Currently, this species of *Borrelia* has only been identified in Wisconsin and Minnesota. At the time of the bacteria's discovery, there were no cases or controls that resided in either Wisconsin or Minnesota, so complications associated with the new species of *Borrelia* were not introduced into the current study.

The second type of matching performed was frequency matching. Frequency matching based on sex was performed so that the sample population did not contain a single sex in cases or controls (Schlesselman, 1982). Females made up 64% of the case population and 69% of the control population. Males made up 36% of the case population and 31% of the control population. In addition, a case (n = 203) to control (n = 388) ratio of approximately 1:2 was used to reduce sampling bias.

Matching on education level was performed. While educational level was not a variable under study, the secondary site was a college campus. Matching on educational level became an important consideration as advanced educational level can change socioeconomic status and effect, specifically, access to health care. Because all but five of the cases came from the primary care site, a matching of cases between collection sites based on educational level was not necessary. Matching between controls based on educational level was explored. While the population was not matched person for person, participants with at least a college degree were matched by percentage of the total population at both sites. In addition, all cases and controls had at least a high school education. With all three types of matching performed to minimize sampling bias, generalizability of study results beyond the sampled population was insured.

An additional limitation of this study was recall bias. Recall bias occurs when cases recall exposures more frequently than controls (Hassan, 2005; Pannucci et al., 2010; Schulz & Courtright, 2002). This differential recall between cases and controls can be intentional or unintentional (Hassan, 2005). This recall difference often occurs because the cases spend more time trying to determine what exposure may have led to their disease state (Pannucci et al., 2010; Schulz & Courtright, 2002). In addition, the amount of time between an occurrence and the remembrance of an occurrence can contribute to the problem (Hassan, 2005; Pannucci et al., 2010). The longer the time frame, the more likely recalled data may be distorted (Hassan, 2005).

To reduce recall bias, all participants were asked to fill out a ROSS scale. The ROSS scale collects symptoms for the week prior to the date the form is filled out, so the amount of time between symptom occurrence and recall is minimized. The ROSS scale is completed at each visit to the primary care clinic, so symptoms in cases were verified by comparison to the previous visit's ROSS scale.

An additional way to address recall bias is to use an instrument with a high degree of validity (Hassan, 2005). Data for this study was collected using a modified Burrascano Symptom Checklist that is based on the Wahler Physical Symptoms Inventory (PSI), which has demonstrated previously to have a high level of internal consistency on test-retest scores over several different subgroups (Wahler, 1968). The PSI collects data on the frequency of general symptoms associated with most any illness, but does not specifically address severity of symptoms. A modified version of the PSI was created by Burrascano (2008) to address the specific symptoms associated with Lyme disease and the severity of those symptoms. A modified version of the Burrascano Symptoms

Checklist (ROSS scale) was created by my clinical partner to address the needs of his practice. The only modification in both the Burrascano Symptom Checklist and the PSI was to reduce the number of symptoms surveyed. Symptoms surveyed were specific to Lyme disease; all other symptoms were removed.

One additional limitation of note was the fact that most cases (n = 198) came from a single clinical practice. The clinical practice treats a large number of Lyme disease patients each year, some of whom are in the later stages of infection. Because this clinical practice specializes in Lyme disease and treats patients in the later stages of infection, reported symptom frequency and severity may reflect long term infection. All cases were not necessarily in the same stage of infection.

The use of the EM rash as a diagnostic tool for identifying cases is also a limitation. Based on the CDC criteria for reporting cases to the NNDSS, presence of the EM rash is a positive indicator of Lyme disease and no further testing is required (CDC, 2015). In addition, both the IDSA and ILADS accept the presence of the EM rash as a positive indicator of Lyme disease and don't require further testing to confirm the diagnosis (Cameron, Johnson, & Maloney, 2014; Wormer et al., 2006). The current study only identified the EM rash in 28% of cases and other studies found the EM rash didn't follow the classic bull's eye pattern (Aucott et al., 2012; Bennet et al., 2007; Stonehouse, Studdiford, & Henry, 2012; Strle et al., 2013). If the presence of the EM rash is considered a gold standard for diagnosis and not all cases of Lyme disease experience the EM rash, other avenues to early diagnosis must be explored.

The final study limitation also leads into areas for further research. All data collected was self-reported. While case data could be confirmed via patient medical

records, control data could not be independently verified. Unfortunately, the study required participant self-reporting of symptoms and severity. All cases and controls received the same forms, were allowed take as much time as needed to fill the forms out, and were assured of the completely anonymous nature of the form as no personal identifiers were included on the form.

Future Research

Symptoms, by definition, are subjective since they are experienced only by the person describing them. The severity of a symptom is also subjective; what one person experiences as debilitating pain may be considered minor by another. This difference in symptom reporting and severity may be related to sex. In a review of the literature conducted by Barsky, Peekna, and Borus (2001), women consistently reported "more intense, more numerous, and more frequent bodily symptoms than men" (pg. 266). Kroenke and Spitzer (1998) found that symptom reporting was significantly higher in women than in men, regardless of the symptom type surveyed.

Some of the symptoms in the current study were included in the Barsky, Peekna, and Borus (2001) and the Kroenke and Spitzer (1998) studies. These symptoms included headache, nausea, fatigue, palpitations, joint pain, and back pain (Barsky, Peekna, & Borus, 2001; Kroenke & Spitzer, 1998). Joint and back pain was assessed as part of the musculoskeletal category of symptoms when analyzing study data and displayed a significant difference between males and females in the current study. Headache, nausea, and fatigue were included in the general category of symptoms and were only included in the total symptom index score. Palpitations were included in the cardiac category of symptoms and was only included in the total symptom index score.

In cases where a specific disease was experienced, these sex differences in symptom reporting disappeared (Barsky, Peekna, & Borus, 2001; Davis, 1981; Eskelinen, Ikonen, & Lipponen, 1994; Katz & Criswell, 1996; Macintyre, 1993; Marshall & Funch, 1986). Katz and Criswell (1996) studied rheumatoid arthritis, an autoimmune disease. Eskelinen, Ikonen, and Lipponen (1994) studied acute appendicitis, caused by a bacterial infection, and Macintyre (1993) studied the common cold, caused by a virus. Lastly, Marshall and Funch (1986) studied colorectal cancer. Since the current study was based on a specific disease (Lyme disease), the sex differences in reporting may also be minimal.

Many of the research studies cited are older studies; the current focus of sex differences in symptom reporting has shifted to specific diseases and variations in reporting based on age group. Macintyre, Hunt, and Sweeting (1996) suggested that gender differences in symptom reporting vary over the course of a person's lifetime. Both sex and age based differences in Lyme disease symptom reporting across a period of time would make an interesting area to explore next.

An additional area for future research focuses on the biological differences related to the immune system response to an infection. Pennell et al. (2012) discussed the differences in immune system responses based on genes contained on the X-chromosome and the presence of miRNA. While genetic influences were not a part of the current study, sex based differences in symptom presentation, frequency and severity could be related to the physical differences in how the immune system reacts to the *Borrelia* bacteria. The X-chromosome contains miRNA; the Y-chromosome does not (Pennell et al., 2012). miRNAs are thought to regulate transcription of certain genes, some of which

regulate the immune system response (Pennell et al., 2012). Genetic mapping of miRNAs from case X-chromosomes may present some insight into the symptom presentation differences found in the current study.

Positive Social Change Implications

The suggested implications of this research for positive social change include increased knowledge of the sex differences found in Lyme disease; prevention of delays in diagnosis and treatment for patients with Lyme disease; decreased expenses associated with late Lyme disease due to increased diagnosis in the early stage of infection; and early access to needed health care services.

Based on the results of the current study, presence of the EM rash and the musculoskeletal symptoms examined in this study displayed a significant difference based on both sex and age variables. The EM rash did not occur as frequently overall as reported in previous studies; the EM rash only appeared in 28% of cases in the current study (Binder et al., 2012; Donta, 2012; Franz & Krause, 2003; Girschick et al., 2009; Miraflor et al., 2016; Wormser et al., 2006). Of the 28% of cases that reported the EM rash, 56% were women over the age of 35 years. Musculoskeletal symptoms were present with greater frequency and severity in female cases over male cases.

In addition, this significant difference extended to case/control differences. Significant differences between cases and controls were also found for the overall symptom index scores, as well as the specific symptoms in the neurological and cognitive categories. Because all of the symptoms of Lyme disease can be associated with another disease or condition, information regarding the occurrence of these *groups of symptoms*

in Lyme disease cases will help with the earlier diagnosis of the disease (Henry et al., 2012).

Based on a recent online survey conducted by Lymedisease.org, only 7% of the 6,000 study participants were diagnosed within the first month after the initiation of symptoms (Lymedisease.org, 2015). Most research suggests that earlier treatment provides the patient's best chance for a full recovery (Cameron, 2007; Donta, 2012; Johnson & Stricker, 2004). Once treatment is initiated, 80-90% of patients significantly improve (CDC, 2015). Delaying treatment can be costly, both in patient health costs and in reduced quality of life (Johnson et al., 2011). Zhang et al. (2006) determined that the annual health care costs associated with late Lyme disease treatment ($16,199 per person) can be 12 times higher than those costs associated with early Lyme disease treatment ($1,310 per person).

During the early stage of infection, treatment with beta-lactam antibiotics is highly successful at killing the bacteria and stopping the progression of the infection (Binder et al., 2012; Cameron, 2007; Donta, 2012; Franz & Krause, 2003; Girschick et al., 2009; Johnson & Stricker, 2004; Muellegger, 2004; Wormser et al., 2006). Late phase infections may require longer term or intravenous treatments with antibiotics (Binder et al., 2012; Cameron, 2007; Donta, 2012; Franz & Krause, 2003; Girschick et al., 2009; Johnson & Stricker, 2004; Muellegger, 2004; Wormser et al., 2006). Unfortunately, antibiotic treatment for late phase infections does not always result in a complete recovery with no symptoms (Johnson et al., 2011). It is in these instances that post-treatment Lyme disease syndrome and/or chronic Lyme disease may be the diagnosis.

Because late phase manifestations can be incapacitating, adults could be burdened with "functional impacts" of Lyme disease (Aucott et al., 2013, p 2). Functional impacts occur when a patient experiences symptoms, and/or increased severity of symptoms, that prevent them from completing daily activities (Aucott et al., 2013). These functional impacts may include loss of job, loss of productivity, lapse of insurance coverage, and disability (Johnson et al., 2011). In fact, loss of productivity makes up more than half the costs of late Lyme disease infection (Johnson et al., 2011). Early, accurate diagnosis of Lyme disease can prevent all of these impacts by stopping the spread of the infection before the serious complications of late Lyme disease occur.

Early, accurate diagnosis of Lyme disease is so important to recovery that the federal government is working on legislation to improve the possibility for early detection. In the House of Representatives, HR 4701 – "Vector-Borne Disease Research Accountability and Transparency Act of 2014" passed in September 2014. This act establishes an advisory committee to examine all aspects of Lyme disease including research, diagnosis and treatment and includes all stages of Lyme disease, including the heavily disputed chronic Lyme disease stage (Lyme Disease Association, 2016). In the U.S. Senate, The Lyme and Tick-Borne Disease Prevention, Education, and Research Act of 2015 was proposed in July 2015. This act provides more federal government action in the development of education and prevention programs, along with enhanced research support for diagnosis and treatment options for Lyme disease patients (Lyme Disease Association, 2016). Vital to creation of federal legislation are research findings to provide insights into the highly complex disease that is Lyme disease. The current study may be

used to provide additional insights into sex and age differences in symptom presentation of Lyme disease.

In addition, 18 states have proposals under consideration to become laws (Lyme Disease Association, 2016). Currently, eleven of those states have some type of law in place to allow extended treatments for Lyme disease patients and to protect physicians from medical hearing and licensing issues related to extended Lyme disease treatments. Proposals that require insurance companies to pay for extended treatments are also being considered. Each of these instances provide Lyme disease patients the opportunity for access to the necessary treatments for complete recovery once the infection has moved beyond the early stages of infection.

Methodological Implications

The current study followed a quantitative quasi-experimental methodology. A case/control prospective study was conducted to determine whether frequency and severity of Lyme disease symptoms were different in males and females or based on the age of the individual. In addition, the current study sought to determine if Lyme disease symptom frequency and severity could be used as a diagnostic tool through calculation of a symptom index score.

This method was well suited for data collection and analysis to answer the research questions posed. A few changes to the study design would improve the outcome slightly. Additional locations for control enrollment would help boost the sample size of the population. Surprisingly, case enrollment was easy. The minimum number of cases required to reach statistical significance was quickly reached. Unfortunately, control enrollment was more difficult. A secondary site was required to locate and enroll enough

controls to reach the 1:2 case to control ratio needed to reduce the sampling bias present in the study.

The ROSS scale has been used by the primary care clinic physician for many years and is a modification of a validated instrument. Despite this fact, the ROSS scale has not been validated on its own. Comparing reported results within the study population (N = 591), participant answers displayed consistent reporting across the entire study population, which provides some support for the validity of the instrument. In addition, evidence of consistency from patient medical records is present. Patients at the primary care clinic fill out the ROSS scale at each visit, so consistency is present from form to form.

Lastly, race/ethnicity information was not collected in the current study, but a recently published study conducted by Nelson et al. (2016) suggested that Lyme disease among the Hispanic population in Lyme endemic states is increasing. Lyme disease displayed the same bimodal age distribution in the Hispanic population that is reported overall for Lyme disease (Nelson et al., 2016). This bimodal distribution was slightly skewed to older age groups (Nelson et al., 2016). In addition, Hispanic males were reported to have Lyme disease more frequently than Hispanic females (Nelson et al., 2016). One interesting finding from the Nelson et al. study was that Hispanic males and females are often diagnosed at a later stage of infection than their Caucasian counterparts. Lack of access to health care was the proposed cause.

In an earlier study conducted in Connecticut, Caucasians made up 82% of the reported cases of Lyme disease and Hispanics made up only 1% of the sample population (Ertel, Nelson, & Carter, 2012). Surveillance data from the CDC list Lyme disease as a

primarily Caucasian disease with 94% of all reported cases for 1992-2006, with Blacks, Asians/Pacific Islanders, and American Natives/Alaskan Natives making up the other 6% (CDC, 2008). In the case of African Americans, inability to recognize the EM rash may lead to misdiagnosis in the early stage of infection (Borchers et al., 2015). No ethnicity data was available at all and data for the time period 2006-2015 was not available. More studies on race/ethnicity variations in Lyme disease are required.

Recommendations

Diagnosis of Lyme disease continues to be a controversial topic. The efficacy of the two-tiered testing method recommended by the CDC is consistently called into question (Donta, 2012; Moore, 2015; Stricker & Johnson, 2011). Typically, the issue is related to when the two-tiered testing is performed. The first step in the two-tiered testing process is an enzyme immunoassay (EIA), which looks for IgM and IgG antibodies to *Borrelia* (Borchers et al., 2015). The second test is the Western Blot (Moore, 2015). Unfortunately, antibody production takes some time after exposure to an antigen – IgM antibodies develop first but can take up to two weeks to be found in measureable quantities (Borchers et al., 2015). IgG antibodies develop later in the infection and are not found in measureable quantities until 4-6 weeks after the initial infection (Borchers et al., 2015). In addition to this delay in production, the EIA and Western Blot tests cannot distinguish between old and new infections (Borchers et al., 2015). Lastly, the cost of the two-tiered testing method has increased in recent years. According to Hinkley et al (2014), the cost of two-tiered testing for Lyme disease in 2008 was $492 million. As the cost of laboratory testing increases, a viable option for diagnosis must be found.

In addition to the problems associated with the two-tiered testing methods, a second imperfect diagnostic method is the presence of the EM rash (Binder et al., 2012; CDC, 2015; Donta, 2012; Franz & Krause, 2003; Girschick et al., 2009; Miraflor et al., 2016; Wormser et al., 2006). The current study, as well as other studies, showed that the EM rash does not occur in all cases of Lyme disease (Aucott et al., 2012; Bennet et al., 2007; Miraflor et al., 2016; Stonehouse, Studdiford & Henry, 2010; Strle et al., 2013). If the EM rash is present, it may have a non-typical appearance (Aucott et al., 2012).

Lastly, PCR and bacterial culturing from infected tissues are alternative testing methods that can be used to confirm diagnosis (Borchers et al., 2015). Both methods are expensive, time-consuming, and not always successful (Borchers et al., 2015). Waiting for results from both PCR and bacterial culturing would delay treatment as well (Borchers et al., 2015).

With these issues in mind, finding alternative methods for diagnosis is important. The symptom index scoring (SIS) system is based on symptom frequency and severity as experienced by the patient. The SIS system can be used at the initial visit independent of when the tick exposure happened or whether enough time has elapsed for antibodies to be produced. The SIS system's only requirement is computer access and the computational Excel spreadsheet developed as part of the current research study. While the SIS system is not a standalone diagnostic tool, it can be used during any stage of infection and will help to identify possible Lyme disease cases.

Conclusions

Lyme disease is a vector-borne disease that is caused by the bacteria *Borrelia burgdorferi* and transmitted to susceptible hosts via the *Ixodes scapularis* and *Ixodes*

pacificus ticks in the United States. Lyme disease is endemic to 14 states, primarily located in the northeastern section of the United States. As the habitat for the tick vector expands, the incidence of Lyme disease will increase in new states along the border for the current endemic region.

Based on the results of the current research study, Lyme disease symptom frequency and severity display significant differences based on biological sex and age. In addition, odds ratio variations support these differences. The likelihood of an individual experiencing musculoskeletal symptoms with Lyme disease is 11 (95% CI: 6.34, 18.49) times higher than experiencing those symptoms in the absence of Lyme disease. The likelihood of an individual experiencing neurological symptoms with Lyme disease is 12 (95% CI: 7.90, 19.48) times more likely than experiencing neurological symptoms in the absence of Lyme disease. The likelihood of an individual experiencing cognitive symptoms with Lyme disease is 10 (95% CI: 5.24, 17.30) times more likely than experiencing cognitive symptoms in the absence of Lyme disease. The likelihood of an individual experiencing cutaneous symptoms with Lyme disease is 144 (95% CI: 19.72, 1048.73) times more likely than experiencing cutaneous symptoms in the absence of Lyme disease. Each of these symptoms can then be used to help diagnose Lyme disease at an early stage of infection, where treatment will be the most successful.

The symptom index scoring (SIS) system can provide assistance in the diagnosis of Lyme disease at any stage of infection. A minimal calculated score of 3.7 or higher in the SIS system is suggestive of Lyme disease infection. The SIS system has 82% sensitivity and 78% specificity using the 3.7 point score. While not a perfect test, it can

be another weapon in the arsenal for early Lyme disease diagnosis. The earlier the diagnosis, the more likely the disease outcome will be positive.

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Appendix A: ROSS Scale

Sex:
- ☐ Male
- ☐ Female

Date of Birth: _____

State of Residence: _____

Menopause:
- ☐ Completed
- ☐ Experiencing symptoms now
- ☐ Have not experienced symptoms yet
- ☐ Not applicable

Occupation: _____

Marital Status:
- ☐ Married
- ☐ Single
- ☐ Divorced
- ☐ Widowed

Education Level:
- ☐ High School Graduate
- ☐ College Graduate
- ☐ Graduate School
- ☐ Technical School

Number of Children Under 18:
- ☐ 0
- ☐ 1-2
- ☐ 3-4
- ☐ 5+

ANSWER THE NEXT QUESTIONS IF YOU ARE A LYME AND ASSOCIATED DISEASES PATIENT

History of Tick Bite: Present:
- ☐ Yes
- ☐ No

Date of Tick Bite (Month/Year) _____

Bull's Eye Rash
- ☐ Yes
- ☐ No

Lyme Test Positive:
- ☐ Yes
- ☐ No

Which Test (if Applicable):
- ☐ ELISA
- ☐ Western Blot
- ☐ Other: _____

Date of Initial Diagnosis (MM/YY): _____

Date of Initial Symptoms(MM/YY): _____

_____

Diagnosis Based on:
- ☐ Clinical Symptoms
- ☐ EM Rash
- ☐ ELISA and Western Blot
- ☐ Other: _____

Please put an X in the box that BEST describes the frequency of each symptom as you experienced it – DURING THE PAST WEEK.

Symptom	Never	1-2 days	3-4 days	5-6 days	Everyday
Fatigue/Tiredness					
Fever					
Chills					
Facial Numbness					
Disturbed Sleep					
Poor Concentration					
Memory Loss					
Irritability					
Crying					
Sadness/Depression					
Headaches					
Blurred Vision					
Eye Pain					
Ear Ringing/Buzzing					
Jaw Pain					
Sore Throat					
Swollen Glands					
Dizziness					
Lightheadedness					
Stiff Neck					
Back Pain					
Chest Pain					
Palpitations					
Nausea					
Diarrhea					
Testicular/Pelvic Pain					
Tingling/Numbness/Burning					
Painful Joints					
Stiff Joints					
Sore Muscles					
Night Sweats					
Other					
Other					

Please put an X in the box that BEST describes the severity of each symptom as you experienced it – DURING THE PAST WEEK. Scale: 0 – Not affected; 1 – slightly noticeable; 2 – Minor problem but noticeable; 3 – Moderate problem that interferes with some daily activities; 4 – Major problem that interferes with most daily activities; 5 – Disabling

Symptom	0	1	2	3	4	5
Fatigue/Tiredness						
Fever						
Chills						
Facial Numbness						
Disturbed Sleep						
Poor Concentration						
Memory Loss						
Irritability						
Crying						
Sadness/Depression						
Headaches						
Blurred Vision						
Eye Pain						
Ear Ringing/Buzzing						
Jaw Pain						
Sore Throat						
Swollen Glands						
Dizziness						
Lightheadedness						
Stiff Neck						
Back Pain						
Chest Pain						
Palpitations						
Nausea						
Diarrhea						
Testicular/Pelvic Pain						
Tingling/Numbness/Burning						
Painful Joints						
Stiff Joints						
Sore Muscles						
Night Sweats						
Other						
Other						

Appendix B: Permissions

Permission to modify the Burrascano Symptom Checklist to align with the ROSS Scale

Hi Dr. Burrascano,

My name is Vicki Stanavitch and I am an Assistant Professor at Keystone College, but I am writing to you today in my other role as a PhD Candidate at Walden University. I am working with Dr. Daniel Cameron as my clinical supervisor and he gave me this email address.

I am proposing a Lyme disease study to look at whether there are differences in symptom presentation and severity based on sex or age. I would like to use your Symptom Checklist with a slight modification as the measurement instrument. I am looking for either your permission or your confirmation that the checklist is in the public domain. I need this for my IRB application.

You will be given full credit in all publications or presentations for the instrument whether it is in the public domain or not.

The modification that will be made will be two-fold.

1. A few of the symptoms listed have been eliminated to allow for data collection and analysis to be simplified.

2. The severity and frequency scale will be quantified in the following way:

a. frequency will be measured as never, 1-2 days, 3-4 days, 5-6 days, and/or everyday

b. severity will be measured as not affected, slight/barely noticeable, minor problem but noticeable, moderate problem that interferes with some daily activities, or major problem that interferes with most daily activities.

Both of these changes will help in the quantitative analysis of the data collected.

I hope that you will either give me permission to use your checklist or confirm that it is part of the public domain.

Thank you for your consideration.

Vicki

Vicki A. Stanavitch
Assistant Professor of Biology, Chemistry and Public Health
Faculty Coordinator for Undergraduate Research
Keystone College
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La Plume, PA 18440
570-945-8410 (office)
570-499-1997 (cell)
vicki.stanavitch@keystone.edu

****

Joe! [burraj51@bigplanet.com]

Actions

To:

M

Vicki A. Stanavitch

Inbox

Tuesday, September 09, 2014 9:49 AM

You forwarded this message on 9/24/2014 8:33 PM.

Good morning

Thank you for your e-mail. Your work sounds exciting! I would be happy to allow your use of my checklists as you outlined. All I ask is that you send to me a copy of the revised checklists. I would also love to get a copy of the results of your eventual studies- would make me happy to see my work being expanded upon.

Best wishes and congratulations on your Doctorate.

Dr. B

Joseph J. Burrascano Jr. M.D.

Water Mill, NY, USA

Sent from my LapTop

Permission to use the ROSS Scale as the data collection instrument for the study

INFO [info@danielcameronmd.com]

Actions

To:

M

Vicki A. Stanavitch

Inbox

Tuesday, April 28, 2015 10:10 AM

You forwarded this message on 4/28/2015 10:27 AM.

Good news. You can use the ROSS with our name on the scale.

Dr. Cameron

On 4/28/15 9:59 AM, Vicki A. Stanavitch wrote:

Hi Dr. Cameron,

Hope all is well!

I am working on the IRB approval for data collection here at Keystone College so that I can increase the control population for my study.

I need your permission to use the ROSS Scale to collect control data here at the College. **The IRB requires your written permission for use.** I know you had given me verbal permission at the clinic, but I have to provide that permission in writing. I can remove your name from the scale if you prefer.

An email stating that it is fine to use the scale is all I need…nothing more formal is required. Let me know as soon as you can.

Thanks for your help so far with this project! I hope to be finished this summer.

Vicki

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Permission to publish the ROSS Scale as part of my dissertation

ROSS Scale Permission

Daniel Cameron [info@danielcameronmd.com]

Actions
To:
M
Vicki A. Stanavitch
Attachments:
(2)Download all attachments
Chapter 4 Final Results.docx (1003 KB)[Open as Web Page]; Modified ROSS Scale.pdf (262 KB)[Open as Web Page]

Thursday, August 18, 2016 8:58 PM

Vicki Stanavitch

You have my permission to include the modified ROSS scale in your final dissertation. I would appreciate being a contributor to your outstanding research.

Dr. Cameron