# Knowledge and Perception of Lyme Disease in Manitoba: Implications for Risk Assessment

by

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A Thesis submitted to the Faculty of Graduate Studies of

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## Abstract

Understanding the epidemiology and nature of Lyme disease (LD) in Manitoba, and the perception of its risk to the population of the province are important for the development of appropriate provincial public health policies, for guiding clinicians in their practices, and for general public awareness to minimize risk to the population. However, there is minimal research into knowledge levels and resulting perceptions of Lyme disease, and how those perceptions influence various groups in their practices. The objective of this study was to explore four groups for their knowledge level and perceptions of Lyme disease: scientists, policy/decision makers, clinicians, and disease-specific advocates. A grounded theory approach was used as the research framework. Semi-structured interviews were completed with a sample of 23 key informants from the four groups. One major category: knowledge and understanding of LD, and 5 sub-categories emerged from the data. These were: lack of knowledge and understanding of LD, application of LD knowledge, LD transmission, individual roles, and personal perspectives. The findings indicate the importance of the critical current science of Lyme disease being disseminated across the four groups, to enable the development of risk perceptions conducive to effective disease control. Identification of barriers in communication between the groups can be used to develop strategies to facilitate further research, public health, and public education in the province.

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# Chapter I: Introduction

This thesis seeks to examine the current understanding of the epidemiology of Lyme disease, the nature of the disease in Manitoba, and the perception of its risk to the population of the province.

Lyme disease (LD), or Lyme borreliosis is a zoonotic infectious disease resulting from a bacterial *Borrelia* infection maintained in a variety of animal hosts and reservoirs, and thought to be vectored primarily by certain *Ixodes* tick species. Zoonotic infectious diseases in general exist within a host and parasite continuum between wildlife, domestic animal and human populations (Artsob, 1997; Daszak, Cunningham and Hyatt, 2000). Serious systemic health impacts result from Lyme disease, and Aultman, Walker, Gifford, Beard, Scott and Severson (2000) include this disease with other worldwide arthropod-borne pathogens such as malaria and African sleeping sickness that continue to pose enormous health threats.

For infectious diseases like LD with fairly high global incidence, studying the epidemiology and ecology of the disease is important, since there are important regional variations in the disease cycle (Pleasant, 2000). Lyme disease is noted for its wide geographical distribution in the northern hemisphere (Gray, Kahl, Lane and Stanek, 2002), yet there are few reported cases of Lyme disease in Manitoba and throughout Canada, compared to other northern countries where the *Borrelia* pathogen is ecologically successful.

The understanding of the disease, and perception of LD risk in Manitoba are important for the development of appropriate provincial public health policies, for guiding clinicians in their practices, and for general public awareness to minimize risk to the population.

Literature Review

The general thread through the literature indicates that a lack of knowledge and understanding of Lyme disease, and difficulties associated with risk assessment might influence the perception of risk held by individuals and various groups. Lyme disease is documented as one of the most controversial illnesses in the history of medicine (Johnson and Stricker, 2009). "Lyme disease is a growing public health threat" (Stricker, Lautin and Burrascano, 2006), yet the slim Manitoba literature indicates the risk is low, and could be influencing the perception of risk in the province.

A health risk is defined as a chance of receiving a negative impact to health, as a result of exposure to a hazard (Hunter, 2004). In this case, the Lyme disease bacterium is the biological hazard, and exposure to the pathogen is dependent upon the presence of a number of ecological factors. Understanding the diversity of the bacterium, its vectors, and their hosts, in the context of a dynamic environment is critical to evaluating the potential exposure and resultant health risk presented by this infectious disease. Research implicates *Borrelia* as one of the most successful pathogenic bacterial groups, with scientists and medical practitioners only recently starting to explore its wide-ranging human health impacts and associated risk. Research has postulated that other ticks besides *Ixodes*, and other arthropods may also be able to serve as competent vectors for Lyme disease. Furthermore, vector competence studies and the methods guiding them may fail to identify avenues of potential pathogenesis, since the traditional laboratory analysis model for vector competency may overlook ecological factors found in nature. This has significant implications for public health surveillance in jurisdictions where the conventional vectors are not present in large numbers. A lack of recognition of potential vector presence might influence regional risk assessment and perception of the disease. Research has also indicated that the significant variety of small and large mammal bacterial and vector hosts further complicates the issue of risk assessment (Gray, Kahl, Lane and Stanek, 2002). Additionally, Lyme disease is difficult to diagnose since symptom mimicry of other diseases has itself created significant confusion, and questions surrounding testing reliability have been raised. Research has suggested that Lyme

disease cases may be overlooked or misdiagnosed in geographical regions where the perception of risk is low (Lang and Territo, 1997). Collectively, these issues suggest that Lyme disease may be a more serious public health issue in Manitoba, and in Canada, than it has been considered to be to date.

Risk assessment cannot be initiated without a thorough understanding of the basic etiology and epidemiology of the disease within Manitoba. Furthermore, the ongoing evolution of the pathogen, vectors, hosts and reservoirs within the province must be considered. Complications in public health surveillance have been identified in the U.S., since in order to meet the U.S. Lyme disease Centers for Disease Control (CDC) case definition criteria, exposure to an endemic geographic region supporting both the pathogen and the vector must be met (Cromley and McLafferty, 2002). Since Manitoba in general is not considered an endemic geographic region, and only a handful of locations in Canada are, low risk has been reported to date in the literature. Serious concern for human health clearly arises if risk is not reported accurately, since it forms the basis from which individuals including the policy makers, public, and practicing physicians develop their perception of risk.

Risk perception, defined by Health Canada as the way that individuals intuitively see and judge risks (Hunter, 2004), hinges on thorough risk assessment. Significant evidence has been presented to support that ecological and epidemiological factors, vector competency issues, and associated diagnostic issues in Lyme disease require further exploration, and form the core of a potentially important public health issue.

Risk assessment requires an evaluation of the recipient's exposure to a hazard (Hunter, 2004), and is therefore part of the task in assessing LD risk. An examination and understanding of the nature and process of health risk assessment is important before attempting to determine risk perception of LD in the province, and generally throughout the country. "The concept of risk cannot be limited to simply knowing the probability of occurrence and the seriousness of the damages caused. It's a matter of social construction, and numerous elements contribute towards its perception and acceptability.

These elements have been studied for 20 years or so" (Hergon, Moutel, Bellier, Herve and Rouger, 2004). Willis, DeKay, Fischhoff and Morgan (2005) reported that while laypeople's perceptions of health and safety risks have been widely studied, only a few studies have addressed perceptions of ecological hazards or disease, such as Lyme. Risk assessment and risk perception present a relatively new field of study, and this qualitative research sought to address the issue of risk perception for LD in Manitoba.

Renn's 2005 article on risk perception clarifies risk 'perception' as "the mental processes through which a person takes in, deals with, and assesses information from the environment (physical and communicative) via the senses. Bickerstaff (2004) reviewed the field of risk perception research, using examples primarily from work relating to air pollution issues, and identified social and cultural factors that influence the way in which people interpret and make sense of risk. Beecher, Harrison, Goldstein, McDaniel, Field, and Susskind (2005) indicated an individual's perception of risk develops from his or her values, beliefs and experiences, and identified factors that affect perceptions of risk include whether the risk is knowable, voluntary (can the individual control exposure?), and equitable (how fairly is the risk distributed?). They also stressed the existence of measurable differences in how technical experts and citizen stakeholders define and assess risk. No research to determine how different groups define and assess risk for LD is evident in the literature. Additionally, no research has been conducted to determine how differences in a person's LD knowledge base might influence their perception of risk.

Research indicates that the bacteria that cause Lyme disease are one of the most successful groups of bacteria on the planet, with scientists and medical practitioners just recently starting to explore its wide-ranging human health impacts, and associated risk (Gray et al., 2002). This accomplished traveler and ominous pathogen is a heterogeneous group of spirochetes (Pachner, 2004) collectively known as the *Borrelia burgdorferi* sensu lato complex. Recognition of a few "competent" or important *Ixodes* tick vectors for the bacterium, has set the stage for establishing human risk. Research has postulated that other ticks besides *Ixodes* however, and other arthropods may also be able to serve as

competent vectors for Lyme disease (Gray et al., 2002). Furthermore, vector competency studies and the methods guiding them, may fail to identify avenues of potential pathogenesis, since the traditional laboratory analysis model for vector competency may overlook ecological factors found in nature. This has significant implications for public health surveillance in jurisdictions where the conventional vectors are not present in large numbers. A lack of recognition of potential vector presence might influence regional risk assessment, and resulting perception of the disease. Research has also indicated that the significant variety of small and large mammal bacterial and vector hosts further complicates the issue of risk assessment. Additionally, Lyme disease diagnoses are fraught with difficulty, and symptom mimicry of other diseases has itself created significant confusion (Lang and Territo, 1997). Research has suggested that Lyme disease cases may be overlooked, or misdiagnosed in geographical regions where the perception of risk is low (Gray et al., 2002).

Health Canada's risk assessment framework includes identification of the issue and context, assessing risks and benefits, identifying and analyzing options, selecting a strategy, implementing a strategy, and monitoring and evaluating results while involving interested and affected parties throughout the process (Hunter, 2004). Risk assessment controls include interception of disease at the source, along the path, or at the person. When risk is deemed negligible or acceptable by Health Canada, no action or controls are implemented. This is currently the case with Lyme disease, and if greater risk exists than is currently recognized, risk controls are not in place to protect people. The literature strongly suggests that risk of Lyme disease in Manitoba and in Canada is low. The Canadian Public Health Laboratory Network, responsible for setting guidelines for Lyme disease testing and diagnosis in Canada, reported in March 2007 "Lyme borreliosis is uncommonly seen in Canada". Health Canada reports "for most Canadians, the risk of exposure to Lyme disease is fairly low, and is highest in the regions where blacklegged and western blacklegged ticks are established" (2007). However, Health Canada also indicates "surveillance has shown that migratory birds can carry these ticks to other parts of Canada, and researchers believe the ticks may be establishing themselves in areas that are not identified yet. This means there is a risk that people in other regions of Canada

may also be exposed to infected ticks" (2007). This clearly suggests that exposure may be nation-wide, yet risk has been defined to date as "fairly low". No studies have been implemented to determine professional practices in light of the message that we are a "fairly low risk" nation.

Lyme disease is an infectious disease under national surveillance (Public Health Agency of Canada, 2000), and although not listed as a "national notifiable disease" according to the Public Health Agency of Canada (2003), it will be in 2009 (Ogden et al., 2009). Reported goals of surveillance to facilitate control include identification of prevailing incidence levels, identification of epidemiologic patterns and risk factors associated with the disease to assist in the development of intervention strategies, and identification of outbreaks. Additional goals include "satisfying the needs of government (e.g. regulatory programs), health care professionals, voluntary agencies and the public for information on risk patterns and trends in the occurrence of communicable diseases" (Public Health Agency of Canada, 2000).

Health Canada's mandate for decision making in health risk management is focused on maintaining and improving health, and striving for prevention rather than control with the best available information from the scientific, economic and technological communities (Hunter, 2004). Additionally, Health Canada's decision-making process is reported as a flexible approach where possible, with risk management strategies designed to be feasible, effective, and of reasonable cost. When analyzing risk management options, consideration is given to the perceptions and concerns of affected parties, and to the indirect social, cultural and economic impacts. Furthermore, communicating information regarding both the decision-making process, and public health risk is crucial to risk management (Hunter, 2004). Morrison, Kukafka and Johnson (2005) stressed that health messages are crucial to the field of public health in effecting behaviour change. Clear and appropriate messaging of information to the public regarding their health risk is vital. Risk perceptions are developed from health risk information. Risk communication brings perceived risk and technical risk together, and in the issue of public health, "perception is reality" (Hunter, 2004). If Lyme disease is indeed a greater risk than is currently

perceived, future strategies for risk management and communication will require modification to ensure an accurate picture of risk is painted for improved public health.

Unfortunately, reliability issues surrounding risk assessment and communication of Lyme disease globally (Edlow, 2003), and within Manitoba (Fallding, 2003) have created headlines. Ongoing risk assessment can only be initiated with a thorough understanding of the basic etiology and epidemiology of the disease within the province, and constant review of new science. Information and awareness, coupled with preventative behaviours and controls can help to reduce risk, but only after it is accurately identified. If risk is minimized, the likelihood of prompt and effective diagnosis and treatment critical to avoiding chronic health problems is also minimized (Lang and Territo, 1997). Given the relative newness of this disease to the medical community, coupled with the incredible complexity of LD, a thorough understanding of LD risk cannot be reached without extensive and ongoing epidemiological research (Gray et al., 2002). Epidemiology is the study of the determinants of disease in a population, with the practical purpose of controlling the spread of disease either by limiting microbial transmission in infectious diseases, or altering the susceptibility of the population (Snydman, 1989). Manitoba's Environmental Health Risk Assessment Team is designed to "identify, assess and address important and emerging health issues to reduce the threat of environmental public health risks and protect the health of Manitobans" (Manitoba Health, 2007). Surprisingly, priority areas identified for this team do not include emerging infectious diseases such as Lyme disease, enhancing the importance of the ecological and epidemiological risk factors considered in this study. Manitoba Health provides updates to physicians, and posts public information regarding Lyme disease in the province, however this information is generally brief, and does not include much of the current global research. The Lyme treatment protocols for physicians for example, were prepared in 2001, and updated in 2003 (Manitoba Health, 2001; Manitoba Health, 2003).

The perceived seriousness of Lyme disease seems to be directly tied to the perceived prevalence communicated to the public. Risk perception in public health is reportedly an

important issue, and is influenced by a variety of factors including an individual's age, gender, education, values, and previous experiences (Hunter, 2004), along with race and income (Kalkstein, 2007; Fleming, 2007). Purvis-Roberts (2007) indicated the importance of determining the difference in perception of risk between experts, or more educated professionals, and laypeople, so that a potential hazard can effectively be communicated to the public. Beecher et al. (2005) stressed that a two-way communications dialogue between experts and the public is necessary in risk communication, and that the credibility of the purveyor of information coupled with the public's useful knowledge base and their concerns, lay the foundation for risk perception. Interestingly, Chauvin, Hermand and Mullet (2007) discovered key personality facets that are predictive of risk perception associated with health and medical care. Grasmuck and Scholz (2005) also pointed out that risk perception in their environmental study was mainly determined by emotional concerns.

The key influences on perception however include an understanding of the risk, and dreaded outcomes (Hunter, 2004). Studies in risk perception of acquiring HIV (Norman, 2007), Malaria (Pistone, 2007) and diabetes (Kim, 2007) support these key influences, most notably how knowledge and understanding influence risk perception, and furthermore influence behaviour geared to minimize risk. Brewer, Chapman, Gibbons, Gerrard, McCall, and Weinstein (2007) report that consistent relationships between risk perceptions and behaviour suggest that risk perception is rightly placed as a core concept in health behaviour theories. Furthermore, in an interesting Lyme disease vaccine study on risk perception, it was noted that not only do risk perceptions affect protective behavior, but protective behavior can also affect risk perceptions (Brewer et al., 2004). A study linking risk perception to behaviour was presented by Kalkstein and Sheridan (2007), which indicated that increased risk perception of the health effects of heat, resulted in increased citizen response to warning. Weinstein, Kwitel, McCaul, Magnan, Gerrard, and Gibbons (2007) reported "accurate measurement of beliefs about risk probability is essential to determine what role these beliefs have in health behaviour", and present another study linking risk perception to behaviour. Their study was interview based, with questions asking for agreement or disagreement with statements about risk

probability, and questions asking respondents to estimate the magnitude of the risk probability on a 7-point verbal scale. They concluded that risk perception predicted risk-reducing behaviours, which is an important message for public health. A general lack of concern, or perceived low risk of Lyme disease, potentially places the population at greater risk of disease. An accurate portrait of communicated risk needs to be painted so that precautionary behaviours can be promoted if they are necessary. Interestingly however, research in areas where Lyme disease is endemic has demonstrated that despite adequate knowledge about its symptoms and transmission, many people do not perform behaviors to reduce their risk of infection (Corapi, White, Phillips, Daltroy, Shadick and Liang, 2007). The authors suggested in light of this research that new prevention strategies directed at patient education and confidence, need to be explored in the future.

Hilden (1989) highlighted some key issues in environmental health risk research and the ensuing political decision process, and urged that politicians be taught the facts of scientific and statistical life, and in turn, scientists should be made aware of the nature of political decision processes. This research aimed to determine the perceptions of these particular groups, decision makers and scientists, and assess the flow of information between the two, along with two additional groups. Pfeiffer (2006) indicated that scientific evidence is a key factor to be considered in the development of disease control policies, however pointed out that there has been a reduction in public trust in scientific evidence. Pfeiffer (2006) further suggested that response and commitment toward policies amongst stakeholders was influenced by their risk perception. The two additional groups identified as stakeholders and interviewed in this study were clinicians and disease-specific advocates amongst the public. Lyme disease knowledge stems from the scientific community, and both the political and medical communities' response to the scientific information sets the stage for public health risk perception. The level of knowledge and communication between all participant groups, and the overall perception of LD risk to Manitobans is critical to LD control.

Although new to the modern medical arena since 1975, the presence of the Lyme disease bacterium in North America and Europe by the 1890s was historically established by

analyses of museum specimens of ticks and mammals (Mawby, 1998). "Lyme borreliosis is a serious infectious disease of humans and some domestic animals in temperate regions of the northern hemisphere" and people have likely suffered from this disease long before its 1970s identification (Grubhoffer, 2005). Curiously, we have continued to 'escape' this disease in Manitoba while the local ecology appears on all levels to support the disease. The scientific research required to completely understand how this disease operates in nature today, and to accurately assess human risk, relies on the fields of entomology, ecology, wildlife biology, medicine and epidemiology. Edlow's U.S. focused "Bull's Eye: Unraveling the Medical Mystery of Lyme disease" concludes with "we still have a lot to learn about Lyme disease, and more importantly, we still have a lot to learn about the scientific process" (2003, p. 253), prompting questions about this disease in our country, and province. Why is Lyme disease a "medical mystery", and what do we need to learn about in Canada, and in Manitoba? The science behind LD has been widely documented, yet much cutting-edge research is not seen in the Manitoba health literature or protocols.

Elsewhere, Lyme disease has been highly misdiagnosed as a number of other illnesses due to the forty or more symptoms victims might display (Edlow, 2002). As a result, it has earned the nickname 'The Second Great Imitator' (second to syphilis), and in some cases has been initially misdiagnosed as multiple sclerosis (MS), Parkinson's, Chronic Fatigue Syndrome, ALS, lupus, fibromyalgia, depression, schizophrenia, and Gulf War Syndrome among others (Lang, 1997), before a correct diagnosis of Lyme disease has been established. Are there cases of Lyme disease being misdiagnosed in Manitoba? What is the true risk of disease in Manitoba, and is this information appropriately funneled to the medical community? If the message received in the medical community sets the foundation for physicians to establish their personal perceptions of risk, which influence or govern their practices to a certain degree, do these perceptions affect the number of Lyme disease cases diagnosed in Manitoba?

Risk assessment helps to form the basis for public health interventions (Michalsen, 2003). Risk assessment, and the perception of Lyme disease risk cannot be thorough without

investigation of all potential exposures and pathways of disease, and the questions are clear. Do vectors other than those currently recognized transmit Lyme disease? Are potential Lyme disease vectors being overlooked or dismissed due to strict vector competency requirements, and a lack of appropriate research? Understanding both pathogen and vector physiology, along with host preferences and resulting pathogen reservoirs is critical to evaluating the comprehensive ecology and potential pathogenesis of this disease. A look beyond the scope of conventional vectors has not been considered in Manitoba, or in Canada to date.

## Research Questions and Objectives

Does the scientific knowledge base contribute to the perception of local LD risk, and is it possible that some patients are overlooked due to either a lack of knowledge, or as a result of clinical perceptions? It is clear that a multitude of significant factors play a role in creating the picture of Lyme disease risk in the province and country, one which might be very different from that currently acknowledged.

This study explored the LD literature, and the understanding of LD by various groups in the province, and the perception of risk associated with this knowledge and understanding. This manuscript is the report of a two-year qualitative study, driven by the following specific research objectives.

This thesis has three main objectives:

First it will review the current scientific understanding of the ecological and epidemiological issues influencing the risk of Lyme disease, in order to set the stage for the analysis of Lyme disease risk perception. The aspects of most concern include: the pathogen *Borrelia burgdorferi*; Lyme disease vectors; life cycles and competency; and the hosts and reservoirs of the disease. The science behind LD has progressed tremendously in recent years, and is perhaps much more complex than generally understood.

Secondly, it will provide a descriptive spatial analysis of the reported incidence of Lyme disease from a global, regional and local perspective, with reference to LD symptoms and other regional diseases that might invite confusion.

Thirdly, this thesis will investigate risk perception for this disease in the province from the perspective of regulatory and/or academic scientists, decision/policy makers, medical clinicians, and disease-specific advocacy groups, through key informant interviews. The global knowledge base, from which foreign risk perceptions and policies develop, will be compared with the local knowledge base, upon which local risk perceptions and policies are developed.

## Chapter II: Research Methods

To evaluate the current understanding of the epidemiology of Lyme disease, the nature of the disease in Manitoba, and the perception of its risk to the population of the province, two different strategies were adopted in order to satisfy the three research objectives. First, a review of existing scientific and epidemiologic literature on the nature of the disease and its distribution was conducted to fulfill the first two objectives: to review the current scientific understanding of LD and spatial distribution of the disease. In Chapter Three the extensive scientific literature on pathogens, vectors, hosts and reservoirs is reviewed and presented for its association with risk and risk perception. Ecological conditions supporting the pathogenesis of Lyme disease in Manitoba are also reviewed through the literature. In Chapter Four, the spatial distribution of Lyme disease in Manitoba is compared to the global pattern, patterns found throughout Europe, and disease incidence throughout North America. Lyme disease symptoms, and other regional diseases presenting with similar symptoms are referred to comparatively. The general symptoms of Lyme disease, and difficulties in disease diagnosis due to symptom mimicry are also examined, and described.

Secondly, to attain the third objective of risk perception of LD within the province and the implications for risk assessment, interviews with twenty-three key informants belonging to the categories of regulatory and/or academic scientists, decision/policy makers, medical clinicians, and disease-specific advocacy group representatives were conducted, with the aim of analyzing the respondents' understanding of Lyme disease. These data were analyzed using grounded theory, and the results of this analysis are presented in Chapter Five. Grounded theory is currently the most comprehensive qualitative research methodology available since it was introduced by Glaser and Strauss in 1967 (Morse and Field, 1995), and focuses on gathering data from a variety of sources, including interviews, in a particular area of study (Haig, 1995). The original goals of Glaser and Strauss were to produce research that would be of value to both professional and lay audiences, and to develop solid theory that fit with reality (Morse and Field, 1995). Morse and Field (1995) indicate grounded theory is difficult to describe because

it is nonlinear in nature, and point out the primary purpose of grounded theory is to generate explanatory models of human behaviour that are grounded in the data. Grounded theory ultimately allows a researcher to generate a theoretical statement to enable an explanation or prediction of theory (Hunter, 2005). Grounded theory takes an inquiry based approach, and is considered a problem-solving endeavor geared to encompass the human perspective in the area of study (Haig, 1995).

Grounded theory has been described as an important qualitative strategy with the researcher acting as the instrument, while participants' words and experiences are collected and coded as data, and are used to discover themes or develop theories (Sinuff, Cook and Giacomini, 2007). This methodology has been successfully employed by a variety of disciplines including these in the health care field (Haig, 1995). For instance, Jensen, Lassen, Robinson, and Sandoe's 2005 study of expert and lay perceptions of zoonotic food risks utilized a series of qualitative interviews with both lay people and experts. The interviews were analyzed for values underlying some of the dominant perspectives or themes, making certain consequences for risk communication clear. Andersson, Furhoff, Nordenram, and Wardh (2007) employed a qualitative study into medical practitioners' perception of oral health in their elderly patients. Informed consent was given, and eleven in-depth interviews with both semi-structured questions, and questions of perception were administered. Analysis by grounded theory comprising three stages of coding was employed. Three categories, (including one core category), each containing subcategories, were identified and labeled. The most significant category was identified as the core category, and explained the central meaning of the respondents' perceptions of the oral health of their elderly patients. Analysis revealed the practitioners had little or no awareness of the oral health of their elderly patients. The interviews disclosed several contributing factors, some which may be detrimental to the general health of the elderly population. A qualitative study investigating Lyme disease risk perception clearly lent itself well to this research method. Data collection and analysis through a qualitative grounded theory similar to the 2007 Andersson et al. study described above was employed.

Interviewing participants directly involved with receiving and channeling scientific information regarding Lyme disease to the health care field, and those in the medical field acting on this information was key to assessing risk perceptions in Manitoba. Theoretical sampling, as opposed to random sampling, ensured the use of participants who would best inform the research according to the conceptual requirements of this study. Key informants, those considered experts in their area, were selected, to minimize invalidity in randomly selecting scientists who may not be versed in the topic. A good key informant is able to express thoughts, feelings, and his or her perspective on the topic (McKillip, 1987), and those selected were extremely capable of completing the task. Appropriateness and adequacy are necessary to guide qualitative interview sampling, and an adequate range of participants is required to provide a full range of variations in the phenomenon so that definitions and meanings are grounded in the data (Morse and Field, 1995). Adequacy in this study was achieved through rich and full interviews, and a better understanding, and apparent saturation of 'what is going on here?' regarding the issue of risk was achieved. The key informant groups are outlined below.

Key Informant Groups

1. Regulatory and/or Academic Scientists: This group of key informants works directly with Lyme disease, and included entomologists, zoonotic experts, and wildlife biologists in assessing risk associated with the bacteria, disease vectors and hosts involved in the pathogenesis of Lyme disease. Given limited research on Lyme disease being conducted in the province, interviews with these key informants was critical to ensure the science associated with all ecological aspects of this disease, and its risk were covered. It is recognized that certain scientific key informants are government employed, but their scientific expertise regarding Lyme disease was specifically sought, independent of their employer. Further, it is recognized that there was some overlap with key informant categories, and certain individuals in this group wear 'two hats', and may also play a role in some capacity of public health, or in developing policy. Consequently, this group formed the largest group of interviewees with nine participants. The intention of

interviewing this group was to collect scientific information associated with assessing the risk of Lyme disease in the province, and to explore risk perception as this information is channeled into the realm of public health.

2. Decision/Policy Makers: Interviews with four risk-communicating government officials were conducted. A larger group was approached, however Manitoba Health agreed to have only one representative interviewed. This group of key informants included individuals who do not work directly as scientists or experts in Lyme disease, although they have a certain level of expertise with the disease. These individuals were interviewed collectively for their roles in interpreting the scientific information channeled from the scientific community, and in making and communicating risk management decisions.

3. Medical Clinicians: Interviews with five medical practitioners and specialists in the field of infectious disease and neurology were conducted. These key informants were interviewed for their role as non-government clinicians whose practices are based on communicated risk.

4. Disease-Specific Advocacy Groups: Interviews with representatives of select advocacy groups were conducted. These five key informants were selected due to the specific groups they represent, the specific diseases most associated with Lyme disease, along with LD advocacy group representatives.

Interview schedules were prepared in advance, and sent to participants prior to the interview. Letters of consent were prepared, signed and received from all participants. Fifteen key questions formed the core of the interview for all participants, with further questions extending into the participants' areas of expertise, as the interview allowed. Question sequence was developed to guide the interview and allow for a logical flow of discussion.

Core Interview Questions

1. What is your level of expertise/knowledge regarding Lyme disease?
2. What do you know about the pathogen that causes Lyme disease?
3. How common is Lyme disease in Manitoba? In Canada?
4. How familiar are you with the ecology and life cycles of the vectors involved in Lyme disease transmission?
5. How familiar are you with the ecology and life cycles of the hosts involved in Lyme disease transmission?
6. What is the risk of contracting Lyme disease while living in Manitoba?
7. Are you aware of any difficulties associated with diagnosing Lyme disease?
8. Are you aware of any difficulties associated with treating Lyme disease?
9. Do you believe the risk of Lyme disease is appropriately portrayed in the province?
10. Are you confident that information regarding Lyme disease risk in Manitoba as delivered through the public health care system reflects current knowledge?
11. Do you suspect the risk of Lyme disease to increase or decrease in Manitoba during the next decade?
12. What preventive methods are currently employed to minimize Lyme disease risk to Manitobans and Canadians?
13. Do you have a role in assessing the risks associated with this disease in Manitoba, and in Canada?
14. Do you feel at risk for Lyme disease living in Manitoba?
15. Do you worry about you or your family contracting Lyme disease, and have you taken any personal precautions in the past year to prevent yourself from exposure to Lyme disease?

The semi-structured interviews were audiotaped with an MP3 portable recorder. Field notes were recorded in handwritten form during interviews, as were observer's comments (O.C.) which included pertinent details of the interview such as date and location, along with interviewees' gestures, emotion, and behaviours.

Grounded theory served both methodology and data analysis in this qualitative study. Morse and Field (1995) indicated that data collection and analysis of data should occur simultaneously, closely linked at every step of the way, and the generation of theory is based on comparative analyses between or among groups within a substantive area. Every piece of data is compared with every other piece of relevant data, as suggested by Morse and Field (1995). The extensive volume of current scientific information regarding the pathogen, vector, hosts and reservoirs pertinent to assessing risk in Manitoba was explored to provide necessary background information before data analysis could occur. Thorough reading and understanding of descriptions of reported Lyme disease cases and reported risk in Manitoba, generally in Canada, and in neighbouring jurisdictions was also necessary prior to interview analysis. Reported information on the symptoms of Lyme disease, and symptom mimicry of other diseases such as MS, was also read for understanding before moving ahead to analyze the interviews.

Researchers using grounded theory seek to identify patterns, and analyze the data they collect to develop tentative theories from interviews and observations. The literature cites a number of qualitative interview based health care studies that have been successfully analyzed using grounded theory. Fletcher, Furney and Stern (2007) analyzed interview transcripts using grounded theory principles in a recent study on patient interactions with their physicians. An interview-based study by Hurley, Sargeant, Duffy, Sketris, Sinclair and Ducharme (2007) explored perceptions of medical treatments using grounded theory methodology, and interviews were transcribed verbatim and analyzed for emerging themes. A number of other qualitative interview-based health related research studies focusing specifically on perceptions, and analyzed through grounded theory principles have been cited. For example, Lloyd-Williams, Kennedy,

Sixsmith, A. and Sixsmith, J. (2007) studied elderly persons perceptions on death and dying; Lewis, Kersten, McCabe, McPherson and Blake (2007) studied perceptions of patients' pain and related body parts; Newton studied the perceived relevance of oral health (2007); and Charleston and Happell (2006) studied nurses and nursing students' perceptions while working with patients in the mental health field. Beaton (2009) explains that qualitative research is a useful tool to explore perplexing or complicated clinical situations, indicating at least fifteen qualitative studies in total knee replacement surgery alone from 1996 through 2009 have employed grounded theory as an analysis tool to develop rich understanding of textual data.

Key informant interviews in this study were analyzed using grounded theory after being transcribed verbatim, and checked. Crang (1997) recommended using square brackets, [ ] to offer words adding clarity to a quote such as [surprised tone] or [nods], and "..." to signify pauses in conversation (Limb and Dwyer, 2001). Additionally, Crang suggested [....] be used to indicate removal of words from a quote, and that transcribed interviews be shared with interviewees so they can see how their quotes are used and if they are making the points the want(ed) to make. Butler added that following transcription, many qualitative researchers return a copy of the typed interview transcript to an interviewee for further comments to be added (Limb and Dwyer, 2001). Both Crang's and Butler's suggestions were applied to this research throughout the analysis, and copies of the transcribed interviews were returned to the interviewees, along with a cover letter requesting that they check over the transcript, and edit or add comments to ensure their points were precise. Most of the participants responded, with some offering additional comments.

Next, a data check was conducted assessing the quantity of the data, data quality and any uncertainties, in order to establish the integrity and usefulness of the data. Following the participants' transcript checks and their addition of further comments, transcripts were printed for analysis. The twenty-three interviews ranged in duration from twenty-five minutes to two hours, and the resulting transcriptions ranged in length from five to twenty typed pages.

Grounded theory is described as "a repetitive process where the analyst is required to return constantly to data sources, and to check aspects of the emerging interpretation and to gather new data where appropriate" (Hunter, 2005). Smith (1997) referred to grounded theory as a process of constant comparative analysis (Hunter, 2005). With a constant comparative method, formal analysis begins early in the study, and might almost be completed by the end of data collection (Bogdan and Biklen, 2007). In this study constant comparison analysis began early in the interview process and was an ongoing process. Field notes from all completed interviews were re-read and checked, prior to subsequent interviews. Memos were written, and utilized in refining further interview questions for each subsequent interview.

For further analysis, the data were then coded and categorized systematically, and theorizing was reserved until patterns in the data emerged from the categorizing operation. Morse and Field's general description of analysis requires open categorizing, memoing, determining a core category, recycling earlier steps in terms of the core category, sorting memos, and writing up the emerging themes and theory (1995). Crang (1997) indicated the production of something like a collage of sorted quotes and categories in segments and fragments will often offer a path through the material (Limb and Dwyer, 2001).

Crang's line by line coding (Limb and Dwyer, 2001) was the first step taken to best allow critical factors in the data to be revealed, to assist in the development of categories, and ultimately themes. This line by line coding was applied to this data, and included highlighting meaningful words and phrases, followed by coding in the margin, and written notes or memos identified as O.C. (observer's comments). Glaser first referred to this type of coding as substantive or open coding (Morse and Field, 1995). Morse and Field (1995) however, refer to this type of coding as "first level coding", and recommend all interview transcripts be analyzed line by line, with descriptive code names or abbreviations written in the margin. LeCompte and Schensul (1999) refer to this as "item level analysis", but regardless of the term all apply to looking at individual segments and

chunks of dialogue. Coding involves reading, and rereading the interviews in their entirety and reflecting on them as a whole, and eventually themes emerge when there are significant concepts that link substantial portions of the interviews together (Morse and Field, 1995). Schensul et al. (1999) indicated that identifying types of codes might be helpful in the code-design process, and the types of codes chosen for this study included method codes (e.g. research), event codes (e.g. diagnosing LD), perspective codes (e.g. concern or worry), content codes (e.g. known risks), strategy codes (e.g. education), relationship codes (e.g. policy makers and medical community), and behaviour codes (e.g. disease prevention).

The codes used in this data analysis were derived based on recommendations by Schensul et al. (1999), who indicated they should be operational, have single word or phrase names which are close to the concept they describe, should be distinctly different from one another, are initially kept at a low level of inference, are related to chunks of data, should reduce data to a manageable form, and may often be organized hierarchically. Bogdan and Biklen (2007) recommended limiting codes to 30 to 50 per study, and after creating the code list, recommended it be tested to see if they work for the study, and suit the goals. This procedure was applied to this study, and created the framework for analysis. They further indicated that decisions to limit codes are imperative, and at some point in the analytic process, the codes should become fixed. 35 codes were originally created after reading, and then re-reading the transcripts. Two were dismissed on further analysis however because they were not necessary, and another single code was added at this time, resulting in 34 fixed codes used in the transcript analysis. Listing codes and assigning an abbreviation or a number to each code is considered helpful for working with the codes (Bogdan and Biklen, 2007), and abbreviations were chosen for this study.

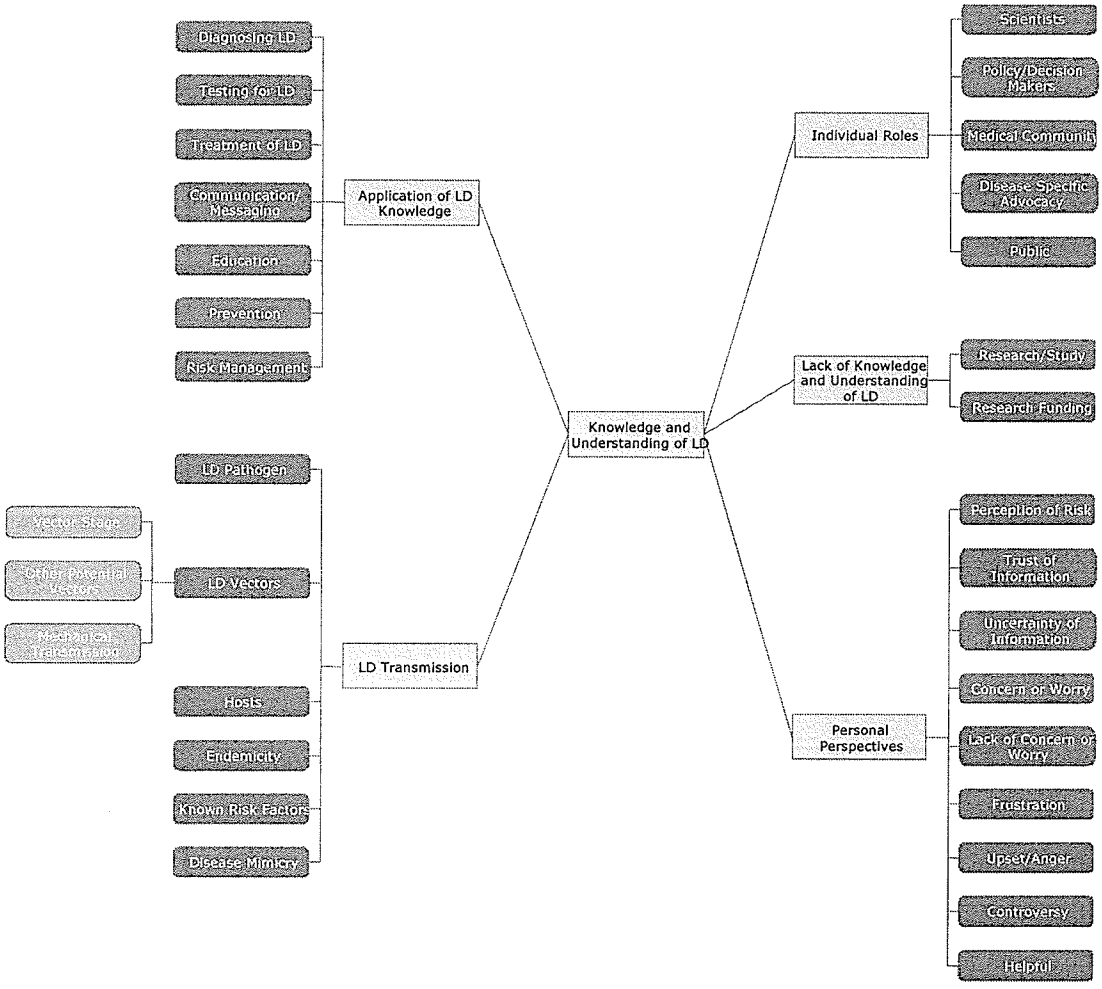
Table 1. Codes and Abbreviations used in transcript analysis

| Abbreviation | Code | Abbreviation | Code |
|---|---|---|---|
| rs | research | sc | scientists |
| $ | research funding | po | policy makers |
| co | communication/ messaging | md | medical community |
| tr | transmission of LD | ad | disease specific advocacy |
| dx | diagnosing LD | pu | public |
| ts | testing for LD | wy | concern/worry |
| tt | treating LD | fr | frustration |
| pa | pathogen | up | upset or anger |
| ve | vectors | pc | perception of risk |
| ho | hosts | in | trust of information |
| rk | known risk factors | un | uncertainty of information |
| en | endemicity | kn | knowledge /understanding |
| opv | other potential vectors | lk | lack of knowledge /understanding |
| vs | vector stages | vy | controversy |
| mt | mechanical transmission | ed | education |
| dm | disease mimicry | rm | risk management |
| pr | prevention | he | helpful/caring |

Codes were applied to phrases, sentences or groups of sentences within the data that shared a common idea, and a descriptive label or category was ultimately assigned to groups of codes. In order to categorize and subcategorize effectively, Mayring (2000) suggested interpreting text based on the content of the research questions. Categories were developed with this in mind, subcategories followed, and a core category was very readily identified throughout the analysis process. Knowledge and understanding of LD

was at the core of the phenomena under study, while lack of LD knowledge and understanding, LD transmission, application of LD knowledge, personal roles, and personal perspectives formed 5 subcategories. Morse and Field (1995) also suggested that the content of semi-structured interviews in which the participants have been asked the same questions throughout the course of the study might be sorted and analyzed by item number. This method proved to be most useful for analyzing data in this study. In addition to sorting by question number, the coded key words or phrases from the four key informant groups were extracted and categorized, in content analysis style by topic or question number (Morse and Field, 1995) to search for commonalities within the groups and/or differences amongst the groups.

Figure 1. Code Category Map



All patterns, trends and variations in the data were sought following categorizing of the data. Schensul et al. (1999) refer to this stage of analysis as pattern level analysis, indicating it is similar to building a jigsaw puzzle where once the builder has found all of the yellow pieces, and all of the blue pieces, or all of the pieces with a pattern on them for example, they can begin to assemble those pieces into a coherent chunk. Schensul et al. elaborate, indicating that taking this to the next level of seeing how the yellow chunks are related to the blue chunks, or where they fit into the overall puzzle is the process by which patterns or themes emerge from the data. Chunks in this data emerged from question specific responses, which led to these labeled categories and subcategories, which ultimately unfolded into themes. Morse and Field's (1995) recommendation not to exceed ten larger categories per any given study in order to maximize effective and efficient data sorting was followed. Five major categories were established based on commonalities in the qualitative research stemming from the research questions, and open codes were clustered based on similarity or dissimilarity of content (Morse and Field, 1995). Krippendorf (1980) reported "How categories are defined...is an art. Little is written about it" (Mayring, 2000). First level coding was considered complete when the core category "Knowledge and Understanding of LD", based on facts from the data, emerged. Throughout the open coding process, ideas and insights were recorded in memo (O.C.) form ("think pieces", Bogdan and Biklen, 2007), to help search for themes, to preserve ideas that may be currently premature but important later on, and to note thoughts about similarities and contradictions in the data regarding any emerging theory. Jackson pointed out that the researcher should also be sure to explore the significance of humour, hesitation and non-verbal cues while analyzing transcripts (Limb and Dwyer, 2001), since themes are usually quite abstract, and often difficult to identify (Morse and Field, 1995). This was taken into consideration in this study, particularly given the vague controversy surrounding this disease, and incorporated into the larger category of personal perspectives.

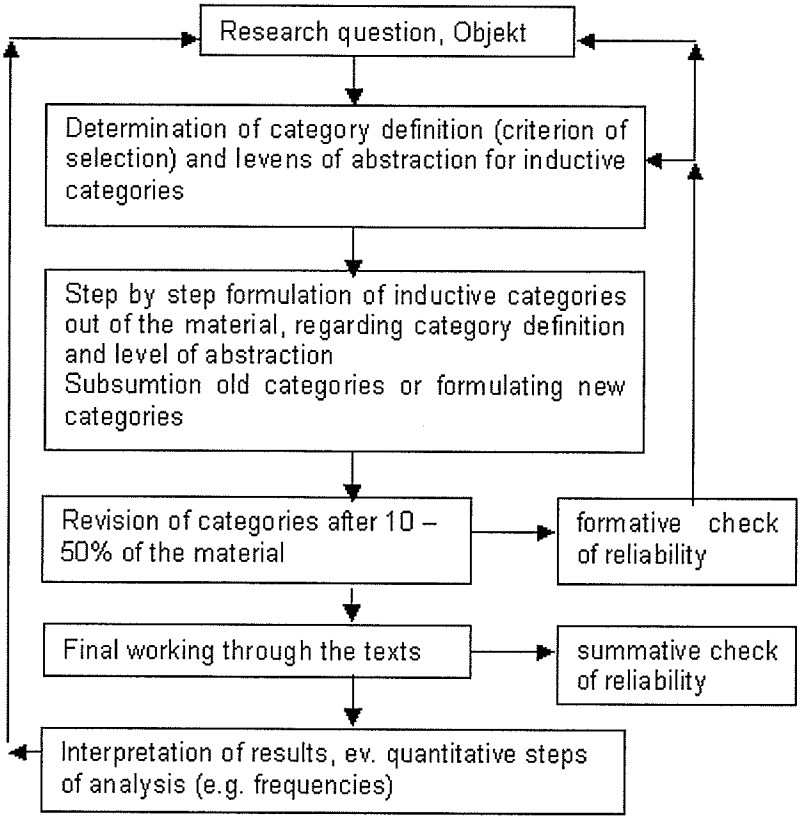
Following first level coding and the identification of categories, a further review of the literature at this point lead to the generation of further research questions, and interconnections between the categories were sought as certain patterns and linkages

emerged, as Morse and Field indicated might be expected (1995). Schensul et al. (1999) indicated that patterns emerge in a variety of ways which include: declaration, frequency or omission, similarity, co-occurrence, corroboration, sequence, or in congruence with prior hypotheses. Patterns in this study were identified primarily through their emergence via frequency or omission, and similarity.

Certain main categories sorted naturally into two or more subcategories, when the category had more than ample data. Beaton and Clark (2009) pointed out that the researcher should strive for deep interpretation and understanding of the categories, and tease out different levels of meaning, identifying new concepts and themes emerging. Given data collection and analysis were deeply coupled in this study, interview questions were extended during the interviews to pry for deeper understanding. Morse and Field (1995) further indicated that often themes do not immediately "jump out" of an interview, or the coding/categorizing process, but may be more apparent if the researcher steps back and considers "What are these folks trying to tell me?" They further indicated that once the theme has been identified, it often appears obvious. During the grounded theory analysis process of these key informant interviews, the eventually identified themes appeared concrete after re-reading the data, through a defined model outlined as follows.

Mayring (2000) indicated after categories are formulated in the context of the material, 'feedback loops' such as modeled below assist in the process of analysis, when categories are assigned.

Figure 2. Step Model of Inductive Category Development




Mayring, 2000

Mayring's model was utilized throughout the categorizing process of this study, working toward the goal of identifying themes. Miles and Huberman (1994) indicated flow charts such as Mayring's assist the researcher in drawing conclusions, since the data are compressed and organized, and a new way of thinking about the more textually embedded data might follow. Furthermore, data display allows for additional, higher order categories or themes to emerge from the data that go beyond those first discovered during the initial process of data analysis. Diagrams of the process can assist in illustrating the relationships of the various concepts, or the process of moving through the various stages and phases (Morse and Field, 1995). Given the nature of analyzing interview transcripts in hard copy, and applying codes, post-it notes to develop hierarchical relationships were used extensively to organize and re-organize the categories. Themes were identified in part through the Coding Category Map designed to visually display summarized data.

A study focusing on the relationship between the perceived risk of contracting SARS and reported compliance with health orders and protocols followed a qualitative design similar to this study (Cava et al, 2005). Semi-structured interviews were used, and grounded theory data analysis was completed through reading and re-reading the transcribed interviews, categorizing and identifying common themes, and comparing and contrasting the data. Their research concluded that there is a need for greater credibility in public health communications to increase compliance with health protocols, and to contain outbreaks of new infectious diseases. This research investigating the perception of Lyme disease risk in the province followed a 'recycling steps' (Morse and Field, 1995) as described, or 'feedback loop' (Mayring 2000) as displayed above, or 're-reading' of data as was utilized in the Cava et al. (2005) study throughout the interpretation of the transcribed interviews.

This qualitative research focused on content, analyzing additional pieces of evidence as being consistent with the other observations. Reliability is determined when the observations are not being contradicted, but rather being repeated in detail. The reading and re-reading of the data and field notes allowed for any patterns, phrases, events or ideas of interest to emerge, and be loosely counted in the data if they occurred repeatedly (Schensul et al., 1999). As an alternative to research replication reliability, analyzing this qualitative research required searching for supportive, similar, or omitted evidence as repeated items. Multiple qualitative observations act like multiple items or repeated measurements of a quantitative scale – they demonstrate that the construct exists, and demonstrate the reliability of the observations (Collins and Brewer, 1981). The coding system developed and utilized in this study allowed for the observation of repetitive items in the data, which became the skeletal framework to flesh out emerging themes.

As data were analyzed and interpreted, a written summary of each interview was prepared, along with important direct quotes from interviewees, a process suggested by Crang (1997), which was helpful for comparing ideas emerging from the interviews (Limb and Dwyer, 2001). Comparative analysis was employed between transcripts, via codes, to enable the interpretation of links, relationships, and emergent themes. A

comparison of themes emerging from specific questions, and across the four groups of key informants was critical to subsequently develop a theory regarding the perception of risk in Manitoba. We read that a good grounded theory is one that is inductively derived from data, subject to theoretical elaboration, and judged adequate to its domain with respect to a number of evaluative criteria (Haig, 1995). Morse and Field (1995) pointed out that theoretical sensitivity is absolutely essential in grounded theory, more specifically that the researcher is able to recognize what is important in the data and give it meaning. They maintain that continual interaction with the data, and being well grounded in the technical literature permits theoretical sensitivity to be realized. Furthermore, they indicate this is necessary to formulate a realistic theory that is loyal to the phenomenon under study. The extensive length of time devoted to this research allowed an increased opportunity to continually interact with the data, and formulate a theory loyal to the data, and the topic.

Sahlsten, et al. (2007) were able to achieve saturation following constant comparative analysis using a grounded theory approach to tape-recorded data collected via interview. A search for saturation of content in this study followed the achievement of reliability in quantity. Morse and Field (1995) suggested that categories emerging from key informant interviews may lend themselves to saturation when no new information about the characteristics of the category emerge, and a feeling of 'completeness' is reached. The achievement of saturation was realized toward the end of data collection in this study, when it became clear that all details pertinent to this Lyme disease investigation had materialized repetitively, and three themes and an eventual theory surfaced through data analysis.

The data were shared post-analysis, and confirmed for reliability and trustworthiness, as summaries were sent out to the participants for further comments. Similarly, Kempainen et al. (2007) demonstrated trustworthiness in their qualitative analysis of health care recipients' perceptions, via feedback from their sample group on the grounded theory analysis.

A significant amount of LD science and new research has been introduced since Lyme was first identified in North America, but much of this information has yet to channel into the perceptions and practices of those involved in Canada. The depth of knowledge and understanding worldwide of the pathogen(s), the vector(s), the hosts and reservoirs, and the overall ecology and epidemiology of Lyme disease is immense. A review of the complex nature of each of these factors and their multifaceted relationships in Chapter Three allows us to recognize that our participant groups could not possibly be fully versed in all details. Key concepts offering critical information for our participants are evident in the scientific literature however, and the flow of this key information to the groups, and the impact on their perceptions is important to this study.

## Chapter III: Ecology and Epidemiology of Lyme Disease

The ecological complexity of Lyme disease pathogenesis, issues in vector competency, and the potential for misdiagnosing Lyme disease are well reported in the literature. Global research of the pathogens, the vectors, the hosts and reservoirs, and ecology of LD is vast, yet minimally incorporated into Canadian risk assessments and perceptions.

The group of pathogens which cause Lyme disease known as *Borrelia burgdorferi* sensu lato are firmly established on a global scale. Several competent *Ixodes* vector ticks have been identified for this pathogenic group. Given numerous studies (including Netusil 2005, Gray et al. 2002, Piesman 2001, Magnarelli and Anderson, 1988) supporting the presence of *Borreliae* species in tissues of other ticks, and various other arthropods, investigation into potential vectors within Manitoba is important to determining risk in the province. The tick studies in Manitoba have been focused on *Ixodes* species alone, and have not included our abundant *Dermacentor variabilis* species, or others (MB Health, 2004). Furthermore, research on the hosts and reservoirs of *Borreliae* species has not been conducted in Manitoba aside from some recent and minimal work at Buffalo Point in the southeast, and in isolated locations in Canada.

### The Lyme Disease Pathogen: *Borrelia burgdorferi* sensu lato

Vector-borne infectious diseases involve a causative or etiologic agent, which is usually some type of microorganism that acts as a pathogen or causes disease in the host, and is transferred to the host by a vector. The Lyme disease pathogen is a spirochetal prokaryotic bacterium of the *Borrelia burgdorferi* sensu lato complex. The vector is a living organism, usually an arthropod, responsible for transmission of the pathogen, and resulting disease to humans. The Lyme disease vectors identified to date are restricted to several species of ticks (Gray et al., 2002). Hard ticks of the *Ixodes ricinus* complex are largely responsible for vectoring Lyme disease. Intermediate hosts are present in some vector-borne diseases, Lyme disease included, and reservoir populations often exist and maintain the pathogen population in nature (Gray et al., 2002). Lyme disease

pathogenesis at the genetic, species and ecosystem levels on a local, regional, national and global scale is a new and largely unexplored medical science, and warrants much attention. The ecology of LD is much more complex than is commonly acknowledged, and the implications for risk assessment and risk perception would appear to be significant. To effectively portray the complexity of LD ecology, an in-depth review of the literature of the pathogen, the vectors, and the hosts and reservoirs is required. In this chapter I will present the current LD science associated with each of these critical components of disease transmission, as they are important to the rest of this study.

Although the LD pathogen is likely ancient, it has quite recently surfaced as an agent of disease in North America. *Borreliae* spirochetes were studied significantly along with their vectors for the first time in Connecticut mid-May through September 1983. Spirochete-infected *Ixodes scapularis* larvae and nymphs were removed from nine different species of birds, and from white-footed mice (Anderson and Magnarelli, 1984). Spirochetes were detected in the midguts of *I. scapularis*, *I. dentatus, Dermacentor variabilis, Haemaphysalis leporispalustris,* and two species of insects (*Cuterebra fontinella* and *Orchopeas leucopus),* and Anderson and Magnarelli indicated at the time that possibly other arthropods other than *I. scapularis* vectored Lyme disease in the northeastern United States.

Twelve known genospecies comprised the *Borrelia burgdorferi* s.l. complex worldwide in 2003, with only three known to be pathogenic in humans (Lagal, 2003): *B. burgdorferi sensu stricto (*s.s.*), B. garinii,* and *B. afzelii* which were later confirmed by culture in 2005 (Grubhoffer, 2005). A potential fourth species *B. bissettii* was also under investigation at the same time (Oliver, Lin, Gao, Clark, Banks, Durden, James and Chandler, 2003; Gray et al., 2002). Very recently in Europe, a new human infectious species *B. spielmani* was described (Wilske, 2007), making then a total of 13 confirmed genospecies responsible for causing Lyme disease. Baranton and De Martino recently confirmed *B. spielmani* as a human pathogen (2009). The other *B. burgdorferi* s.l. species included: *B. valaisiana, B. lusitaniae, B. japonica, B. miyamoto, B. tanuki, B. sinica, B. turdii,* and *B. andersoni.* Pathogenicity for *B. valaisiana* and *B. lusitaniae* remained

uncertain in 2002 (Gray et al., 2002), and it was expected that additional genospecies would continue to emerge (Gray et al, 2002; Vanderhoof-Forschner, 1997). Bertolotti, Tomassone, Tramuta, Grego, Amore, Ambrogi, Nebbia and Mannelli (2006) confirmed *B. lusitaniae* strains from patients in Portugal and Italy, and indicated the dominance of this species in the Mediterranean basin, and its role in causing Lyme disease. Most recently, a 14th *Borrelia* species was identified in the southern United States in a comparative isolate study against the 13 other known genospecies, and named according to its geographic location: *B. carolinensis* (Rudenko, 2008).

Table 2. *Borrelia burgdorferi* sensu lato genospecies and their distribution (as of 2002)

| ***Borrelia* species:** | **Geographical distribution:** |
|---|---|
| *B. burgdorferi* sensu stricto | Europe, North America |
| *B. garinii* | Europe, parts of Asia |
| *B. afzelii* | Europe, parts of Asia |
| *B. valaisiana* | Central Europe, Ireland, Great Britain, Netherlands |
| *B. lusitaniae* | Portugal, Tunisia, rare in Central and Eastern Europe |
| *B. spielmani* | Europe |
| *B. bissettii* | Slovenia, North America |
| *B. japonica, B. miyamoto, B. tanuki* | Japan |
| *B. sinica* | China |
| *B. turdii* | Japan |
| *B. andersoni* | North America |

Gray et al., 2002

Burgdorfer reported that the survival mechanism of spirochetes is responsible for the diverse pathology of these organisms (1999), and the molecular basis of how *Borrelia burgdorferi* maintains itself in nature via a complex life cycle in ticks and mammals is poorly understood (Yang, Pal, Alani, Fikrig and Norgard, 2004). We read in February 2005 from the same lab in which Willy Burgdorfer identified *Borrelia burgdorferi* in 1981, that despite the fact that Lyme disease is the most commonly reported vector-borne disease in North America and Europe, we know little about which components of the causative agent, *B. burgdorferi* are critical for infection or virulence (Rosa, Tilly and Stewart, 2005). The authors also pointed out that certain features of the pathogen itself have hampered the development of an effective system of genetic analysis, which will be necessary to advance our understanding of the infectious cycle and pathogenesis of Lyme disease. Krinsky reports that "spirochetes by nature are insidious organisms, and the complexity of their relationship with different mammals and birds only complicates an understanding of their biology. Add to that, different person's immunological responses to spirochetes, and the confusion increases" (2009).

Soon after Burgdorfer's identification of spirochetes, comparative analyses against other known *Borreliae, Treponema* and *Leptospira* spirochetes were initiated (Johnson, 1984). Lyme disease spirochetes were found to most closely resemble *Borreliae* in their microaerophilic, catalase-negative characteristics, their utilization of carbohydrates, plasmid concentrations, and mole percentage guanine plus cytosine values (27.3 – 30.5%). Furthermore, this study showed that the three Lyme disease spirochetes identified constituted a single species, and clearly separated them from the other *Borreliae*, an important piece in LD pathogenesis.

*Borreliae* are thin, elongated, motile corkscrew or wave-like spirochetal bacteria (Gray et al., 2002). Six outer surface proteins (Osps) A through F have been characterized, which play an important role in the pathogenesis of the bacterium in human disease (Gray et al., 2002). Outer surface protein C (OspC) is a major surface lipoprotein of *B. burgdorferi* with critical importance in the invasion of tick salivary glands, and transmission of spirochetes from the arthropod vector to the mammalian host (Pal, Li, Wang,

Montgomery, Ramamoorthi, Desilva, Bao, Yang, Pypaert, Pradhan, Kantor, Telford, Anderson and Fikrig, 2004). A tick receptor (TROSPA) that is required for spirochetal colonization of *I. scapularis* and effective pathogen transmission to the mammalian host was recently identified in November 2004 (Pal et al., 2004). This receptor is essential for pathogen adherence to the vector, and was a crucial find. Further evidence of outer surface protein variations and resulting differences in disease presentation is found in the Malawista, Montgomery, Wang, Fu and Wiles report (2000) focusing on geographic clustering of variant surface protein.

Speciation of *Borrelia* is mainly based on molecular methods such as phylogenetic analysis of the 5S-23S rRNA intergenic spacer sequence, 16S rRNA gene sequence, whole genome DNA reassociation experiments, analysis of rRNA gene restriction patterns, protein electrophoresis patterns, and differences in reactivity to specific murine monoclonal antibodies (Gray et al., 2002). One of the most striking features of *B. burgdorferi* s.l. however is its unusual genome, which includes a linear chromosome of 910,725 base pairs and 853 genes, and at least 17 linear and circular plasmids (Fraser, 1997). Skotarczak (2009) reported *Borrelia* spirochetes have an incredible ability to adapt in different host environments due to these large numbers of plasmids. Successful adaptation to different hosts means greater risk to people in the future. Recent molecular and genetic studies have confirmed that *B. burgdorferi* is one of the most complex bacteria known (Stricker, 2006). Because of its importance as a pathogen of humans and animals, researchers undertook the important task of mapping the complete genome sequence to help understand its life cycle, and to assist in advancing drug and vaccine development to interrupt the pathogenicity of the bacterium. Barbour and Zuckert (1999) reported that we are not even close to understanding the pathogenesis of Lyme disease, given it is the first genome of any parasite that infects both invertebrates and vertebrates, and displays peculiar morphology, physiology and behaviour. Hyde, Trzeciakowski and Skare (2007) recently reported that while *Borrelia burgdorferi* adapts to the distinct environments of its arthropod vector and mammalian host during its complex life cycle, it alters gene expression and protein synthesis in response to temperature, pH, and other uncharacterized environmental factors. Host adaptation and gene regulation are crucial to

the pathogen's survival, which in turn play a role in disease transmission and identification.

The distribution pattern of Lyme disease appears as a multifocal worldwide epidemic, with endemic regions extending across the continents, however most species of the *B. burgdorferi* s.l. complex are limited in their range, leading to the question "from where and how have the *Borreliae* species migrated?" (Gray et al., 2002). Through several molecular methods, researchers explored the genetic variability, and performed a phylogenetic analysis comparing American to European *Borreliae* species. The results suggest the possibility that *Borrelia* evolved in North America, were introduced to Europe, and the two different populations evolved separately (Gray et al., 2002). During molecular analysis, researchers revealed a horizontal gene transfer that must have occurred across the kingdom a very long time ago, since the particular gene sequence is found across the entire genus (Gray et al., 2002).

The first evidence of large-scale genetic exchanges between Lyme disease spirochetes in nature, particularly through plasmids of the cp32 family was recently identified (Stevenson, 2003). Entire plasmid exchange between two different bacteria was observed, which leads to considerable diversity within the genus. Comparative genomics of related bacterial isolates is a powerful tool in understanding the Lyme pathogen, and observations of *B. burgdorferi* s.l. undergoing genome-wide genetic exchange, including plasmid transfers were observed by Qiu et al. (2004) through multilocus sequence typing. Qui and his colleagues concluded that frequent recombination implies a potential for rapid adaptive evolution and a possible polygenic basis of *B. burgdorferi* s.l. pathogenicity. Baranton and DeMartino (2009) further establish that recent studies on the genus indicate that the genetic variability of the plasmid genes is responsible in large part for the adaptability of the bacterium, and its resulting pathogenesis.

The genetic polymorphism of *B. burgdorferi* s.s. and *B. afzelii* was estimated by sequence typing of four loci from two tick sources in the U.S. and in Sweden (Bunikis, 2004). The genetic variants of *B. burgdorferi* and *B. afzelii* among the samples from the field sites

accounted for greater diversity than previously reported from larger areas of the U.S. and Europe. Cladograms were utilized to reveal at least three monophyletic lineages within *B. burgdorferi,* with the authors concluding that *B. burgdorferi* sensu stricto and *B. afzelii* have greater genetic diversity than had previously been estimated. As the pathogen implicated in human disease is seemingly a collective group of several representative genospecies within the genus *Borrelia,* speciation of the pathogen, and research focusing on nucleotide substitution rates and evolutionary rates of change should lead to a better understanding of disease transmission.

Anderson and Norris' 2006 study examined the genetic diversity of circulating *Borreliae* in the reservoir population from a large region of the western coastal plains of southern Maryland, where moderate numbers of human LD cases are reported. They found the spirochete diversity in Maryland was "not as high as that observed among northern tick populations". Given the northern populations are closer to the Canadian border, this might suggest significant spirochete diversity in Canada, and resulting complications in diagnosing disease.

The challenges of determining multiple and often correlated environmental effects on the rate of evolution are enormous (Pawar, 2005). Evolutionary studies on the genus are ongoing, with a recently developed and interesting hypothesis that has been partially substantiated, specifically that *Borreliae* species spirochetes have co-evolved together with their different arthropod vectors (Gray et al., 2002). Several phylogenetic analyses of *B. burgdorferi* s.l. and its different arthropod vectors have been documented to support this theory.

Comparisons of infection patterns between two American isolates of *B. burgdorferi sensu stricto* and three European isolates, two of species *B. garinii,* and one of species *B. afzelii* were correlated to the difference in human Lyme disease symptoms in the United States and Europe. *B. burgdorferi* s.s., *B. garinii* and *B. afzelii* all cause different clinical symptoms of Lyme disease, with comparative genomics playing an important role in elucidating the underlying differences in *Borreliae* species (Glockner, Lehmann,

Romualdi, Pradella, Schulte-Spechtel, Schilhabil, Wilske, Suhnel and Platzer, 2004). For example, *Borrelia garinii* is reported to be the most neurotropic of the genospecies of *B. burgdorferi s.l.* that cause Lyme disease in Europe, where it is transmitted to avian and mammalian reservoir hosts and to humans by *Ixodes ricinus* ticks (Smith, Muzafarr, Lavers, Lacombe, Cahill, Lubelczyk, Kinsler, Mathers and Rand, 2006). This particular species is linked to more neurological symptoms and clinical presentations than some of the other *Borreliae* species.

Studies on *B. burgdorferi* gene regulation have indicated that much gene activity is regulated at the level of transcription during *B. burgdorferi* passage from ticks to mammals, and further studies will lead to a better understanding of spirochete transmission dynamics (Piesman, 2003). It was recently identified that OspC is crucial for dissemination of *B. afzelii* from the tick midgut to the salivary glands (Fingerle, 2007), which helps to determine the potential pathogenesis of this species through its vector(s). Further, environmental conditions may also play a role in the complex appearance of the disease, as Nadelman (1998) observed that environmental triggers such as temperature and tick feeding influence gene expression in *B. burgdorferi* s.l., which may in turn relate to virulence properties. Another recent study suggests that dissolved oxygen modulates gene expression in *B. burgdorferi*, and may be an important environmental signal along with temperature and pH, as the spirochete cycles between the arthropod vector and mammalian host (Seshu, Boylan, Gherardini and Skare, 2004). Understanding transmission dynamics of the pathogen is crucial to developing a clear picture of Lyme disease risk.

Santino et al. (1997) described the geographical incidence of *Borrelia* infection in Europe collated from various seroepidemiological studies done in several European countries, with at-risk populations, blood donors, or control subjects. The highest incidence was found in Southern Europe, with 43% of Croatians seropositive to *Borrelia burgdorferi* antibodies, Central European countries such as the Netherlands and Switzerland reported lower incidences of 28% and 26% respectively, while in Northern Europe, Sweden was sampled at 19%, and Estonia 2.7% (Santino, Cammarata, Franco, Galdiero, Oliva, Sessa,

Cipriani, Tempera, DelPiano, 1997). The authors noted that these types of comparisons, and the drawing of acceptable conclusions remains difficult, however for our purposes, this is one example of the extensive research literature documenting the infectious success of *Borrelia burgdorferi* s.l.

Hanincova, Kurtenbach, uk-Wasser, Brei and Fish (2006) demonstrated that *B. burgdorferi* is a "generalist microparasite" and concluded "efficient cross-species transmission of *B. burgdorferi* is a key feature that has allowed the rapid spread of Lyme borreliosis across the northeastern United States. Change in this pathogenic group will require constant attention for its associated role in human disease." Species diversity has been studied extensively in different geographical regions. *B. burgdorferi* s.s. accounted for 76% of 46 genetically characterized *B. burgdorferi* s.l. infections from *I. Pacificus* nymphs in a 1997 – 2001 California study (Eisen, Eisen, Chang, Mun, and Lane, 2004), while *B. garinii* and *B. afzelii* are reportedly seen more frequently in Europe than in America (Hengge, 2003). 157 *Borrelia* isolated from *I. persulcatus* ticks and rodents in the far eastern part of Russia were determined as *B. garinii* and *B. afzelii,* which was a similar finding to surveys of *I. persulcatus* and wild rodents in Hokkaido, Japan (Masazuwa, Kurita and Yanagihara1997). Another Asian report indicates 55 adult *I. persulcatus* ticks were collected from the northwestern People's Republic of China in May 1999, with 40% testing positive for *B. burgdorferi s.l.* spirochetes, with *B. garinii* dominating, followed by *B. afzelii* (Takada, Masuzawa, Ishiguro, Fujita, Kudeken, Mitani, Fukunaga, Tsuchiya, Yano and Ma, 2001). A recent Japanese study revealed a similar species to *Borrelia tanukii*, and another novel *Borrelia* species closely related to *B. valaisiana* (Hiraoka, Shimata, Sakata, Watanabe, Itamoto, Okuda, Masazuwa and Inokuma, 2007).

In addition to the diversity of species associated with Lyme disease pathogenesis, sub-species spirochetal diversity has also been observed to play a large pathogenic role. A November 2004 report indicates that some *B. burgdorferi s.s.* strains, such as BL206, may be preferentially maintained in transmission cycles between ticks and white-footed mice, whereas other strains may be more effectively maintained in different tick-

vertebrate transmission cycles (Derdakova, Dudioak, Brei, Brownstein, Schwartz and Fish, 2004), indicating further enzootic complexity in pathogenesis. 53 southern U.S. *Borrelia burgdorferi* s.l. isolates were characterized with DNA analysis, yielding 29 as *B. andersoni* strains, 7 *B. bissettii* strains, and 7 types among *B. burgdorferi s.s.* strains (Lin, Oliver and Gao, 2003). Phylogenetic analysis was also utilized in this study, which allowed for the separation of a distinct pathogenic group, relapsing fever *Borreliae.*

In addition to a spirochetal species' infectious success being geographically linked, the success of the spirochete also depends on its ability to colonise host tissues and counteract the host's defense mechanisms (Singh and Girschick, 2004). Antigenic diversity and molecular mechanisms allow for such success, and further studies on spirochete host interaction in their habitats are needed to understand this complex interplay. With *Ixodes scapularis* as the principal vector in the northeastern United States, and *Ixodes pacificus* as the principal vector in California, studying the different and complex patterns of pathogen transmission in these two distinct regions might be useful in locating regions lacking a recognized vector.

Documentation of other *Borrelia* species not yet proven as Lyme disease agents is recognized. Garden dormice in Central Europe serve as the main reservoir hosts of a novel genospecies of *Borrelia, B. spielmani,* which causes Lyme disease in people (Richter, Schlee, Allgower, and Matuschcka, 2004). Another novel, fast-growing spirochete was isolated from the hard *Ixodid* tick *Hyalomma aegyptium* during the summer of 2000 from the Istanbul area in northwestern Turkey (Guner, Hashimoto, Kadosaka, Imai and Masuzawa, 2003). Upon further investigation, the findings suggested that the unique spirochete was a member of the genus *Borrelia*, and differs from previously described *Borreliae* species. A new genospecies of *B. burgdorferi* s.l. was also recently isolated from various mammals, and identified on the southernmost Japanese islands by RFLP analysis and clustering on a phylogenetic tree, and is most closely related to *B. valaisiana* (Masuzawa et al., 2004).

In 1999, the southeastern United States developed its own version of the tick-borne ailment Lyme disease, with a different tick species transmitting an organism that was different from other *Borrelia* species identified at the time (Baker, 1999). Dr. Felz reported on the species, indicating it was likely a genetically variant strain of *B. burgdorferi s.l.* Southern tick-associated rash illness (STARI) is the Lyme disease-like infection that was recently named in the southeastern and south-central United States (Varela, Luttrell, Howerth, Moore, Davidson, Stallknecht and Little, 2004) following the initial 1999 query. The first successful cultivation of the newly identified *Borrelia* species, *B. lonestari* from *Amblyomma americanum* (lone star) ticks was reported by Varela et al. in 2004, along with indication that future studies investigating the role of this species in human disease were still necessary. A 2007 Georgia study by Varela-Stokes suggested for the first time, successful transmission of *B. lonestari* from aggressive lone star ticks to white-tailed deer, and its successful entrance to an enzootic cycle of disease. *B. lonestari* has been primarily reported in southern states, but recently has been identified in northern ticks, and for the first time in ticks among birds (Jordan et al., 2009). Clearly this is a *Borrelia* species to keep our eye on.

Another *Borrelia* species recently implicated as a disease agent is *B. bissettii*, which has been highly associated with human disease, but has yet to be confirmed in culture. Burkot, Maupin, Schneider, Denatale, Happ, Rutherford and Zeidner (2001) suggested that *I. spinipalpis* has potential to transmit *B. bissettii* to humans, particularly in regions of the western United States where *I. pacificus* has not been found, following a 2001 study of wood rats and deer mice in Colorado. This is clinically significant for people suffering with Lyme disease symptoms who reside in regions qualified as non-endemic due to the lack of a known vector. Across our province and nation, most of our regional geography is defined as non-endemic. It is critical that we are aware of studies like this Burkot et al. study and others, and pay attention to their value in teaching us to look for the opportunities this amazing bacterium might find locally.

Lyme Disease Vectors

The scientific literature tells us the haematophagous arthropods are the key link between the spirochete and human, and are typically responsible for vectoring Lyme disease. The only competent vectors of Lyme disease recognized to date are several species of ticks, and it is important to identify features that make them successful vectors of this disease. Since ticks are obligate haematophagous (blood-sucking) arthropods parasitizing every class of vertebrates in almost every region of the world, their vector potential is enormous (Gray et al., 2002). They must find a blood meal from a vertebrate between all life stages: larva → nymph → adult → eggs. Vanderhoof-Forschner (1997) reported that ticks carry more kinds of microorganisms than any other arthropod, including mosquitoes, while Parola and Raoult (2001) reported ticks to be second only to mosquitoes as vectors of human infectious diseases. An infected tick could transmit the agent of the infection to the vertebrate from which it feeds – whether human or animal (Edlow, 2003). Members of Arthropoda of known concern belong to the family Ixodidae, and Genus *Ixodes, Dermacentor,* or *Amblyomma,* and the most important species belong to the *I. ricinus complex: I. ricinus, I. persulcatus*, *I. scapularis,* and *I. pacificus* (Gray et al., 2002). The diverse and highly successful Acarid Arthropods have been found in wide ranging locations that include hot springs, caves, harsh deserts and tundras throughout the world in almost every conceivable habitat (Encyclopedia Britannica, 1999). Acari specifically surpass all other arthropods, with the exception of mosquitoes, in the number of diseases they transmit to humans as external blood-feeding parasites (Gray et al., 2002). Ticks are well documented in their capabilities as important vectors of *Borrelia burgdorferi* s.l. The classification of these ticks is as follows (Edlow, 2003):

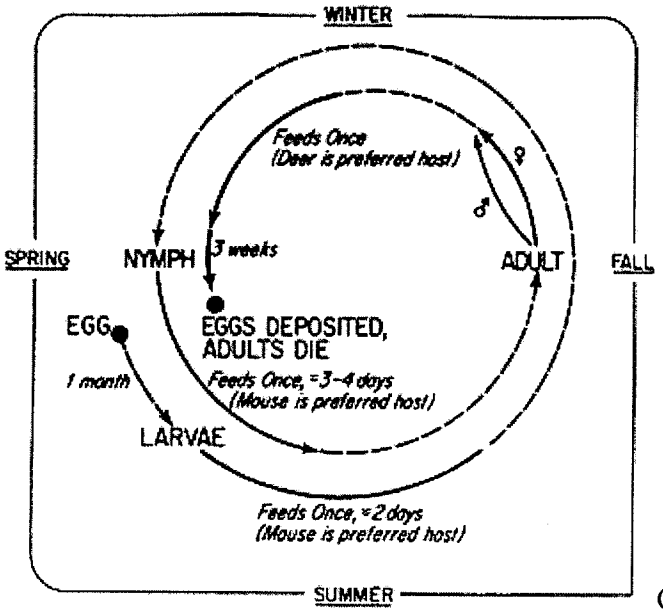
Table 3. Tick Classification

| | |
|---|---|
| Kingdom | Animalia |
| Phylum | Arthropoda |
| Class | Arachnida |
| Subclass | Acari |
| Order | Parasitiformes |
| Suborder | Ixodida |
| Family | Ixodidae ("hard" ticks) |
| Genus | *Ixodes, Amblyomma, Dermacentor* |
| Important Species | *I. scapularis, I. pacificus, I. ricinus, I. persulcatus* |

Tick vectors of LD feed on a wide variety of mammals, birds and reptiles during their life cycle stages, and transmit the spirochetal pathogen during feeding, and also transstadially through the tick's developmental stages (Mawby and Lovett, 1998). An infected tick might transmit the agent of the infection to the vertebrate from which it feeds – whether human or animal (Edlow, 2003), during any stage of its life cycle. Hard ticks, such as *I. scapularis* (deer tick) and *D. variabilis* (the common wood tick / American dog tick), attach to their hosts and feed continuously for several days. When engorged, the female drops from the host, finds a suitable site to rest, lays her eggs in a mass of several thousand eggs in the spring, and dies (Lindsay, 2004). Six-legged larvae hatch in late summer/fall, climb up a blade of grass, and drop onto and attach to a mammal host, which has released the odour of butyric acid stimulating the larvae to do so (E.B., 1999). Following a blood meal in which pathogens might be ingested, the larvae detach and moult, becoming eight-legged nymphs also in search of a host. Following the nymph's blood meal, again usually from a mammal for 3 to 5 days, they fall off and moult into adult males or females (Lindsay, 2004). Adults may wait for hosts for as long as three years (E.B., 1999). The success of ticks relies on the density of all biological events. Out of approximately 2000 eggs laid per adult female, only several survive, given a 99% mortality rate (Lindsay, 2004). Tick hosts include all vertebrates that ticks feed on in nature (Gray et al., 2002). "Reservoir hosts" are proven natural hosts of vector ticks, where ticks might become infected while feeding on them. Scientists indicate that the

two key factors for the ecological success of ticks are their microclimate, and their hosts (Lindsay, 2004). Furthermore, density dependent issues and probabilities seem to play a large role in keeping the *Ixodes* tick populations down, making Canadian tick populations look like "thousands of dots on a map", with few successfully breeding populations (Lindsay, 2004). The pathogenic success of ticks relies on the density of all organisms, and biological events.

Figure 3. Ixodid Life Cycle




Gray et al., 2002

The *Ixodidae* or hard ticks with three life stages: larva, nymph and adult, display qualities which enhance their ability for successful feeding, allowing great success of pathogen transmission. The *Argasidae* or soft ticks are less recognized for their vectoring capabilities, however are nevertheless important as vectors of disease in some regions. Non-vector ticks are unable to transmit spirochetes to the host, while vector ticks are successful in passing on the pathogen (Gray et al., 2002). Bridging vectors transmit the infection to humans without necessarily maintaining the agent in nature (Gray et al., 2002).

The first direct spirochete-vectoring test was carried out on rabbits (Habicht, Beck and Banacht, 1987), in a traditional epidemiological study. Spirochete-infected *Ixodes scapularis* ticks were placed on the shaved skin of albino rabbits, where they could be observed feeding on the blood of their hosts. After several weeks, characteristic Lyme lesions appeared, and the presence of live spirochetes on the skin was observed via microscopy. Classic epidemiological studies identified that certain ticks were indeed competent vectors of the Lyme disease pathogen, very soon after the initial identification of the Lyme bacterium in 1981. What was not known however, was that numerous other potential vectors in addition to *I. scapularis* would be identified in the years of research ahead.

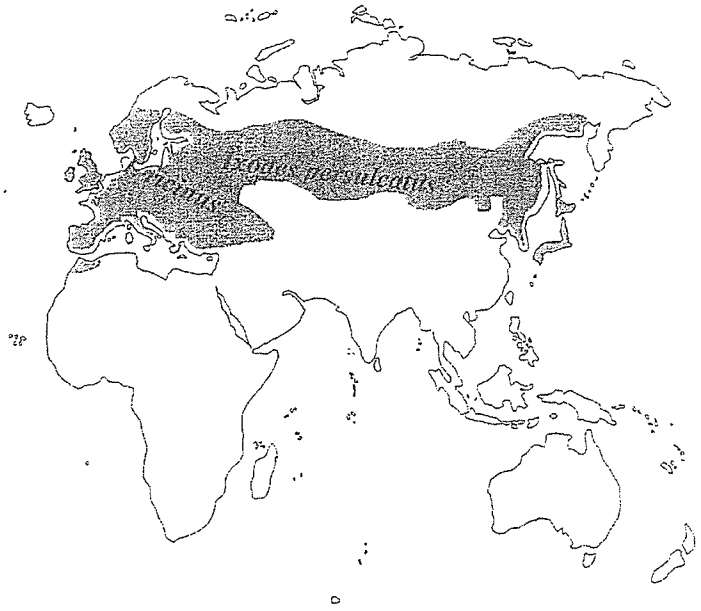
Given that each tick species has an environmental preference, their geographical distribution, and associated risk of disease varies around the globe. Woods and forests are described as preferred tick habitat, with ground vegetation, grassy areas, meadows, weeds, leaf litter and caves also providing homes for ticks. Dense transition areas between manicured lawns and forests are conducive to large populations of ticks. Most ticks prefer to feed off of non-human mammals such as deer, mice, chipmunks, rabbits and birds, which can maintain infection for life. When ticks feed, the mouthparts, consisting of a hypostome and the chelicerae release anticoagulants and antiinflammatories from the salivary glands into the host, and may transmit a pathogen at this point (Vanderhoof-Forschner, 1997). Further discussion of the salivary glands in several contexts will provide a basis for evaluating the competence, and incompetence of certain vectors.

Parola and Raoult (2001) explored the epidemiology of tickborne diseases, and the three general pathways of tick infection: by feeding on bacterimic animals, or by transstadial, or transovarial transmission. The authors pointed out that tick infection may also occur through cofeeding – where an infected tick feeding on an animal infects another tick which is feeding close by. Each stage of Ixodid tick feeds only once, and bacteria ingested by a tick during feeding may be transmitted to another host only if it is capable of passing the infection to the next life stage transstadially, through moulting.

Vanderhoof-Forschner (1997) points out that not every microorganism acquired by a tick during feeding can be deposited into fluids of another host. Not all species of Ixodids are capable of transmitting bacteria transstadially, and therefore are not considered to be competent vectors. She points out that dog ticks can become infected with the Lyme bacterium, but cannot maintain the infections as they moult, thereby eliminating the ability to transmit it to others, and eliminating the ticks' ability to act as a "competent vector". Transovarial transmission in the tick may occur, however the bacteria must then migrate to the salivary glands in order for the vector to be considered "competent". Parola and Raoult (2001) point out that transovarial transmission is rare, and *B. burgdorferi* is more successfully transmitted through the salivary secretions of feeding ticks, and also through regurgitation of midgut contents of various species of vectoring ticks. Concern for disease transmission via transovarially-infected ticks, despite qualification as minimal, should still be considered in assessing Lyme disease risk.

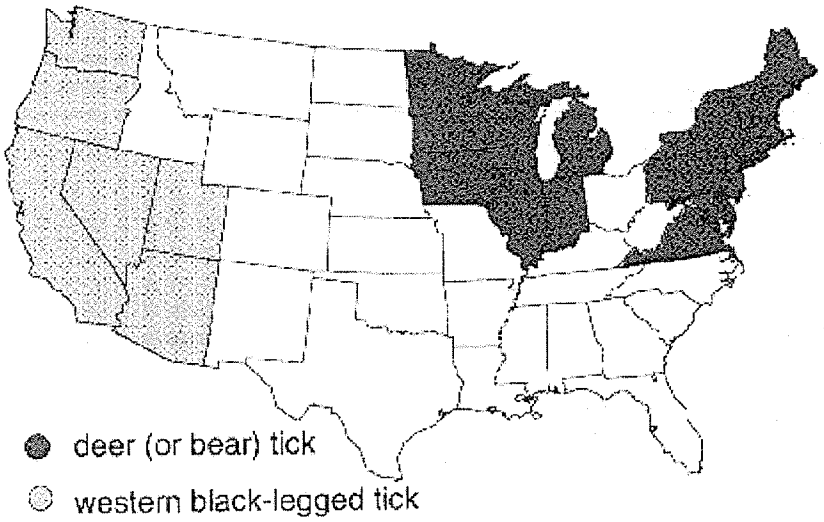
Gray et al. (2002) cite the four *I. ricinus complex* ticks epidemiologically most important in transmitting *Borrelia burgdorferi* sensu lato to humans as *Ixodes scapularis* (eastern North America), *Ixodes pacificus* (western North America), *Ixodes persulcatus* (northern mid-Asia), and *Ixodes ricinus* (Europe and some adjacent areas). They identify the different vector species, occurring in regions with strongly varying macroclimatic conditions and differing host spectra as one of the two ecological puzzles Lyme disease poses. In addition to the four primary bridging vectors of Lyme disease, which transmit the *Borrelia burgdorferi* sensu lato spirochetes to humans, the authors report a literature review revealing that natural infections with *B. burgdorferi* s.l. have been recorded from at least 25 species of *Ixodes* ticks, and some 15 other ticks spanning eight genera: *Amblyomma, Boophilus, Dermacentor, Haemaphysalis, Hyalomma, Rhipicephalus, Argas and Ornithodoros.*

Figure 4. Distribution of Ixodes ticks in Europe and Asia




Coyle, 1999

Figure 5. U.S. Range of *I. scapularis* and *I. pacificus*

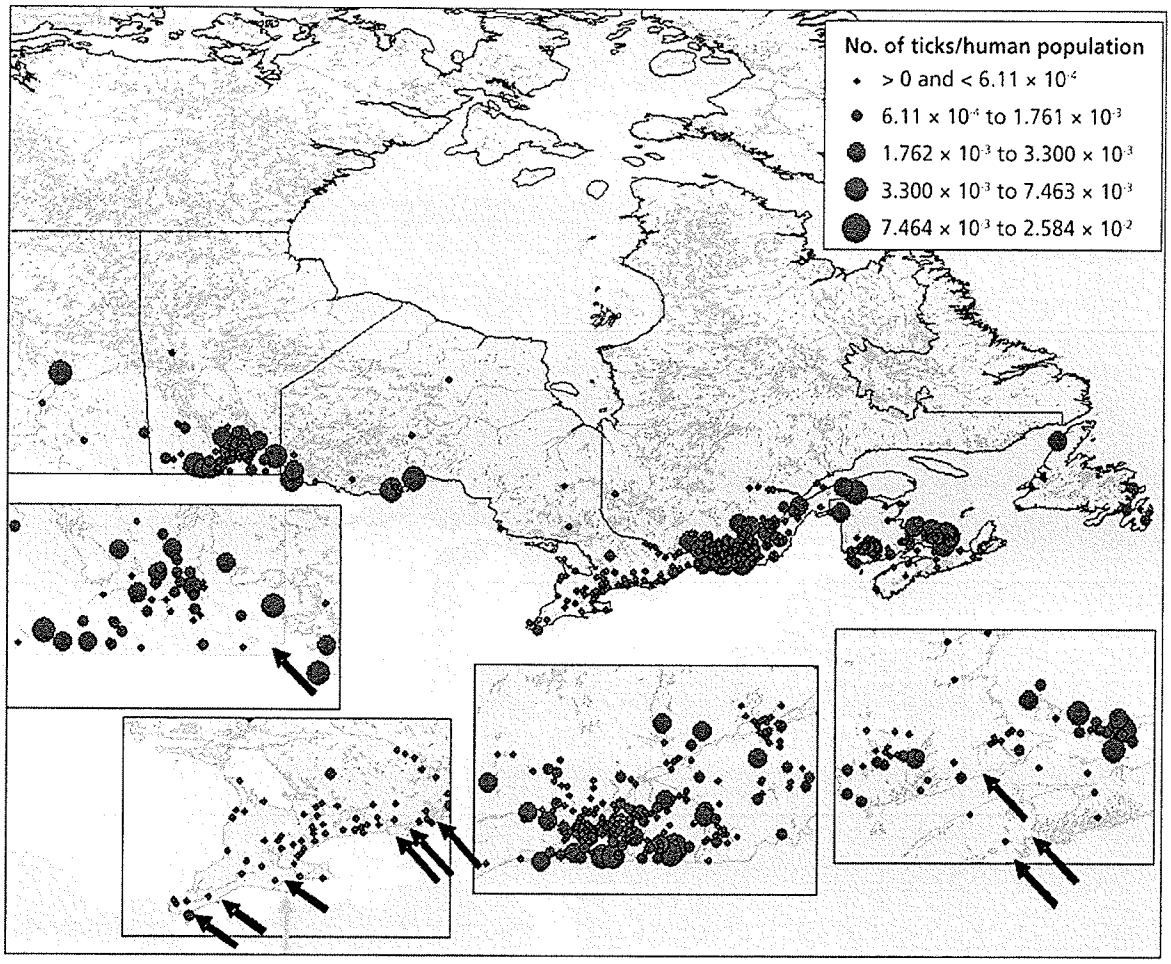



Edlow, 2003

In Canada, the ticks associated with Lyme disease have been found in every province, and were reported to be endemic at Long Point and Point Pelee on Lake Erie; and in the Fraser River delta, the Gulf Islands and Vancouver Island (Regush, 2000). "Lyme has been detected in ticks all over the province of Ontario" (CityNews, 2007). The Toronto

*Star* reported that "the deer tick is found throughout Ontario, mainly on the north shore of Lake Erie, particularly at Long Point, Turkey Point and Rondeau Provincial Park, as well as in northwest Ontario near the Manitoba border, and in St. Lawrence National Park near Kingston (Talaga, 2007). The areas of Lunenburg and Bedford, Nova Scotia have also been identified as an area of *Ixodes* tick endemicity (Artsob, 2004; CPHLN, 2007), and recently Midgeville, New Brunswick and Buffalo Point in southeastern Manitoba have been added (Artsob et al., 2009).

Figure 6. The distribution of *Ixodes scapularis* reflecting information submitted to provincial and federal public health agencies from January 1990 to December 2003 and to the Lyme Disease Association of Ontario for 1993 to 1999




Ogden et al., 2009
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The identification of this important vector in Canada, and the mapping of its distribution are critical to establishing recognized endemic locations, but the geographical distribution of disease will be discussed in chapter three.

The several tick species identified as competent vectors of *B. burgdorferi* acquire the bacterium while feeding on infected reservoir hosts, which sustain the pathogen in their bloodstream and tissues. The spirochetes are maintained in the tick midgut while they moult, disseminating throughout the tick into the salivary glands where they can be passed on to the next host. The spirochete load of bacteria differs from one tick species to another, and plays a significant role in vector competence. Infection rates in ticks are correlated with disease frequency in humans (Walk et al., 2009). Wang, Liveris, Brei, Wu, Falco, Fish and Schwartz (2003) estimated that a minimum of 300 pathogenic organisms (spirochetes) may be required in a host-seeking nymphal tick to be able to transmit infection. These quantitative data can be used for ecological and epidemiological surveillance.

The fact that certain ticks may be able to transmit the disease more readily than others complicates research, and risk assessment. Ticks were recognized as vectors of human disease in the early twentieth century, and an association between tick feeding time and risk of infection was seen soon after, however this varies between species. For example, soft *Ornithodoros* ticks can transmit the bacterium in 15 minutes, while others need to feed for 1 – 2 days to transmit infection. Most hard ticks, depending on the life stage, will transmit infection in 24 – 36 hours (Gray et al., 2002). The author notes that one of the least studied, yet the most intriguing feature of vector ticks is that some are systemically infected with pathogens, while others have local infections, which is an extremely important distinction for transmission success. Infection in the salivary glands, midgut or both, may dictate the time needed to transfer infection to a host, with systemic tick infections correlated to reduced transmission times. Clearly the significance of this minimally studied feature in risk assessment should not be overlooked.

Gray et al. (2002) describe the three main detection methods for *B. burgdorferi* in ticks: dark-field microscopy (DFM), phase-contrast microscopy (PCM), and culture. Polymerase chain reaction (PCR) and immunofluorescence assay (IFA) are additional identification tools. Each of these tools is widely used in vector research, and sensitivity and reliability issues have been minimized from early disease research in the 1980s according to Canadian scientists (Artsob, 2004). Once ticks are identified as carriers of the pathogen, they can be analyzed for their ability to vector, or transmit the pathogen to a host, and this information is then used in risk assessment. Canadian studies however have been solely focused on known *Ixodes* vectoring species.

Vector capacity describes the absolute contribution made by a vector species to the natural prevalence of infection of vertebrates in a given area. Vector potential is described as a subjective term, involving all vector-related variables affecting the stability of pathogen transmission (Gray et al., 2002). Eisen and Lane indicate that vector potential (from factors that increase or decrease vector efficiency) is taken into consideration in assessing the importance of tick species as bridging vectors to humans. Gray et al. (2002) presented a powerful collection of data and information on vector competence. To be considered as a competent vector of Lyme disease, a tick species must feed on infectious vertebrates, be able to acquire the pathogen during the blood meal, maintain it through one or more life stages (transstadial passage), and pass it on to other hosts during the next feeding. Again, if it cannot, it is considered to be a non-vector tick. Vector competence is reportedly confirmed in the lab for 12 tick species: *Ixodes affinis, I. jellisoni, I. pacificus, I. persulcatus, I. ricinus, I. scapularis, I. angustus, I. dentatus, I. hexagonus, I. minor, I. muris,* and *I. spinipalpis.* It should be noted that several genospecies of *B. burgdorferi s.l.* appear to have a low vector specificity, while certain genospecies may be found infecting only one representative tick species. Detection of spirochetes in unfed nymphal or adult ticks clearly indicates transstadial passage, and these are the vector ticks that maintain the pathogen's natural cycle. Surviving the tick moult seems to be a critical physiological challenge for the *B. burgdorferi* pathogen within ticks, and is used extensively as a marker for vector competence (Gray et al., 2002). Alternately, the presence of *Borrelia* in fully fed ticks

does not indicate vectoring ability, since all ticks feeding off infected animals ingest spirochetes in their blood meal. No consideration is given in the literature to the ability of a partially fed tick disrupted from its meal, to pass spirochetes to a second host, since most feedings are uninterrupted. However, the complete realm of situations in nature should be considered to obtain an accurate picture of disease risk. Also, male ticks have been cited to infect female ticks on occasion while mating, and females might transovarially infect their offspring via this route, however Gray et al. (2002) indicated this plays a minimal role in pathogenesis. Again however, minor potential pathways of disease should be weighted accordingly, and incorporated into the risk assessment process.

It is important to note that none of the static tests capable of identifying carriers of *Borrelia burgdorferi* is able to predict with certainty whether a given tick can act as a vector of disease, and this can only be determined by carrying out additional transmission experiments with a well-defined pathogen (Gray et al., 2002). Because of the enlarging spectrum of *Borrelia* genospecies implicated in Lyme disease, it is extremely difficult to draw the right conclusions from transmission experiments with a negative result. Furthermore, experimental models of vectoring a single isolate of the pathogen may not always be representative of the specific tick infectivity for the whole genospecies. The *Borrelia burgdorferi* sensu lato complex responsible for human Lyme disease consists of at least 14 genospecies worldwide, and Gray et al. (2002) emphasize that finding out if a particular tick species in a laboratory does or does not transmit a given genospecies, is not indicative of its abilities under natural conditions. Experiments by Rathinavelu and de Silva (2001) demonstrate that tick-transmitted *Borreliae* display a gene expression and antigen profile that differs from spirochetes cultured in vitro, indicating a necessity to explore variability in laboratory versus natural vectoring scenarios.

Certain laboratory "experimental vectors" have been shown to be capable of acquiring a pathogen while feeding, maintaining it until the following blood meal, and transmitting the infection to a host (Gray et al., 2002). It seems reasonable to assume that these experimental vectors might acquire *Borrelia burgdorferi* in the field through contact with

reservoir hosts, and they should be considered to be vector ticks. It is stressed that vector competence or "importance" is not merely synonymous with experimentally proven tick feeding transmission, and that the situations in natural habitats must be taken into account. The choice of tick hosts in the laboratory does not reflect the situation in nature for example (e.g. hamster vs. white-footed mouse). They also suggested that vectors be classified as primary and secondary to account for their apparent importance in pathogenesis, rather than simply being acknowledged, or dismissed as vectors of disease.

Tick preference for human attachment seems to favour the head and neck region, or the groin, with different tick species showing different preferences. *Dermacentor variabilis* favours the head and neck (59%), *Amblyomma americanum* favours the groin area (54%), while *I. scapularis,* the principal Lyme disease vector in North America attaches at a wide variety of sites. Possible reasons for these preferences have yet to be explored, however the behaviours of questing ticks provide clues for these preferences (Parola and Raoult, 2001). Furthermore, these preferences may play a role in vector potential with human hosts. Seasonality of questing activity, questing behaviour, host range and time elapsed from tick attachment to spirochete transmission may influence vectoring capability. Meiners, Hammer, Gobel and Kahl, (2006) reported the risk of *Borrelia burgdorferi* sensu lato transmission from an infected vector tick to a host increases with increasing duration of tick feeding. It has been recently identified that this delay in transmission may depend on the specific Borrelia species infecting the tick, where *B. afzelii* can be transmitted during the first 24 hours of tick attachment, *B. burgdorferi* requires 48 hours, and nothing is known about the other Borrelia species except that success of transmission always increases with tick attachment duration (Gern, 2009). It follows that if *B. afzelii* is present in the tick population, risk of disease is higher with lower attachment times. In *Ixodes ricinus* nymphs, the main vector of *B. burgdorferi s.l.* in most parts of Europe, the transmission risk appears low to moderate within the first 24 hours of feeding but increases to >70% after only 36 hours. In their study, they used the "so-called scutal index, the ratio between tick abdominal length and scutum width, a very good measure of the level of tick engorgement", for its potential to assess the feeding duration of detached *I. ricinus* nymphs, thereby indicating the level of human infection

risk with *B. burgdorferi s.l.* In an unrelated study assessing human risk, Alekseev, Jensen, Dubinina, Smirnova, Makrouchina and Zharkov (2000) compared questing behaviour in *I. ricinus* complex ticks using methods exploring the effects of temperature and humidity, and also the differences in locomotor activity. Mixson, Campbell, Gill, Ginsberg, Reichard, Schulze and Dasch (2006) reported that *Amblyomma americanum* is an "aggressive tick that feeds on humans during all postembryonic life stages, and in many regions of the United States, it is the tick most commonly found attached to humans". They indicated that public health interest regarding this tick has grown recently, due to the recognition of new human pathogens transmitted by *A. americanum* such as *B. lonestari,* and the tick's expanding distribution.

Humans are not part of the LD natural maintenance disease cycle. Incidental human exposure to the pathogen occurs when enzootic tick vectors bridge the gap from a maintenance cycle of spirochete-vector-host in nature (Gray et al., 2002). The primary bridging vectors of Lyme disease spirochetes to humans are the four hard tick species in the *Ixodes ricinus* complex mentioned earlier: the castor bean tick, *Ixodes ricinus* and the taiga tick, *Ixodes persulcatus* in Eurasia, and the blacklegged tick, *Ixodes scapularis* along with the western blacklegged tick, *Ixodes pacificus* in North America (Gray et al., 2002; Wang et al., 2003). Identification of the close correlation among the distribution of spirochete-infected *I. dammini* (*I. scapularis*) ticks, and human cases of Lyme disease was made in the early 1980s (Magnarelli, Anderson and Chappell, 1984), prompting much attention to these ticks. Blacklegged ticks (commonly called deer ticks) exist in the literature under two different names: *I. scapularis* and *I. dammini.* Identification and classification of this LD tick vector in the early 1980s led to the incorrect assumption that these were two different species, with molecular analysis proving they are a single species (Artsob, 2004). Today, the accepted species name was the first assigned, *I. scapularis.* The biodiversity of this tick, and other vectors is critical to the pathogenesis and management of the disease, and is overwhelmingly more diverse than initially suspected.

The potential of tick species, or their different life stages to act as enzootic vectors or bridging vectors to humans is influenced by numerous factors both intrinsic to the tick such as host preference, and extrinsic such as host abundance (Gray et al., 2002). A 1996 – 2001 Russian monitoring study showed correlation of human tick-borne Lyme borreliosis with high abundance of *I. ricinus,* and high infection rate with *B. burgdorferi s.l.* Tick populations of 19.5 – 38.4 per 1 km of route, and infection rates of 18.0 +/- 1.7 to 22.5 +/- 1.5% were associated with high risk of disease (Kislenko and Korotkov, 2002). DeMeeus, Lorimier, and Renaud (2004) showed that male and female ticks are not equivalently infected by *Borrelia*, and that Lyme is consequently vectored in a much more complex way than usually thought, changing the epidemiological perception, and suggesting new co-evolutionary pathways between the ticks and the *Borreliae*. Are documented infection rates used in risk assessment accurate, given gender specificity of the ticks was not incorporated? The life cycle of the vector, the number of hosts involved, and other unknown variables will require the development of complex models, which consider different routes of pathogen transmission (Rosa, Pugliese, Norman and Hudson, 2003). This again prompts the question of whether the *Borreliae* pathogen complex has been successful in parasitizing more vectors and hosts than have been recognized to date, and if projected risk is accurate.

Willy Burgdorfer indicated back in 1989 that the persistence of the Lyme disease spirochete in the midgut of its tick vectors, and its invasion of other tissues during the ticks' feeding, are unique and differ from the behaviour of all other arthropod-borne *Borreliae* (Burgdorfer, 1989). This phenomenon raises the question as to whether this disease should be investigated as it has been the past two decades, using similar methodologies used to explore other tick-borne diseases. Future directions in vector studies must take the unique behaviour of *Borreliae* species into account. Research to date has been vast in the study of the most prominent vectors, is expanding into closely related potential vector species, and is yet limited in scoping out distantly related species as potential vectors. This alone lends curiosity to potential vectors in Manitoba and Canada that simply have not been studied for their potential role in disease transmission.

In examining the realm of ticks alone, natural infections with *B. burgdorferi s.l.* have been identified in at least 25 species of *Ixodid* ticks spanning several genera: *Ixodes, Amblyomma, Dermacentor, Boophilus, Haemaphysalis, Hyalomma* and *Rhipicephalus*, and two *Argasis* ticks from two genera: *Argas* and *Ornithodoros* (Gray et al., 2002). Vector competence for *B. burgdorferi s.l.* has been experimentally confirmed for 12 tick species, including six of the 14 members of the *I. ricinus* complex: *I. affinis, I. jellisoni, I. pacificus, I. ricinus, I. persulcatus,* and *I. scapularis*, and seven other *Ixodid* species*: I. angustus, I. dentatus, I. hexagonus, I. minor, I. muris, I. spinipalpis* (Gray et al., 2002) and *I. uriae,* the most recently acknowledged (McCoy, Boulinier, Tourard and Michilakis, 2003). Could human exposure to any one of these ticks in nature, if carrying a *Borrelia* infection, potentially lead to human disease despite recognition and acceptance of only four of these tick species as competent vectors?

The *I. ricinus* species complex group of ticks spans almost all geographic regions of the world, and it had been hypothesized that the acknowledged Lyme vector ticks to date are closely related, representing a monophyletic group (Xu, Fang, Keirans And Durden, 2003). Xu tested this hypothesis using a molecular phylogenetic approach, drawing conclusions that the *I. ricinus* complex is not a monophyletic group, and the known major vectors of Lyme disease in different areas of the world are not sister taxa, suggesting that acquisition of the ability to transmit *Borrelia* in species of *Ixodes* might have multiple origins. The literature indicates this idea has not been applied to investigate potentially emerging Canadian vector populations for *Borreliae* species. The resulting picture might be important: If *Ixodes* tick species have independently evolved to vector *Borrelia* elsewhere, should we not suspect that tick species in Canada have, or will evolve to vector *Borrelia* efficiently?

Rosenthal and Spielman (2004) sampled alleles from ticks in the geographic extremes of their ranges to determine whether genes flow freely between populations of the *Ixodes ricinus*-like ticks of eastern North America, and to determine whether the abundant northerly populations of these vectors of Lyme disease may have arisen from a small cohort of ancestral founders. Patterns of diversity present in the nuclear satellite marker

alleles indeed show that ticks of the Northeast and upper Midwest are genetically isolated from those in the Southeast, and originated recently in a common founder population (Foley, Foley, Brown, Lane, Dumlers and Madigan, 2004). Clearly, in order to effectively evaluate the potential of such diverse vectors, a broad range of studies to incorporate differences amongst the potential vectors is a necessity.

Both *I. scapularis (I. dammini),* and *I. pacificus* ticks were established as competent LD vectors of the Lyme disease bacterium by 1984 in the United States. Today in the northeastern United States, about 25 percent of blacklegged nymphs (*I. scapularis)* and 50 percent of adults carry *B. burgdorferi* s.l. says David Weld, executive director of the American Lyme Disease Foundation (ALDF) (Pleasant, 2004). Keirans, Hutcheson, Durden and Klompen (1996) described the distribution of *I. scapularis* to be expanding, and included the state of Florida in the southeastern United States north to the provinces of Nova Scotia and Prince Edward Island, Canada, west to North and South Dakota, and south to the state of Coahuila, Mexico. In the upper Midwest, the range is from 25 to 50 percent, while on the west coast infection rates are lower, with 15 percent of nymphal and about 1 to 4 percent of adult ticks carrying *Borrelia* (Pleasant, 2004). *I. scapularis* ticks have been largely documented to feed on white-footed mice while immature, and on white-tailed deer as adults in eastern North America (Artsob, 2001), occasionally finding a blood meal from a human. *I. pacificus* shares a similar disease cycle in western North America. Vector competency of *I. pacificus* was assessed by Eisen, Dolan, Piesman and Lane (2003), and reluctance of this tick species to feed on certain rodents may limit its importance as an enzootic vector of *B. burgdorferi* s.l. spirochetes, in comparison to the success of *I. scapularis.* Risk classification in the U.S. ranges from no risk, through low, medium and high-risk geographical locales, and is based on the presence of *I. scapularis*, and *I. pacificus* ticks, and the density of host seeking nymphal ticks in particular (ALDF, 2007).

*I. scapularis* and *I. pacificus* were collected from 17 sites in 12 states, and fed on experimental rabbits. All exposed rabbits became infected with *B. burgdorferi* (Piesman, Clark, Dolan, Happ and Burkot, 1999), confirming the competence of these ticks in

vectoring the disease. Further results of this study showed that 165/226 (73%) of the northeastern ticks were infected, whereas 29/51 (57%) of the Midwestern ticks were infected. It seems that wherever these ticks are found, it is highly probable that they are carrying a *Borrelia* infection. Using Russia's classification where high risk is quantified at 18 – 22.5% (Kislenko and Korotkov, 2002), anywhere the ticks are found in the northwest and Midwest would not only be an area of risk, but an area of extremely high risk. In Manitoba, the Buffalo Point area in the southeastern corner would also be tagged as a high-risk area on this Russian scale.

An examination of the literature to date on LD vectors indicates that much research has been done to substantiate the early claim that *Ixodes ricinus* complex ticks are largely responsible for vectoring the disease. A 12-year passive survey in Michigan yielded 4755 ticks of 21 species, 16 of which were probably indigenous in the state (Walker, 1998). *I. scapularis* was one of 12 species of *Ixodes,* along with *I. cookei* being the most numerous of the 12. Eleven of the 175 *I. scapularis* ticks were infected with *B. burgdorferi.* This data surfaced from one of many American studies, and powerful data has emerged from much of the research. In a 1999 study, 165/226 (73%) of *I. scapularis* and *I. pacificus* ticks collected from Connecticut, New York, New Jersey and Maryland were infected, and 29/52 (57%) of ticks collected from Michigan, Wisconsin and Minnesota were infected with *B. burgdorferi s.s.* (Piesman et al., 1999). Recognizing that Minnesota shares a significant geographical border with Manitoba, and 57% of the ticks in this study were infected, it seems more than reasonable to question risk in southern Manitoba. A 1998 to 1999 study in Minnesota revealed that 30 counties in the east-central, central and northern areas of the state reported established populations of *I. scapularis* (Sanders and Guilfoile, 2000). A recent sample from Block Island, Rhode Island collected for the purpose of researching hypersensitivity to ticks and Lyme disease risk showed *B. burgdorferi s.s.* infections in 23% of 135 nymphal *I. scapularis* ticks (Burke, Wikel, Spielman, Telford, McKay and Krause, 2005).

In California, *Ixodes pacificus* nymphal ticks have been implicated as the primary bridging vectors of *B. burgdorferi s.s.* to humans (Eisen, Eisen and Lane, 2005). Eisen et

al. reported that woodlands with a ground cover dominated by leaf litter have emerged as a primary risk habitat for exposure to these infected nymphs, and remote sensing has been successful as a predictive tool in the far-western United States with this recent find. Interestingly, on the east coast, in a New York patch study, plots of less than 2 hectares had on average 3 times as many ticks, and 7 times as many infected ticks per square meter as larger patches. In one 1-hectare plot, a staggering 80% of ticks were infected (Eisen et al., 2005). Clearly, *B. burgdorferi s.s.* is firmly established in the vectoring populations in the United States, and correlated landscape issues play a significant role.

Canadian studies on LD vectors have been less intense, however some important studies deserve discussion. *B. burgdorferi* has been isolated in both the blacklegged tick (*I. scapularis)* and western blacklegged tick *(I. pacificus)* in Canada (Lindsay, 2004). *I. scapularis* and *I. pacificus* ticks have been identified in about 250 locations in Canada, although established populations are deemed "focal" and were limited to Ontario and British Columbia in 1999 (dos Santos and Kain, 1999), and have been identified since in other locations. In 2007, Health Canada reported populations of infected ticks established in parts of southern Ontario, the southeastern corner of Manitoba, and in areas along the south shore of Nova Scotia and in BC. The first Canadian record of *I. scapularis* was a specimen removed from a human in May 1904 at Bracebridge, Ontario (Scott, Fernando, Banerjee, Durden, Byrne, Banerjee, Mann and Morshed, 2001), and the first documentation of *B. burgdorferi* found in a blacklegged tick was in 1987 at Long Point, Ontario. The authors also reported that in the early 1990s infected ticks were also found in Nova Scotia and Kenora, and were likely introduced by songbirds from the south. Lindsay et al. (1991) reported that 58.3% of adult, 17.3% of nymphal, and 0.15% of larval *I. scapularis* questing ticks collected by dragging on Long Point, Ontario during the summer of 1990 were confirmed to be infected with *B. burgdorferi* by indirect immunofluorescence assay (IFA). Since then, *I. scapularis* had been detected by passive surveillance in nine Canadian provinces and are likely introduced into Canada by migrating birds (Artsob, 2003). *I. scapularis* ticks are dropped haphazardly by birds during spring migration (Banerjee, Banerjee, Fernando, Scott, Mann and Morshed, 2000), and are widespread in southern Ontario. Ogden, et al. (2006) indicated that passive

surveillance for *Borrelia* infected *Ixodes scapularis* ticks had been ongoing in Canada since 1990, through tick submissions from the public, veterinarians and medical practitioners. The data from 2,319 mostly adult *I. scapularis* were collated, and *B. burgdorferi* was detected in 12.5% of 1,816 ticks, including 10.1% of the 256 ticks collected from humans. The authors reported their study suggests that the geographic range of *B. burgdorferi*-infected *I. scapularis* in Canada is wide, but point out "most may be adventitious ticks carried from endemic areas in the United States and Canada by migrating birds". Their conclusions indicated the risk of Lyme borreliosis in Canada is mostly low, but more geographically widespread than previously suspected (Ogden et al., 2006). Health Canada's CDC reported a decade ago that the blacklegged tick had been found in every province from Manitoba to Newfoundland (1999).

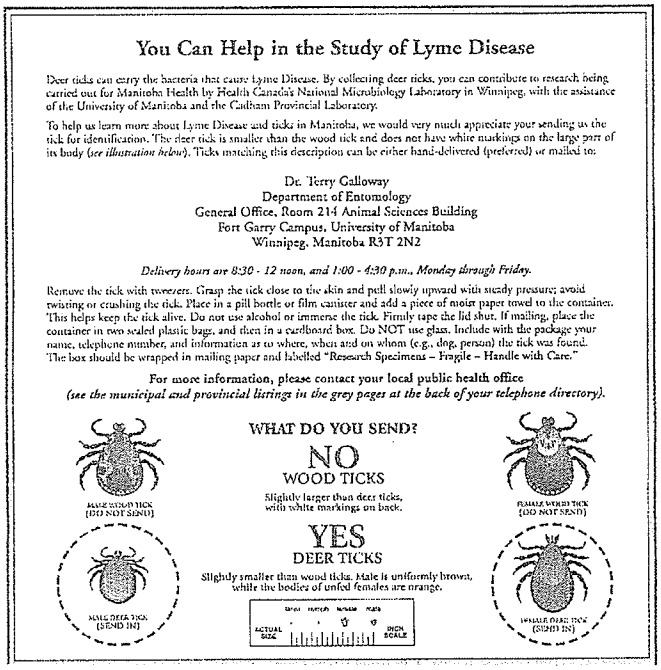
Hosts from which 169 *I. scapularis* were collected by passive surveillance in Atlantic Canada 1999 – 2003 included dogs (95), cats (38), humans (35) and one individual tick from the environment. 24 (14.2%) of these 169 ticks tested positive for *B. burgdorferi* (Artsob, 2003). A specific human LD patient in Lunenberg, NS collected 141 *I. scapularis* ticks from her dog and cat during fall, 2002. *B. burgdorferi* was detected in 25/141 (17.7%) of the ticks. Dr. Artsob (2004) indicated that blacklegged ticks were observed to be breeding in Nova Scotia for the first time. Tick dragging and small mammal collection were helpful in determining the area to be endemic for Lyme disease. During a 2-year study, *B. burgdorferi* collected from blacklegged ticks in Rondeau Provincial Park, ON showed an endemic area for *B. burgdorferi* within an established population of *I. scapularis* (Morshed, Scott, Fernando, Mann and Durden, 2003). The range of *I. scapularis* in the United States was noted to be expanding, and this trend was also apparent in Ontario (Barker and Lindsay, 2000). The blacklegged tick is also endemic at Long Point and Point Pelee on Lake Erie, while the western blacklegged tick is endemic in the Fraser River delta, the Gulf Islands and Vancouver Island (Artsob, 2004; MB Health, 2004). We read that populations of ticks that spread LD were expected to increase in the NE U.S. and spread out across the Midwest and south last summer, appearing in new parts of Canada, Europe and Asia according to experts (MB Health, 2004).

In Manitoba, 275 ticks were submitted in 2001 during a passive surveillance decade-long program on the prairies, of which the majority were *D. variabilis,* but several other tick species were also received, including small numbers of *Amblyomma americanum, D. albipictus, Haemphysalis chordeilis, H. leporispalustris, I. kingi* and *Rhipicephalus sanguineus* ticks (Lindsay, 2004).

*Ixodes* Vectors

It has been recognized that *I. scapularis* is not the most common tick on people and pets in Canada, with *Dermacentor variabilis, Dermacentor andersoni*, and *Ixodes cookei* found in much larger numbers (Barker and Lindsay, 2000). Dr. Lindsay and Dr. Galloway (2004) received 334 ticks (155 from Manitoba; 169 from Saskatchewan; 10 from other locations) in the 1990's surveillance program. The majority of the 334 were wood ticks (*Dermacentor*), 23 were blacklegged (*Ixodes*) ticks, and there were other species as well. The blacklegged ticks were sent in primarily during the months of October and November. The majority were collected from Winnipeg, mostly from dogs, but 3 were on people. 10% of *I. scapularis* collected in the study were infected with *Borrelia*, however less than 1% of ticks collected in Manitoba were blacklegged ticks. The northernmost collection point of blacklegged ticks in Manitoba was near The Pas – their latitudinal distribution parallels the human population in Manitoba, necessitating concerns for sampling technique bias. Furthermore, it should be noted that instructions were delivered for the collection of deer ticks, and not wood ticks, as indicated below:

Figure 7. Public Advertisement: You Can Help in the Study of Lyme Disease



You Can Help in the Study of Lyme Disease

Deer ticks can carry the bacteria that cause Lyme Disease. By collecting deer ticks, you can contribute to research being carried out for Manitoba Health by Health Canada's National Microbiology Laboratory in Winnipeg, with the assistance of the University of Manitoba and the Cadham Provincial Laboratory.

To help us learn more about Lyme Disease and ticks in Manitoba, we would very much appreciate your sending us the tick for identification. The deer tick is smaller than the wood tick and does not have white markings on the large part of its body (*see illustration below*). Ticks matching this description can be either hand-delivered (preferred) or mailed to:

Dr. Terry Galloway
Department of Entomology
General Office, Room 214 Animal Sciences Building
Fort Garry Campus, University of Manitoba
Winnipeg, Manitoba R3T 2N2

*Delivery hours are 8:30 - 12 noon, and 1:00 - 4:30 p.m., Monday through Friday.*

Remove the tick with tweezers. Grasp the tick close to the skin and pull slowly upward with steady pressure; avoid twisting or crushing the tick. Place in a pill bottle or film canister and add a piece of moist paper towel to the container. This helps keep the tick alive. Do not use alcohol or immerse the tick. Firmly tape the lid shut. If mailing, place the container in two sealed plastic bags, and then in a cardboard box. Do NOT use glass. Include with the package your name, telephone number, and information as to where, when and on whom (e.g., dog, person) the tick was found. The box should be wrapped in mailing paper and labelled "Research Specimens – Fragile – Handle with Care."

For more information, please contact your local public health office
*(see the municipal and provincial listings in the grey pages at the back of your telephone directory).*

WHAT DO YOU SEND?

NO
WOOD TICKS
Slightly larger than deer ticks, with white markings on back.

YES
DEER TICKS
Slightly smaller than wood ticks. Male is uniformly brown, while the bodies of unfed females are orange.

MB Health, 2000

During the collection time with the ongoing surveillance program, analysis in the lab was taking place concurrently. Scientists had demonstrated *B. burgdorferi* in 4 of the 40 blacklegged ticks from Manitoba collected that "fall alone", which was a "larger number than they had ever documented, and there were some strange patterns in where they were seeing these ticks" (Artsob, 1999). Human exposure was the primary concern with these patterns. Over 60 blacklegged ticks were documented in Manitoba in 1999, and with several testing positive for *B. burgdorferi* (Artsob, 1999), it was clear at this time that more intensive research into the presence of LD vectors in Manitoba was necessary. Interestingly, *D. variabilis* was simply dismissed in the passive surveillance program, since it had been documented as a very ineffective vector of LD.

A few years later, 13 of the 85 *I. scapularis* ticks sent to the lab in 2000 actually carried Lyme disease, so "if they have established and are reproducing, then there would be more of a threat (of Lyme disease)" (Lindsay, 2004). "We know the ticks are being carried into the province (Manitoba) by migratory birds. People do have a chance of getting bitten by one of these ticks, but there's a higher chance if they are reproducing here"

(Lindsay, 2004). 30 of these 85 ticks were found in Winnipeg, while the remainder were from southern, rural parts of the province (Rollason, 2001). In an April 2002 MB Health (CDC, 2004) report, we read "over 265 blacklegged ticks have been identified in Manitoba since 1996, and 28 have tested positive for *B. burgdorferi*". Clearly there was no denial that both the pathogen, and its vector(s) were present in Manitoba. Health Canada (2007) reported "the risk of contact with ticks begins in early spring when the weather warms up and lasts through to the end of fall. Ticks may also be active in winter in areas with mild temperatures (4°C and above) and no snow", with reference to *Ixodes* species that might carry Lyme disease.

The Lyme Borreliosis Support Group of Manitoba (2000) indicated *Ixodes scapularis* ticks had been identified in the following Manitoba locations prior to their 2000 report, but this does not indicate their endemicity in these areas:

Table 4. Manitoba Tick Sightings 1988 to 1998

| Year | Site |
|---|---|
| 1988 – 1994 | Balmoral, Delta Marsh, Flin Flon, Gunton, Matlock, Morden, Oakbank, Selkirk |
| 1995 – 1996 | Bird's Hill Park, Beaudry or Labarriere Park, Killarney, Matlock, Winnipeg |
| 1997 | Bird's Hill, Bird's Hill Park, Dugald, East Selkirk, Oakbank, Winnipeg |
| 1998 | Argyle, Beausejour, Bird's Hill, Cartier, East St. Paul, Libau, Lundar, Oak Bluff, Selkirk, Souris, Selkirk or Whiteshell, Winnipeg |

Turning our attention to European vectors of LD, Thompson, Spielman and Krause (2001) reported that the frequencies of *B. burgdorferi* s.l. infection in *I. ricinus* complex ticks are high: 36% in Germany, 49% in Switzerland and 37% in the United Kingdom. Comparatively, U.S. figures vary greatly with approximately 55% of ticks infected in western New Jersey representing the highest figure, and European figures also vary

across the continent from 2% to 49% (Mawby and Lovett, 1998). A review of the literature reveals that the Europeans have taken the lead in researching LD vectors over the past decade, and the pathogens they carry. An overview of studies from central, western, southern, and eastern Europe clearly displays this trend.

Several Swiss studies have emerged since the pathogenesis of LD was first described in detail in 1981, which is likely no great surprise since the Swiss physicians were examining clinical symptoms of Lyme in the early twentieth century (Vanderhoof-Forschner (1997). *I. ricinus* ticks collected from 12 different sites in Switzerland in 2003 yielded 3 *Borreliae* species: *B. garinii* dominated, followed by *B. lusitaniae* and *B. valaisiana*, being the first documentation of the presence of *B. lusitaniae* in Switzerland (Jouda, Crippa, Peret and Gern, 2003). Lyme borreliosis was studied on a regional scale in Switzerland from 1999 to 2001 (Jouda, Perret and Gern, 2004). The density of *I. ricinus* ticks and their infection with *B. burgdorferi s.l.* were examined at 11 sites. Infection prevalence varied from 9 to 40% in nymphs, and from 22 to 47% in adults. A correlation between density of ticks, and density of infected ticks was noted. Five *Borreliae* species were identified from the 11 sites: *B. garinii, B. burgdorferi* s.s., *B. afzelii, B. valaisiana, B. lusitaniae,* and six mixed infections were found.

Several other central European countries have also forged ahead with much *Ixodes* research. Spirochetes were isolated from *I. ricinus* ticks in Germany during 1984, soon after the disease was characterized, with an infection rate in adult ticks of 16% (Ackermann, Kabatzki, Boisten, Steere, Grodzicki, Hartung and Runne, 1984). There has been much further research in Germany since. 730 ticks were collected from patients in two areas of southwestern Germany in 1998, with 84 ticks (11.3%) testing positive by PCR for *B. burgdorferi* (Maiwald, Oehme, March, Petney, Kimmig, Naser, Zappe, Hassler and von Knebel-Doeberitz, 1998). Pichon, Kahl, Hammer and Gray (2006) reported *Borrelia* pathogens were identified in 47% of unfed *Ixodes ricinus* nymphs collected from vegetation in a forest on the outskirts of Berlin. *Borrelia afzelii* was the most common species, however other pathogens included *B. valaisiana*, *B. garinii*, and *B. burgdorferi s.s.* From March to October 2003, 2518 host-seeking *I. ricinus* ticks were

collected by blanket dragging all over the city of Bonn, western Germany, to be checked for *B. burgdorferi* infection (Maetzel, Maier and Kampen, 2005). Of 1394 specimens randomly tested by PCR for the spirochete, 250 (17.9%) were infected with *B. burgdorferi s.l.*, with adult infection rates somewhat higher than nymphs. Clearly, exposure within this northern temperate city presents a public health concern, and raises questions about potential urban exposures in north temperate Canada arises.

Denmark has recently been reported to be endemic for Lyme disease (Skarphedinsson, Lyholm, Ljungberg, Sogaard, Kolmos and Neilsen, 2007). In this study, 11% of *I. ricinus* ticks were positive for *Borrelia burgdorferi*. *B. afzelii* was most prevalent, followed by *B. valaisiana*, *B. burgdorferi s.s.* and *B. garinii*. Separating life stages, *Borrelia* was found in 13% of the nymphs, and in 8% of the adult ticks.

A 1998 - 1999 Polish study of deciduous, mixed and coniferous forest in popular recreational areas yielded 1123 *I. ricinus* ticks with a mean infection rate of *B. burgdorferi* at 16.2% (Michalik, Hofman, Buczek, Skoracki and Sikora, 2003). 31.6% of the nymphs in this study were infected, suggesting an increased role of nymphal ticks in vectoring the pathogen. 6817 *I. ricinus* ticks from eight northwestern mixed forest Polish sampling sites during 1998 – 2001 showed 9.4% *B. burgdorferi* s.l. infection rates (Wodecka, 2003). Ticks collected from 2 sites in northwestern Poland during 2003 were PCR tested for *B. burgdorferi* s.l., with 16.7% testing positive for the bacterium (Skotarczak and Wodecka, 2003). A further 701 *I. ricinus* ticks collected from suburban and urban forests in Gdansk, Sopot and Gdynia in northern Poland were examined by PCR, with 12.4% *B. burgdorferi s.l.* infection prevalence (Stanczak, 2004). Most recently, Cisak, Wojcik-Fatla, Stojek, Chmielewska-Badora, Zwolinski, Buczek and Dutkiewicz (2006) collected 1,813 *I. ricinus* ticks from 6 districts in Poland. *B. burgdorferi s.s.* was the dominant genospecies, and found in a total of 62.8% of *I. ricinus* ticks infected with *B. burgdorferi s.l.* This presents a marked increase in tick infection rates in Poland in recent years.

Tsirmpas and Tsirmpas (2006) reported Romania to be endemic, and Khanakah, Kocianova, Vyrostekova, Rehacek, Kundi and Stanek(2006) reported Austria to also be well known as an endemic area of Lyme borreliosis. Evidence was seen from host-seeking *Ixodes* ticks collected from a floodplain forest ecosystem in the Czech Republic and Austria during 1989 – 2002 (Hubalek, Stunzner, Halouzka, Sixl, Wendelin, Juricova and Sanogo, 2003). 797 nymphal ticks and 719 adult *I. ricinus* ticks were examined for *B. burgdorferi* with 16.2% of nymphs, and 29% of adult ticks positive for the spirochete. It was noted that there was a significant increase in the prevalence of *Borrelia* in *I. ricinus* during autumn in this ecosystem. 2398 *I. ricinus* ticks were collected in southern Moravia, eastern Bohemia and the Czech Republic from 1996 to 2000 and examined for spirochetes (Janouskovcova, Zakovska, Halouzka and Dendis, 2004). The prevalence of *B. burgdorferi s.l.* in *I. ricinus* ticks varied with the year, however two epidemiologically important *Borrelia* species were always present: *B. afzelii* and *B. garinii.* Of 209 *I. ricinus* ticks removed from humans in the Czech Republic during 1997 – 2001, 62% of the ticks were nymphs, of which 10.7% were *B. burgdorferi* s.l. infected, along with a 20.3% adult female infection rate (Hubalek, Halouzka and Juricova, 2003). This study supported the conclusions drawn from North America that nymphal *Ixodid* ticks are the main important vector in the transmission of Lyme disease. In a further report on the Czech study, a total of 298 *I. ricinus* ticks feeding on humans in the Czech Republic between 1997 and 2003 were tested for *B. burgdorferi s.l.* infection by darkfield microscopy, and 20% of 74 adult females, and 9% of 203 nymphs were infected (Hubalek, Halouzka and Juricova, 2004).

Tick-borne illnesses, along with annual activities of vector species were studied from 1993 – 2002, leading to the acknowledgement that two epidemiologically significant *Ixodes* ticks are common in Latvia (Bormane, Lucenko, Duks, Mavtchoutko, Ranka, Salmina and Baumanis, 2004). *I. ricinus* ticks dominate in western and central Latvia, while *I. persulcatus* seems to dominate in the eastern part of the country. Both tick species are important vectors in Lyme disease transmission. *B. burgdorferi* s.s., *B. afzelii, and B. garinii* were isolated from 1040 *Ixodes* ticks from all regions of Latvia in 2004, where Lyme borreliosis is endemic, with prevalence of *Borreliae* in *I. ricinus* and *I.*

*persulcatus* of 22.6 and 27.9% respectively (Ranka, Bormane, Salmina and Baumanis, 2004). The use of 16S-23S rRNA PCR RFLP typing is simple, sensitive and fast, allowing one to differentiate among *B. burgdorferi s.l.* species and subspecies with various degrees of pathogenic potential, and was utilized in this recent study. *I. ricinus* ticks have been shown to contain DNA of several spirochetes belonging to the *B. burgdorferi s.l.* complex in Portugal, with major differences in genetic diversity between ecozones (Baptista, Quaresma, Aires, Kurtenbach, Santos-Reis, Nicholson, Collares-Pereira, 2004), indicating a need for researchers to differentiate vector potential of each vector in transmitting each different species of the *Borrelia burgdorferi* s.l. complex. A 2002 study found *B. burgdorferi* to be widespread in *I. ricinus* ticks in Spain, with the authors indicating an increasing occurrence of the pathogen in this region (Barral, Garcia-Perez, Juste, Hurtado, Escudero, Sellek and Anda, 2002).

Data from southern Europe emerges primarily from Italy. The prevalence of 141 *B. burgdorferi s.l.* infected *I. ricinus* ticks collected in an Italian study area in 2003 was 16% in nymphs and 12.5% in adult ticks, with 3 genospecies identified: *B. afzelii, B. garinii* and *B. valaisiana* (Santino, Iori, Nicoletti, Valletta, Cimmino, Scoarughi, Santapaola, Sessa and DelPiano, 2003). 23.2% of sera from 181 forestry rangers in northeastern Italy tested positive for *B. burgdorferi s.l.* in a 2004 study, with incidence clearly associated with working in the foothills, and a history of yearly tick bites (Cinco, Barbone, Grazia, Mascioli, Anguero, Stefanel and Luzzati, 2004). Contrary to the results of this study, a two-year tick dragging investigation conducted in central Italy yielded only a few *I. ricinus* ticks, none of which tested positive by PCR to *B. burgdorferi s.l.* (Curioni, Cerquetella, Scuppa, Pasqualini, Beninati and Favia, 2004). Perhaps we place too much value on tick dragging studies, which might lead to skewed perception of risk in Manitoba and in Canada? Most specimens actually collected in the Italian study were identified as *Haemaphysalis punctata.* 14.3% of adult *H. punctata* collected in a different study in nearby Spain were recorded with *B. burgdorferi* infection. Overall however, *I. ricinus* is the tick species that has been recorded in most Italian regions, particularly in woods and shrubby habitats where the relative humidity will allow the tick to complete

its 3 year developmental cycle, and act as both vector and reservoir for *Borrelia* (Rizzoli, Rosa, Mantelli, Pecchioli, Hauffe, Tagliapietra, Beninati, Neteler and Genchi, 2004).

Epidemiologic data on Lyme borreliosis in southeastern Europe is scarce (Christova and Komitova, 2004). Analysis of 1257 Bulgarian patients between 1999 and 2002 revealed that most of the patients lived in a rural area, or were bitten by ticks during activities in a rural area. *I.ricinus* ticks collected in Turkey in May 2002 yielded all *Borrelia* species known to be carried by *I. ricinus*, and provided the first evidence for the existence of the Lyme pathogen in Turkey (Guner, Hashimoto, Takado, Kaneda, Imai and Masuzawa, 2003).

Moving toward eastern Europe, the prevalence of *B. burgdorferi* s.l. genospecies in West Siberia and in many other regions of Russia remains insufficiently investigated, but a 2003 study showed ticks were infected with *B. garinii* and *B. afzelii* (Beklemishev, Dobrotvorsky, Piterina, Ivanov, Nomokonova and Livanova, 2003). The Kiov Region of Russia is characterized by the highest incidence of *Ixodes* tick-borne borreliosis with incidence ranging from 10.5 to 48.6 per 100,000 inhabitants (Utenkova, Iastrebov, Bondarenko and Oparina, 2004). Infections in the region are from both *I. ricinus* and *I. persulcatus* species. An investigation along the Russia-China border to explore tick species and isolate bacteria from ticks (He, 2007) provided recent evidence that the Heilongjiang region is endemic, and requires Lyme disease prevention and control measures.

Minimal, but significant data on Lyme vectors has emerged from Asia. Lyme disease was not reported in Korea as of 1993, although the spirochete was isolated from the vector tick *Ixodes persulcatus* in the region (Oh, Song, Yoo, Kim and Lee, 1993). Field surveys conducted in northeastern China in May 1996 yielded ticks of 3 genera and 12 species, with *I. persulcatus* dominating, and *I. nipponensis, I. pavlovskyi, H. douglasi, H. megaspinosa,* and other *Haemaphysalis* species (Takada, Ishiguro, Fujita, Wang, Wang and Masuzawa, 1998). *I. persulcatus* ticks were the only ticks infected with *Borrelia,* with 57 different strains identified; 29 *B. garinii* strains and 16 *B. afzelii* strains were the

most prevalent. In China, the ability of *I. persulcatus, H. concinna* and *D. silvarum* to transmit *Borrelia* spirochetes was determined under laboratory conditions (Sun and Xu, 2003). The results showed that all three tick species can acquire spirochetes while feeding on infected mice, however the capability of *I. persulcatus* to maintain spirochetes alive during moulting (transstadially) and subsequent tick stages was superior to the other two species.

Moving around the globe, the first report of a vector, and presence of *B. burgdorferi s.l.* in Morocco, and *B. burgdorferi* in North Africa was made in 2003 after a tick study was conducted from January to June 2002 in the Taza region northeast of Morocco (Sarih, Jouda, Gern and Postic, 2003). 295 *I. ricinus* ticks were collected and analyzed, with the mean rate of *Borrelia* infection at 47.8%. 82 genospecies of *Borrelia* were identified, with *B. lusitaniae* dominating the samples at 92.7%, and *B. burgdorferi s.s. and B. garinii* significantly lower in incidence.

In 2009, a report from northern Mexico indicated clinical Lyme cases were reported, and 18 of 214 *Ixodes, Dermacentor and Amblyomma* ticks pulled from small mammals were *Borrelia burgdorferi* sensu stricto infected, recently identifying this region Lyme endemic (Gordillo-Perez et al., 2009). A study in Mexico to assess the serological evidence of *B. burgdorferi* s.l. infection in residents of Mexico City, and from the northeast region of the country was developed in 2003 with the help of 2346 samples from the National Serum Bank (Gordillo-Perez, Torres, Solorzano-Santos, Garduno-Bautista, Tapia-Conyer and Munoz, 2003). Antibodies against *B. burgdorferi* were detected in 3.43% of the Mexico City residents, and 6.2% of those in the northeast region of the country. The authors concluded that *Borreliae* infections are present in the region, and identification of infected vectors is still required to confirm the presence of Lyme disease in Mexico.

Willy Burgdorfer indicated in 1989 that the geographic distribution of the spirochete may be far greater than explored and assumed, and may include areas where the disease in humans is reportedly absent (Burgdorfer, 1989). Additional species potentially serving

as unrecognized vectors need further study, with attention to other *Ixodes* species, and other tick genera/species that have not been acknowledged to date for their vectoring potential of *Borreliae.* Given that humans serve as occasional hosts to a variety of tick species globally, it is important to determine which tick species other than the competent *Ixodes* LD vectors, carry infections, and parasitize humans. Smith, Lacombe, Rand and Dearborn (1992) reported a 1989 -1990 statewide survey in Maine to identify the most common tick species parasitizing humans, identifying 17% as *I. scapularis (I. dammini),* 34% as *I. cookei,* and 45% as *Dermacentor variabilis,* with occasional other ticks implicated. The entire significance of this find remains to be determined. However, given indication in the literature that *Borrelia* species are extremely adaptable to tick species across the *Ixodes* genus, consideration into *I. cookei's* vectoring potential might be warranted. Furthermore, human exposure to *Dermacentor variabilis* is clearly elevated from other tick species, and if there is any potential for disease transmission, this is important.

One of the first tick studies reported following the identification of the Lyme disease pathogen in 1981 was a 1983 – 1984 tick screening study performed on white-tailed deer in Connecticut, New York and North Carolina (Magnarelli, Anderson, Apperson, Fish, Johnson and Chappell, 1986). All infected ticks collected were either seeking hosts (questing) or feeding on deer. Spirochetes were detected by immunofluorescence in *I. scapularis (I. dammini), D. albipictus, and A. americanum,* indicating the variety of tick species feeding off of a potentially infected regional population. A 1989 statewide survey of ticks parasitizing white-tailed deer in Maryland produced 3,437 *I. scapularis (I. dammini),* 2,013 *Dermacentor albipictus* and 23 *Amblyomma americanum,* with a mean infection rate reported as 8% for all ticks (Amerasinghe, Breisch, Azad, Gimpel, Greco, Neidhardt, Pagac, Piesman, Sandt and Scott, 1992), again indicating a variety of tick species in one region potentially infected from the same source, and perhaps potentially capable of vectoring the pathogen through some pathway. Specific studies on *Ixodes* ticks other than the conventional established vectors followed in the two decades ahead.

*Ixodes sinensis* should be considered a competent vector of Lyme disease in South China after a recent laboratory investigation showed that *I. sinensis* became infected with *B. garinii* while feeding on mice, maintained active spirochetal infection during moulting and subsequent tick stages (Sun, Xu and Cao, 2003), fulfilling all vectoring requirements. Another *Ixodes* species also demonstrated to competently vector *B. burgdorferi* was *I. muris* (Dolan, Lacombe and Piesman, 2000). Larvae were fed onto infected mice, with a 66% infection frequency identified, moulted into nymphs with a decline in infection to 38%, and subsequently fed on mice, which were demonstrated to harbour infection by ear biopsy.

In a 2002 rodent and tick study in South Carolina it was observed that 26% of *I. affinis* ticks feeding off various infected rodents were infected with *B. burgdorferi s.l., a* significantly higher percentage than infected *I. scapularis* ticks in the same area (Clark, Oliver, James, Durden and Banks, 2002). Additionally, 9% of *I. minor* ticks removed from woodrats contained spirochetes, leading to the conclusion that both *I. affinis* and *I. minor* are potentially significant maintenance vectors of the spirochete. *I. minor* was identified as a possible primary enzootic vector of *B. burgdorferi s.s.* in South Carolina following a 1994 -1995 study reported by Clark, Oliver, Grego, Durden, James and Banks (2001), in which this tick species showed a statistically significant positive association with spirochetal infection in rodents. *I. neotomae* with high infection rates, was identified as responsible for maintaining Lyme disease in the woodrat population, but not involved in human disease because it has not been proven to bite people (Cromley and McLafferty, 2002). However, some might question whether it might vector Lyme disease if it had the chance.

Peavey, Lane and Damrow (2000) reported that *I. angustus* is a competent experimental vector of *B. burgdorferi s.s.,* with observations of its success in transstadially passing spirochetes, and transmitting infection. The methods utilized in this study were typical for vector competence studies, where inoculation of mice, followed by larval feeding, moulting, and subsequent nymphal feeding on uninfected mice produced infection. The results showed vector efficiency comparable to that of *I. spinipalpis.*

*B. burgdorferi* was identified in a beaver tick (*I. banksi*) at Sault Ste. Marie and a squirrel tick (*I. marxi*) at Palmer Rapids. Spika and Ashton (1996) indicated that more research was needed to determine if these tick species are competent vectors capable of transmitting spirochetes to animals and humans. *B. burgdorferi* had also been isolated from *I. angustus* in British Columbia (ALDF,1999), and from *I. dentatus* on cottontail rabbits in New York (Anderson, Magnarelli, LeFebvre, Andreadis, McAninch, Perng and Johnson, 1989).

While *Ixodes* tick vectors were first being recognized for their competence in North America, the first observations of LD in Australia occurred, amongst other places around the globe. The lack of the American vectors in Australia at the time left a quest for the link on that continent (Schmid, 1984). In 1991 (Piesman and Stone) it was hypothesized that *I. holocyclus,* the Australian paralysis tick, was a logical Lyme vector candidate, but when tested, the tick ingested spirochetes, but could not maintain the infection transstadially. The authors pointed out that the experiment should be repeated with Australian strains of spirochetes. Even though Lyme disease is reported in Australia, the parallel to the situation in Canada is clear as Russell (1998) points out that its presence is controversial. Dr. Barry Lycka diagnosed two Alberta residents with Lyme disease in 1983 and 1984 (Lycka, 1986). He indicated that the ticks thought to transmit Lyme disease did not inhabit the area of Alberta where one patient contracted his disease, and if definite disease symptoms "can only be transmitted by a tick bite, there must be a tick in northern Alberta that is capable of transmitting the disease". He further indicated that although *I. angustus* and *I. sculptus* were prevalent in the area, they had yet to be established as vector ticks of *B. burgdorferi.* Elsewhere around the world we read about other instances of questionable Lyme disease, or similar illnesses. A recently emerging clinical entity producing clinical manifestations to those observed in Lyme disease has been under discussion in Brazil (Mantovani, Costa, Gauditano, Bonoldi, Higuchi and Yoshinari, 2007). This currently named "LD imitator syndrome" is considered to be a zoonosis transmitted by ticks of the *Amblyomma* genus, in which mobile, uncultivable spirochete-like bacteria, identified through PCR are not part of the *Borrelia* genera.

These researchers describe the "possible existence of a new tick-borne disease in Brazil imitating LD, except for a higher frequency of recurrence episodes observed along prolonged clinical follow-up". There are concerns and issues with vector and pathogen systems, and widely varying disease surveillance programs between various regions, adding to the difficulty of studying vector potential. The Australian scenario is paralleled to varying degrees around the globe, with other continents and countries acquiring regional data and understanding of LD and its potential vectors. Although Lyme disease is thought to be vectored exclusively by the *Ixodes ricinus* complex globally, with significant evidence supporting the presence of these vectors and their *Borrelia* infections on several continents, research supporting other arthropods as potential competent vectors for LD has created controversy in the scientific community, and in the realm of public health.

Possible Tick Vectors other than *Ixodes*

The literature suggests a variety of species from outside the *Ixodes* genus potentially vector disease. Grubhoffer, Golovchecnko, Vancova, Zacharovova-Slavickova, Rudenko and Oliver (2005) indicated that *Borrelia burgdorferi* s.l. species are transmitted mainly by *I. ricinus* complex ticks, in addition to a few additional species not currently assigned to the complex. Lang and Territo (1997) reported growing evidence of infection from *Amblyomma americanum,* and "some reporting of infection from the American dog tick" (*Dermacentor variabilis).* Filippova reported in 1990 that *I. dentatus, Amblyomma americanum*, *Dermacentor variabilis* and *D. andersoni* were potential vectors that needed further study, and that 17 other tick species had been studied at the time for natural infection with *Borreliae* spirochetes. A twelve-year passive survey from 1985 – 1996 in Michigan based on 4755 tick submissions yielded 21 species, the most common being three species of *Dermacentor,* with *D. variabilis* most predominant (Walker, 1998). The rabbit tick, *Haemaphysalis leporispalustris* is also common across Canada, and may be picked up locally by ground-frequenting birds foraging within their nest area (Scott et al., 2001). Isolates of *B. burgdorferi* s.l. have been made from rabbit ticks in Alberta

(Artsob, 1996). Understanding the diversity of all vectors, along with their host preferences is critical to evaluating vector potential, and ultimately human risk of disease.

In 1984, *Amblyomma americanum* was implicated as a likely second vector of Lyme disease in New Jersey when the spirochete was isolated from nymphs and adults of this species (Schulze, Bowen, Bosler, Lakat, Parkin, Altman, Ormiston and Shisler, 1984). From 1994 to 1996 however, lab experiments showed this species failed to transmit *B. burgdorferi s.s.* (Armstrong, Brunet, Spielman and Telford, 2001). James et al. reported further on this species in 2001, indicating its association with Lyme disease in the southern United States, and the presence of *Borrelia lonestari* (a novel species) identified in *A. amblyomma* ticks by DNA amplification techniques (PCR). Furthermore, observation of a patient with an attached *A. amblyomma* tick and early signs of disease confirmed the roles of both the pathogen species, and the vector species in disease transmission. The authors indicated that further research on *B. lonestari* is crucial, since its first citing here as a new tickborne pathogen of humans, and its connection with an unrecognized Lyme vector tick.

An important Japanese study reported by Ishiguro, Takada, Masazuwa and Fukui (2000) on the prevalence of *Borreliae* in ticks carried on migratory birds showed new evidence of competent vector tick species. 361 ticks were collected from 1733 birds of 40 different species, with *Haemaphysalis flava* (94.4%), *H. longicornis, Ixodes columnae, I. persulcatus, I. turdus,* and one unknown Ixodes species representing the variety of tick species. 27 *H. flava* nymphs, 2 *I. persulcatus* nymphs, and one female *H. flava* moulted from a nymph were positive for *Borrelia garinii* in culture. The findings of this study are significant because not only do they confirm the presence of *B. garinii* in other tick species not currently recognized as potential vectors of disease, but the confirmation of transstadial passage of *B. garinii* from nymph to adult *H. flava* indicates vector competency.

Cases of human Lyme-disease-like illness were reported in Itapevi, Brazil during 1992 (Barros-Battesti, Yoshinari, Bonoldi and DeCastro, 2000). Throughout 1995 to 1996,

ticks were collected from small mammals and identified as *Ixodes didelphidis, I. loricatus,* and *Amblyomma cajennense.* No reports on vector competence for these species are seen in the literature.

From 1987 to 1997, 17,000 ticks were collected in China and analyzed for *B. burgdorferi s.l.*, with 8 species of ticks carrying spirochetes including *I. persulcatus* (>80%), *I. granulatus, I. acutitarsus, Haemaphysalis concinna, H. longicornis, H. bispinosis, H. cornigera taiwana* and *Dermacentor silvarum* (Bauch, 1990). The figures reported were impressive, with 16 to 40 percent of adult *H. bispinosis* and 24 percent of adult *I. granulatus* containing spirochetes as determined by direct immunofluorescence, and proven as important vectors in southern China. A 2009 study shows *I. granulatus* in Taiwan to be a unique *Ixodes* lineage, distinct from common vector ticks, yet successful in harbouring *Borrelia burgdorferi* sensu stricto (Chao, et al., 2009). A 1990 German report documented the need for further study into vector potential for *H. concinna* and *D. reticulatus* considering the concentration of these tick species in certain biotopes (Bauch, 1990). In 1996, Alekseev, Burenkova, Vasilieva, Dubinina and Chunikhin added that the successful transmission of *B. burgdorferi* from *D. reticulatus* to mice, soon after attachment to the host, was observed.

In 2004, Montana remained the only state in the U.S. to not have a CDC-confirmed case of Lyme disease (Lyme Disease Foundation (LDF), 2003). Despite a decade of patients visiting their doctors with LD-like symptoms, the lack of western blacklegged or blacklegged ticks in the state (the only confirmed transmitters of "true" Lyme disease in which *Borrelia burgdorferi* is the etiologic agent) leaves the illness unreportable to the U.S. CDC. It seems that Montana's "failure to consider the indigenous Rocky Mountain wood tick *(Dermacentor andersoni)* as the culprit in transmitting the LD-like illness may have cost the state precious time in combating the problem" (LDF, 2003). We might speculate whether or not the extremely low incidence of diagnosed Lyme disease in Manitoba parallels the situation in Montana. Evidence continues to implicate this Montana *Dermacentor* wood tick as the culprit. Dr. Damrow reported "It looks like a Lyme-like agent has adapted to the wood tick, but we don't know for sure", and further

tests will be performed on Montana's ticks at the Rocky Mountain Laboratories (where Willy Burgdorfer first identified *B. burgdorferi)* (LDF, 2003). Other reports that the southeast United States appears to have its own version of tick-borne Lyme disease, with 30% of patients testing positive for the spirochete on closer analysis revealing different *B. burgdorferi* surface proteins than those of the New England and Midwest bacteria (Artsob, 1999). Indication that "the tick species transmitting this illness seems to be very different, and also may be transmitting an organism that is very different" was suggested (Artsob, 1999).

*Dermacentor* species

We cannot dispute the overwhelmingly supportive research documenting the capability of other tick genera to carry, and potentially vector the LD pathogen. Despite this obvious support, there is curious resistance to acknowledge the potential disease risk. Seemingly, the traditional laboratory analysis model for vector competency has hindered research into potential vectors, which may ultimately be overlooked for their role in disease transmission. This detachment between science and public health would appear to influence risk perception where currently accepted vectors are not endemic. A 1998 report (Hubbard, Baker and Cann) indicated the presence of *B. burgdorferi* s.l. in British ticks of eight species, dating more than a century back to 1897! PCR analysis of eight species of anthropophilic ticks: *I. ricinus, I. hexagonus, I. uriae, I. trianguliceps, D. reticulatus, H. punctata, Rhipicephalus sanguineus,* and *Argas vespertilionis* indicated infection, however the authors point out that this in itself is not a measure of vector competency. It does however indicate the need to examine the vector potential of all of these tick species, spanning several genera. In an early 1990 – 1992 study 0.2% of 5,915 ticks collected in Belgrade were identified as *Dermacentor marginatus,* with a 31.7% infection rate (Stajkovic, Drndarevic, Lako, Dmitrovik, Obradovik, Djerkovic, Cekanac and Djordjevic, 1993). The significance of this find should have clearly initiated further study into the potential of *Dermacentor* as a genus of tick vectors. Throughout Canada, and in both rural and urban Manitoba, the enormous populations of *Dermacentor* species lend much curiosity to the potential of this vector. *Dermacentor variabilis*, our common

wood tick or dog tick, and *Dermacentor andersoni,* the Rocky Mountain wood tick, both found in Manitoba share a similar life cycle, as do all *Acari* ticks, with differences primarily in hosts (Elliott, 2002). *Ixodid* Acari, or hard ticks also share a parallel life cycle. *Ixodes scapularis* ticks have been largely documented to feed on white-footed mice while immature, and on white-tailed deer as adults (Artsob, 2004), while *D. variabilis* is commonly found on both animals and humans. Dogs are the preferred hosts of adult *Dermacentor* ticks, while immature stages feed almost exclusively on small rodents, with a preference for mice and voles. *Dermacentor andersoni*, the Rocky Mountain wood tick is much less common in Manitoba, but like the wood tick, is very resistant to starvation. A blood meal is required for maturation in both species; however larvae, nymphs and adults can survive for more than two years without feeding. In Manitoba, adults are usually what people see in the spring, as they are seeking their hosts, whereas immature stages that feed primarily on rodents are never seen (Elliott, 2002). The Public Health Agency of Canada reported in June, 2006 that "most species of ticks found in Manitoba, including the more common American dog ticks (also known as wood ticks), are not effective transmitters of Lyme disease" (Manitoba Health, 2006). The global literature seems to suggest *Dermacentor* and other tick species are hosting *Borreliae* pathogens nevertheless.

We read that Lyme disease spirochetes were detected in 5 adult questing (1.3%) *D. variabilis* tick midguts in Michigan in 1992 (Walker, Smith, DeWitt, Beaudo and McLean, 1994), and in the midguts *of I. dammini, D. variabilis*, and *H. leporispalustris* in an early Connecticut study (Anderson, Johnson, Magnarelli and Hyde,1985). A 1996 study in a multipurpose recreational area in the San Francisco Bay region revealed that adult ticks collected adjacent to trails were infected with *B. burgdorferi*, specifically 1.6% of *D. variabilis* and 0.2% of *I. pacificus* (Lane, 1996). *B. burgdorferi* was detected by PCR in 44 of 776 (5.67%) *I. pacificus* ticks, and in 3 of 58 (5.17%) *D. variabilis* ticks overall.

Feir, Santanello, Li, Xie, Masters, Marconi and Weil (1994) reported spirochetes detected by IFA and PCR in 1.9% of questing *A. americanum* and 2.0% of questing *D. variabilis*

ticks collected in a southeastern Missouri study. Additional studies to determine the role of these ticks in the epidemiology of Lyme disease were suggested. IFA specific for *B. burgdorferi* showed spirochete infection in *I. scapularis* and *D. variabilis,* but not in other species of ticks also examined from a Virginia site (Sonenshine, Ratzlaff, Troyer, Demmerle, Demmerle, Austin, Tan, Annis and Jenkins, 1995). In 1997, of 127 *D. variabilis* ticks removed from humans in Monroe County, Wisconsin and tested for *B. burgdorferi*, 14 (11%) returned positive results (Stromdahl, 2001). Clearly, a concern for human exposure to *Borrelia*-infected *Dermacentor* is necessary. Additional studies are needed to determine the role of these ticks in the epidemiology of Lyme disease in Missouri and neighbouring states (Feir et al., 1994). A small percentage (1.9%) of raccoon host-associated *A. americanum, D. variabilis, I. texanus* and *I. cookei* contained *B. burgdorferi* in a North Carolina study (Ouellette, Apperson, Howard, Evans and Levine, 1997). When *B. burgdorferi* was recovered from the guts of Michigan questing *I. scapularis* adults and nymphs and adult *D. variabilis* in 1992, spirochetes were cultured successfully with the following infection rates: 57/179 (31.8%) *I. scapularis* females, 62/204 (30.4%) *I. scapularis* males, 9/54 (16.7%) *I. scapularis* nymphs, and 5/383 (1.3%) *D. variabilis* adults (Walker et al., 1994). Most noteworthy is that these ticks were questing. We should also note that *D. variabilis* nymphs were not mentioned in this study, and we might wonder where they were, and if they were also infected.

In a Canadian Communicable Disease Report issued in May 1999, results of a passive surveillance study program for ticks in Saskatchewan reports 1505 of the 1522 ticks collected, or 98.9% were *Dermacentor variabilis* species.

Table 5. Ticks collected in Saskatchewan during a passive surveillance program conducted in 1998

| Species | Sex or instar | | |
|---|---|---|---|
| | Males | Females | Nymphs |
| *Amblyomma americanum* | 0 | 1 | 0 |
| *Dermacentor albipictus* | 1 | 4 | 0 |
| *Dermacentor andersoni* | 2 | 2 | 0 |
| *Dermacentor variabilis* | 682 | 822 | 1 |
| *Ixodes scapularis* | 1 | 3 | 0 |
| *Ixodes sculptus* | 0 | 1 | 0 |
| *Rhipicephalus sanguineus* | 0 | 2 | 0 |

Health Canada, 1999

Scientists indicated that the occasional positive for *B. burgdorferi* s.l. occurred from *D. variabilis* collected at Lyme disease endemic Long Point, Ontario (Artsob, 2004). Given the numbers of *D. variabilis* are so high in Canada, it seems likely that the overall number of infected ticks is also high. It was suggested that if we look closer at *D. variabilis* in Canada, we might find something (Artsob, 2004), however although some *D. variabilis* are infected with *B. burgdorferi,* they seem to be dependent upon *I. scapularis* populations, which are proven as more competent vectors (Artsob, 2004). A 1989 report on Canadian data indicated intensive sampling efforts for *I. dammini* (*I. scapularis*) in other regions of Manitoba, following the collection of the first tick of this kind in Winnipeg in June 1989, found only *D. variabilis* (U.S. CDC, 1989), clearly

indicating the need for vector potential research on this species simply because of its prevalence in Manitoba. In correspondence with Dr. Galloway regarding the Manitoba Health Passive Surveillance Programme 2001, 65.1% of tick submissions from Manitoba were *Dermacentor variabilis* (American dog ticks, or wood ticks), 15% were *I. scapularis*, and small numbers of other species were also collected. Most were collected from within the City of Winnipeg. 8.6% of the collected *I. scapularis* ticks were infected with *B. burgdorferi*, yet surprisingly, the other ticks were not screened for infection (Lindsay, 2004). The significant percentage of *Ixodes* ticks sent in from urban Winnipeg, with an 8.6% infection rate, clearly indicated exposure and risk within the city in 2001. Outside the city, recreational activity such as cottaging and camping potentially alter risk in increasing exposure to outdoor environments. If *Ixodes* was present within the city, it seems more than likely it would be present, and likely in larger numbers, in rural habitats.

Information from an intriguing U.S. study exploring *B. burgdorferi* uptake in tick larvae (Soares, Zeidner, Beard, Dolan, Dietrich and Piesman, 2006), adds to the curiosity of *Dermacentor variabilis'* potential role in transmitting Lyme disease. The researchers indicated that feeding *D. variabilis* larvae presented with an average of 16 spirochetes per larvae acquired after 4 days of feeding, which represented 1/195 of the counts of feeding *I. scapularis* larvae. However, during the first day after feeding, the spirochete growth rate in *D. variabilis* reached 0.076 generations per hour, 7.7 times greater than the highest growth rate detected in *I. scapularis*, and intense spirochete growth continued up to four days post-infection. At this time, a significant average of 282 spirochetes per *D. variabilis* larvae reached 1/8.5 of the *I. scapularis* count. Despite identifying a significant bacterial load in *D. variabilis* larval ticks, the researchers could not demonstrate the transmission of *B. burgdorferi* by *D. variabilis* (Soares, et al., 2006).

Parola and Raoult (2001) cite a wide range of mammalian hosts for a variety of *Ixodid* tick species, along with a high affinity in general for humans. To note, they also cite the same range of mammalian hosts for *Dermacentor variabilis* and other *Dermacentor* species, again coupled with a high affinity for humans. Until vector competence has been demonstrated for *Dermacentor* however, and 7 other tick genera, Gray et al. (2002)

reminded us that they should not be assumed to serve as bridging vectors for *B. burgdorferi s.l.* to humans. However, the authors also pointed out that problems in the design of experimental vector-competence studies have been explored, and that vector (and reservoir) competence are "definitely not synonymous merely with the experimentally proven capability of transmission of a particular agent during tick feeding". The situation in nature must also be accounted for, which has not yet been incorporated into analyses on competency, and the different life stages of the vectors are an example of one situation in nature to explore further.

Characteristics of ticks, their distribution, associated ecology and feeding hosts all contribute to their vectoring potential of disease. The geographic distribution of ticks depends on specific climatic and ecological conditions, and Lyme disease has been distributed worldwide for some time (Boire, 1991). The factors controlling the activity of the vector, such as the latitude of their habitat, or weather changes that disturb natural seasonal rhythms, may influence the spreading of the spirochetes and their survival rates in ticks (Wodecka, 2003). In a U.S. Mid-Atlantic study of 663 *I. scapularis* ticks collected in 1997 and 1998, significant associations between tick abundance and land cover, distance to water, distance to forest edge, elevation and soil type were made (Bunnell, Price, Das, Shields and Glass, 2003). Stunzner, Hubalek, Halouzka, Wendelin, Sixl and Marth (2006) reported the risk of acquiring Lyme borreliosis in habitats at higher altitudes to be limited, because of the lower density and infection rates of ticks compared to those at lower altitudes in central Europe, but nevertheless indicated the risk does exist. In Canada, we have not considered how local ecology and altitude for example support disease factors, and how this is correlated to LD risk.

Tick Vector Life Cycles and Competence

In addition to the diversity of tick species that vector Lyme disease, the different life stages: larva, nymph and adult display varying degrees of vector competence, which is important in determining risk. In early studies, Edward M. Bosler of the New York State Department of Health found the spirochete in all stages of *I. dammini (I. scapularis)*

(Habicht, Beck and Benach, 1987), indicating the presence of *Borrelia* in immature ticks. He was not aware however of the significance of his find, and the importance and success of immature ticks in their ability to transmit disease. The ability of infected nymphs to vector infection is well documented in the literature. Studies such as the one reported by Woodrum and Oliver (1999) where infected *I. scapularis* nymphs were fed on uninfected hamsters, and 3 of 4 males, and 6 of 6 female hamsters became infected, are commonly described.

The relative importance of life stages, such as nymphal versus adult female questing ticks is difficult to evaluate since human cases of Lyme disease are seasonally distributed in accordance with peak nymphal activity, and also with peak adult feeding times. However, it can be reasoned that the larval stage is of little significance as a bridging vector since larvae are not aggressive human biters. Adult ticks can transmit Lyme disease, but since they are larger, and more likely to be removed from a person's body within a few hours, they are less likely than nymphs to have sufficient time to transmit the infection (CDC, 1999). Furthermore, research in the U.S. has indicated that for the most part, ticks transmit Lyme disease to humans during the nymphal stage, probably because nymphs are more likely to feed on a person, and are rarely noticed because of their small size (less than 2 mm). Nymphs consequently have ample time to transmit infection after approximately 2 or more days of feeding (National Centre for Infectious Diseases (NID), 1999). Since immature ticks are small, and feeding usually goes unrecognized by human hosts, it follows that a history of tick bite is rare when a clinical diagnosis of Lyme disease is assigned (Edlow, 2003).

What is not recognized in most of the literature, is that these nymphal stages of tick life cycles carry the greatest risk of disease transmission (Gavloski and Elliott, 2002). The nymphal stage is considered the primary bridging vector for *I. scapularis* in North America, and for *I. ricinus* in Europe, with nymphs feeding longest and associated with 85% of disease (Gray et al., 2002). In far-eastern Europe and Asia however, adult females of *I. persulcatus* are more aggressive than nymphs, and likely vector a larger volume of disease (Alekseev, Jensen, Dubinina, Smirnova, Makrouchina and Zharkov,

2000). We read "80% of LD cases are due to the bite of nymphal ticks from May through August. Because nymphal ticks are so tiny and their bite is not painful, patients may be unaware of them" (Rollason, 2001). "The nymphal tick population – which causes 90% of all infections – was extremely high last year", reported the American Lyme Disease Foundation (ALDF, 2003). Lang and Territo (1997) reported a lower figure, stating 80% of human disease cases result from the nymphal stage. A public website posted by the Insect Control Branch of Winnipeg indicated "the blacklegged tick that can be as small as the head of a needle transmits Lyme disease. Because of its size, often people do not realize they have been bitten" (2000). Peavey and Lane (2002) reported infection prevalence in *I. pacificus* nymphs to parallel that of the adults, and given factors influencing a greater likelihood of hosting a nymphal tick, humans were at greater risk of infection from this tick life stage.

Although specimens of *I. scapularis* have been collected from all provinces in Canada, only small numbers of adult (usually female) ticks have been found, usually on dogs or people (CDC, 1998). The rare chance of actually finding a nymphal tick makes identification of infection rates in nymphs an impossible task, and prompts the question of where the nymphs are feeding, when the adults are found on people and their pets. Ticks do not travel far in their life cycles, unless hitchhiking on birds occurs (ALDF, 1999). Because the nymph stages of *I. scapularis* are so small in size (less than 3 mm), they are primarily responsible for transmission of Lyme disease (dos Santos and Kain, 1999). Nymphal behaviour was studied by Vassallo and Perez-Eid (2002), with the authors concluding that nymphs are more responsive to a human than adults of the same *Ixodes* species. Nymphs typically peak in May and June, resulting in a peak of clinical illness in July. This leaves Canadians at risk earlier in the season, but generally from May to September. In a Health Canada report detailing the first identification of blacklegged ticks in Saskatchewan, the evidence of *D. variabilis* dominating the tick populations in Saskatchewan was powerfully illustrated (Canadian Communicable Disease Report (CCDR), 1999). The lack of nymphs of either species collected in this study invites the question of where they are feeding – for where there are adults, there once were immatures.

All studies and subsequent reports on *Dermacentor* have focused on the potential of the *D. variabilis* adult as a competent vector, which according to the literature, it is likely not. However, effort needs to be directed to the studies and reports of larval and particularly nymphal vectoring capabilities, given their likelihood of competence or importance in comparison to other ticks in similar stages of their life cycles. Perhaps it is the "grown–up wood ticks that people worry about" (Galloway, 2002), but it's the immature stages that perhaps they ought to worry about if there is a parallel to *I. scapularis or I. pacificus*. Early evidence of *B. burgdorferi* in white-footed mice and eastern chipmunks in the U.S. in 1985, with infected *D. variabilis* nymphs feeding on these hosts (Anderson et al., 1985) should perhaps have received more attention than it did.

Large numbers of *Dermacentor* adults were collected from both the passive surveillance program, and in a variety of Canadian drag sampling tests, and remained in the Health Canada lab to be tested for infection (Lindsay, 2004). However, immature ticks of this species were not collected. Gray et al. (2002) reported that in drag sampling for *I. pacificus,* adults, but very few nymphs are collected from woodlands, or from open grasslands, perhaps explaining the lack of *D. variabilis* nymphs collected. Furthermore, in discussions of *I. pacificus* ticks, Gray et al. reported that humans are exposed to nymphs, but rarely to adults when leaf litter dominates ground cover. Apparent difficulties in the collection of immature ticks, and the resulting difficulties in reliable analysis of infection prevalence are exemplars of the problems in accurately identifying vector potential.

Additionally, Gray et al. (2002) reported that *B. burgdorferi s.s.* is readily detected in North American *Dermacentor* species during the first 2 weeks after detachment from an infective host, but is usually absent after 3 weeks. This phenomenon is potentially related to the timing of tick immune responses, and may play a role in transmission capabilities. Strong borreliacidal activity was recorded in the haemolymph of *D. variabilis,* but not in *I. scapularis,* leading to the conclusion that *D. variabilis* is a less competent vector than *I. scapularis* (Gray et al., 2002), but nonetheless remains infected for 2 weeks. It follows

that a tick being exposed to a human host, through any avenue during this time, may facilitate infection. Given our enormous populations of *Dermacentor,* why do we not include some potential for disease transmission through this vector in determining local disease risk?

Numerous studies exploring the reasons behind vector incompetence have been documented in the literature. Gilmore and Piesman (2000) reported on one such study focusing on the potential for *Borrelia* species to migrate from the midgut of infected ticks, to the salivary glands, and potentially into a host animal. Tick-to-host transmission mechanisms were explored, with researchers identifying a relationship between *Borreliae* OspC and effectiveness in transmitting the pathogen to a host. They indicate that further understanding of the mechanism of the outer surface protein expression shifts in the bacterium when exposed to a blood meal will help us to further understand vectoring potential.

The dynamics of vector competence, and explanations for vector incompetence were explored by Eisen and Lane (2003). In moulted, unfed *Ixodes* tick species, the spirochetes are usually isolated in the midgut, then multiply and migrate through the gut wall into the haemocoel and salivary glands during feeding, and into the host. The time elapsed from tick attachment to spirochete transmission differs between tick species, and the authors cited several studies indicating such differences. Other differences contributing to the success of the pathogen in the tick species may be related to immunosuppressive factors present in tick saliva. The inability of spirochetes to escape the midgut during the blood meal, or spirochete mortality caused by exposure to the haemocoel during migration to the salivary glands may result in vector incompetence. However the authors note that the factors ultimately determining vector competence are still unknown. Nuttall, Paesan, Lawrie and Lang (2000) detailed compelling evidence of pharmacological activity of tick saliva as having a profound effect on pathogen transmission, both from infected tick to uninfected host, and from infected host to uninfected tick. They indicated that understanding key events at the tick vector-host

interface will provide a better understanding of the epidemiology and ecology of *Borreliae* as important human pathogens.

Seven tick species evaluated in the lab for vector competence appear to be unsuccessful: *Dermacentor andersoni, D. occidentalis, D. variabilis, Ixodes cookei, I. holocyclus,* and *I. ovatus* (Gray et al., 2002). These ticks acquired infection when feeding on infected hosts, but were unsuccessful in transstadial passage of the bacterium. *Haemaphysalis leporispalustris* is reported also to be a poor potential vector in one study, and the authors reminded the reader that until vector competence has been demonstrated for these species in a laboratory setting, they should not be assumed to play any role as bridging vectors of disease to humans. Piesman, Oliver and Sinsky (1990) reported a study of spirochete numbers in ticks following a moult. They observed a five to tenfold drop in spirochete abundance during each *I. scapularis* moult, but unlike these seven species, the numbers maintained were sufficient for transmitting infection to the next generation.

In a significant 2001 study, Johns, Ohnishi, Broadwater, Sonenshine, DeSilva and Hynes recorded strong borreliacidal activity in the haemolymph of *D. variabilis,* however note that spirochetal infection prevalences as high as 5.4% for *A. americanum* and 11.3% for *D. reticulatus* have been recorded in questing ticks, indicating that transstadial passage in these ticks may be more common than lab studies indicate. Further research is indeed necessary.

Intrinsic tick factors associated with tick vector potential include tick questing behaviour, tick immunity and host preference, while extrinsic factors associated with vectoring potential include climatic conditions, host abundance and behaviour, host immune response, host susceptibility to spirochetes, and genetic variation in infectivity of spirochetes (Gray et al., 2002). Clearly much data is required to accurately assess risk of disease from just one tick type; include other vectors and the task becomes enormous. Intrinsic factors have been studied extensively, and de Silva et al. (2009) report that the exciting new discoveries on *Borrelia*-tick interactions might lead to novel preventions such as transmission blocking vaccines.

Questing nymphal *I. scapularis* ticks seem to have localized midgut infections of *Borreliae* (Piesman et al., 2001). As tick feeding begins, the spirochete population multiplies rapidly in the midgut, increasing the concentration of spirochetes, and shifting the dominant outer surface protein expression from OspA to OspC. Spirochetes travel from the midgut through the hemolymph to the salivary glands, and to the host during feeding (Piesman, Schneider and Zeidner, 2001). It has been suggested that this shift in outer surface protein expression facilitates detachment from the midgut and migration to the salivary glands, but further studies are necessary. Schwan and Piesman (2002) described how *B. burgdorferi* changed its outer surface during their alternating infections in ticks and mammals. Furthermore, Piesman et al. (2001) pointed out that European *Borreliae* genospecies present a more complex pattern of Osp expression in their tick vectors. The results indicated that spirochetes in tick midguts increased sixfold, from 998 per tick before attachment to 5,884 at 48 hours of attachment. Spirochetes in tick salivary glands increased even more significantly, over 17 times, from 1.2 per salivary gland pair before feeding, to 20.8 at 72 hours postattachment. The authors reported small numbers of spirochetes found in tick salivary glands during the first 2 days of feeding. Fikrig, Feng, Barthold, Telford and Flavell (2000) reported finding two proteins expressed by feeding ticks, bbk32 and bbk50 which can interfere with spirochete transmission at various stages of the vector-host life cycle, and require further research. We read by Shih, Chao and Yu in 2002 that the actual mechanism that affects spirochete migration to the tick feeding site was still unknown, yet their observation of certain attractants to the feeding site provided a foundation for further study on the mechanism. Valenzuela, Francischetti, Pham, Garfield, Mather and Ribeiro (2002) reported a study on salivary composition in *I. scapularis,* and analysis of the proteins expressed for their involvement in pathogen transmission. Tick saliva may enhance pathogen transmission, host hypersensitivity to saliva may modify the site of inoculation of pathogens, and it may promote non-viremic transmission of pathogens by cofeeding. According to Valenzuela et al. (2002), each of these possibilities needs further exploration to fully understand LD vector-host dynamics.

Extrinsic tick factors have been explored by a number of scientists. One particularly interesting study revealed that *I. ricinus* larvae feeding alongside infected feeding nymphs on uninfected hosts, can acquire infection. The authors noted that cofeeding transmission and its association with vectoring potential of ticks requires further study (Johns et al., 2001). Piesman and Happ (2001) developed an experimental model to test cofeeding dynamics, yielding the conclusion that an important role for cofeeding in the ecology of Lyme disease has yet to be established. Could cofeeding *D. variabilis* acquire infection from infected *I. scapularis* in Manitoba's endemic Buffalo Point, and potentially transmit LD? If so, the large population of *D. variablis* in the area would clearly increase risk.

The literature on transmission studies indicate varied laboratory results. 133 *P. leucopus* mice were collected in 1986 and 1987, with infected *I. scapularis* and *D. variabilis* nymphs removed from 30 of the mice (Magnarelli and Anderson, 1988). 30.2% of *I. scapularis* larvae and 46.5% of nymphs were infected, compared to 21.9% of *D. variabilis* larvae and 15% of nymphs. Adult ticks of both species were screened by IFA methods, with approximately 45% of adult *I. scapularis* carrying the spirochete, whereas no spirochetes were found in midgut analyses of *D. variabilis.* The authors report that transstadial transmission in *D. variabilis* is likely inefficient, and given there are no convincing reports of wood tick bites and the development of Lyme lesions on humans, that adults of this species do not appear to be vectors of *B. burgdorferi.*

In 1989 Piesman reported that *Amblyomma* and *Dermacentor* were inefficient vectors of *B. burgdorferi* spirochetes. Wright and Nielsen (1990) reported that in studies of immature species of both *I. scapularis* and *D. variabilis* ticks, only *I. scapularis* transferred spirochetes to uninfected mice. In 1990 Mather and Mather reported on three vector ticks tested in Boston for their competence in transmitting *B. burgdorferi* s.l.: *I. scapularis, A. americanum and D. variabilis,* all collected by flagging and on infested mice. The results indicated that *I. scapularis (I. dammini)* was the only species to remain infected following the transstadial moult, and thereby implicating it as the only competent vector of the three. During the summer of 1990, 593 adult, 2 nymphal and 4

larval *D. variabilis* were collected while questing, none of which were infected (Lindsay, Barker, Surgeoner, McEwen, Elliott and Kolar, 1991). In a Georgian study, *A. americanum* and *D. variabilis* were unsuccessful in transmitting a *B. burgdorferi* cotton rat isolate (MI-6) from inoculated hamsters to uninfected laboratory mice, and nymphal ticks did not maintain the isolate transstadially (Sanders and Oliver, 1995). In a similar Oklahoma study, *I. scapularis, A. amblyomma and D. variabilis* were fed on New Zealand white rabbits and infected with *B. burgdorferi* (JDF strain) (Mukolwe, Kocan, Barker, Kocan and Murphy, 1992). *I. scapularis* was the only tick of the three to transmit the pathogen to an uninfected rabbit, and to carry the infection successfully through the moult. Piesman and Happ (1997) reported on vector competency studies with 2 strains of *B. burgdorferi* extremely infectious to *I. scapularis* larvae, indicating that *I. scapularis* was the only competent vector identified, with *D. variabilis* and *A. americanum* proving incompetent in maintaining the infection transstadially. A 2001 study by Johns et al. indicated that *D. variabilis* is highly immunocompetent with borreliacidal factors that clear infection from its tissues, leaving few spirochetes intact following ingestion. In China, *I. persulcatus* has been proven as a competent vector under laboratory conditions, whereas *Haemaphysalis concinna* and *D. variabilis* failed to maintain live spirochetes during moulting (Sun and Xu, 2003). Similarly, 10% of *D. occidentalis* fed on infected hamsters acquired *B. burgdorferi* infection in a 1994 study reported by Lane, Brown, Piesman and Peavey (1994), however none successfully maintained the spirochetes transstadially. Global assessments of risk dismiss the possibility of infection based on transstadial passage of spirochetes, despite indication that it might be possible for an infected tick to pass infection in certain cases through non-traditional vectoring.

Possible Non-tick Arthropod Vectors

Although *Ixodes* ticks are considered the chief vector for *B. burgdorferi* with other ticks earning some recognition as potential vectors, *B. burgdorferi* s.l. has also been found in 30 different species of *Arthropoda*: 13 species of mites *(Acarina),* 15 species of flies *(Diptera)* including horse flies, and deer flies (Suffridge, 1999; Hunt, 1996), two species of fleas (*Siphonaptera)* (Pokorny, 1989; Gray et al., 2002), tabanid flies (Gray et al.,

2002), and mosquitoes such as *Culex pipiens* (Halouzka, Wilske, Stunzner, Sanogo and Hubalek, 1999; Gray et al., 2002 and Hunt, 1996). Zakovska, Nejedla, Holokova and Dendis (2002) reported that the ecology of the Lyme disease agent has been investigated mainly in Ixodid ticks, but less often in other blood-sucking arthropods, and infections in deer flies, black flies and mosquitoes have been largely overlooked. Since "most patients with erythema migrans, the pathognomonic rash of Lyme disease, do not recall a deer tick bite" (Feder, Abeles, Bernstein, Whitaker-Worth, Grant-Kels, 2006) questions of rash origin emerge. Insect bites have been documented as vectors of Lyme disease, however Gray et al. (2002) throughout their extensive review of the disease do not present evidence that insects can serve as vectors for Lyme spirochetes. We read in a 1991 Canadian Medical Association journal that "biting flies have also been identified as possible culprits, and given the debilitating nature of Lyme disease, the proximity of Canada to the United States, and the expanding geographic distribution of carriers, serious questions and concerns about vectors require further research" (CMA, 1991). Biting flies are so common in Canada, and despite this 1991 report, they are excluded entirely in risk assessment.

There are numerous reports of various non-tick vectors, with mosquitoes carrying obvious intrigue for Manitobans. One case of LD in Europe was reported in 1984 following mosquito bites (Schmid, 1984), and Dr. Stephen Zinner, a Harvard Medical School Professor was quoted in July 2000 (in a focus on West Nile virus being discovered in Massachusetts) "In this area, we are used to protecting ourselves from mosquito bites because of Lyme disease" (Pope, 2000). If Lyme disease is carried by local mosquitoes, and if it is remotely possible for them to infect people, then the risk in Manitoba would be clearly elevated.

Two mosquito species, *Aedes canadensis* and *Aedes stimulans* were placed into screened cages with anesthetized, uninfected Syrian hamsters with shaved heads and backs (Magnarelli and Anderson, 1988). Blood-engorged mosquitoes were dissected and IFA stained for spirochetes. Mosquito species fed on the hamsters, and of 101 mosquitoes taking in blood meals, *B. burgdorferi* was detected in the head tissues of 11, albeit at

relatively low numbers. One of the uninfected hamsters that was fed upon by *A. canadensis* had antibodies to *B. burgdorferi* at a titer of 1:32, however no spirochetal isolates were obtained. *B. burgdorferi* was shown to live less than 6 days in the insect's digestive system. The evidence of infection was clearly documented despite its failure to persist for extensive periods of time.

*Borrelia burgdorferi* sensu lato had been detected in, or isolated from ticks, human tissues and wildlife by 1988 (Magnarelli and Anderson, 1988). At this time, they cited a study of 18 species of ticks, mosquitoes, horse flies and deer flies collected in southeastern Connecticut and tested for *B. burgdorferi,* indicating other species aside from the chief vector *I. scapularis,* harbour the bacterium. The efficiency of these insects in transferring the pathogen to hosts is unclear, and the authors reported on a study initiated to determine the infection prevalences among ticks and biting insects, and to determine whether *B. burgdorferi* can in fact, be vectored by these insects. Mosquitoes, deer flies and horse flies, were gathered from Lyme endemic Connecticut during 1986 and 1987. Horse flies were either fed a beef blood medium mixed with live *B. burgdorferi* culture, or an uninfected control meal on feeding devices. Females were dissected and analyzed for spirochetes by dark-field microscopy and IFA staining. 28 of the 57 *T. nigrovittatus* which ingested infected blood from feeding devices had living spirochetes in their heads and anterior digestive tract tissues. At least two of the females harboured live spirochetes for 2 to 3 days after ingesting the infected blood. The results of the 1988 Magnarelli and Anderson study indicated that ticks, mosquitoes and tabanids of 12 species all harboured *B. burgdorferi.* The highest infection rate was noted at 36.2% for *I. scapularis* immatures (consistent with the current literature), with immatures of *D. variabilis* recording the second highest rate at 19.2%. Prepared tissues of each species revealed a varying number of spirochetes. In one region in Connecticut, females of *Aedes stimulans* (mosquito), *Chrysops callidus* (deer fly), and *H. lasiophthalma* also contained *B. burgdorferi* with an infection prevalence of 10.5% or less.

3580 culicine mosquitoes of six species collected in the Czech Republic were examined for *Borreliae* by darkfield microscopy during 1993 – 1995. Females of *Aedes cantans, A.*

*sticticus, A. vexans, Culex pipiens and C. pipiens* biotype *molestus* all harboured spirochetes ranging from 0.7% to 7.8% (Halouzka, Postic, and Hubalek, 1998). *Borrelia afzelii* was indicated as one of the isolates of *Aedes vexans,* with the authors indicating a need for further studies on the potential role of mosquitoes in the epidemiology of Lyme borreliosis. In a 1995 – 1996 study report that 1,743 overwintering *Culex pipiens* female mosquitoes were tested for the presence of spirochetes in South Moravia of the Czech Republic, spirochetes were observed in 5% of the mosquitoes, with one of five isolated strains identified as *B. afzelii.* The conclusion was that "the potential role of mosquitoes in the ecology and epidemiology of Lyme disease (LD) *Borreliae* should be further investigated" (Halouzka et al., 1999). 0.8% of 947 mosquitoes of the genus *Aedes* collected between 2000 and 2001 in the Bukowa forest in Poland were infected with *B. burgdorferi* s.l., suggesting that in comparison to ticks, *Aedes* does not pose a serious epidemiological threat in spreading Lyme disease, "but still poses some risk" (Kosik-Bogacka, Kuzna-Grygiel and Bukowska, 2004). The literature suggests that Manitoba's significant mosquito population, which includes *Aedes vexans,* has never been studied for its potential role as a vector, or carrier of *Borrelia.*

During the summers of 2000 and 2001, 439 *Culex pipiens pipiens* larvae were collected from a barrel of rainwater near a holiday area by Brno City, Czech Republic, and their midguts were observed by darkfield microscopy revealing 10 (2.28%) positive for *Borrelia* (Zakovska et al., 2002). These ten were further analyzed by PCR for a flagellar gene specific to *Borrelia burgdorferi* s.l. with 1.14% testing positive. The authors concluded that a low percentage of *Borreliae* can be found in mosquito larvae. Their report also indicated that spirochetes have been detected in a variety of mosquito species' larvae and pupae: *Anopheles maculipennis, Culicine, and Theobaldia spathipualpis.* They cite the first observation of spirochetes in mosquito larvae and pupae by Sinton and Shute (1939). The presence of the pathogen in the midguts, other parts of the soma, and especially in salivary glands prompts the question of the potential vectoring capabilities for Lyme disease. Furthermore, they propose this might help to explain the epidemiology of Lyme disease. The maintenance of infection throughout individual developmental stages of the mosquito were apparent to Zakovska et al. (2002) in study, and they

indicated the need for further studies on potential transovarial transmission amongst other research needed to explore the role of mosquitoes as potential LD vectors.

Other evidence cited includes the presence of *B. burgdorferi* in 14 species of hematophagus insects during 1985 in Norwich, Connecticut, and that infection prevalence was highly variable among all vectors (Magnarelli and Anderson, 1988). The presence of the spirochete in ticks and biting insects displays wide distribution of the pathogen amongst the haematophagous arthropod population. The authors indicated the source of infection for mosquitoes and tabanids in nature as unknown, however reported that the number of infected specimens varies with population density fluctuations of large mammals such as white-tailed deer, horses or cattle, and that further research on vectoring capability is needed. *Culex pipiens* overwintering in southern Ontario have been found to maintain West Nile virus though the winter (Artsob, 2003), drawing a question about their capacity to overwinter with *Borreliae* species, and potentially vector the disease in Canada.

Other arthropods and insects questioned for their vectoring potential have been studied, with Suffridge, Smoller and Carrington (1999) reporting on an Arkansas study of wolf and brown recluse spiders. Both species were collected and fixed in formalin (10 wolf spiders and two brown recluse spiders), and sections were taken in paraffin and examined for spirochetes by staining, using the modified Steiner spirochete staining method. All 12 spiders were negative for spirochetes, with researchers indicating a need for a larger sample size, more sensitive testing methods, and collection of spiders from Lyme endemic areas before any conclusions could be drawn regarding the vectoring potential of these species. The significant number of 'unknowns' makes establishing LD risk difficult.

Mechanical Vector Transmission of LD

Fleas and mosquitoes across Europe have been documented to harbour *B. burgdorferi* s.l. (Hubalek and Halouzka, 1997). 1 of 322 fleas (*Orchopeas leucopus)* removed from a

white-footed mouse in a 1990 Long Point, Ontario study was infected with *B. burgdorferi,* with the authors suggesting this species to be a poor vector for the LD spirochete (Lindsay et al., 1991). However, it is important to recognize that "although insects may prove incompetent to serve as vectors for *B. burgdorferi* s.l. by laboratory definition, the possibility of occasional mechanical transmission should not be discounted" (Gray et al., 2002). Vectoring opportunities in nature may be quite different from those in the laboratory. Mechanical transmission such as crushing an infected mosquito, or inappropriately digging out a tick and introducing *B. burgdorferi* spirochetes into a bite site remind the reader of the problems associated with laboratory vector competency studies. In the case of typhus for example, it is not the bite from a louse that causes disease, but scratching the louse on the skin. This allows the blood of the louse, containing the infectious agent of disease access to the human, and causes disease (Edlow, 2003). Parola and Raoult (2001) cited indirect transmission routes as possible sources of LD infection, such as contamination of abraded skin or the eyes following crushing of ticks with the fingers. This important method of transmission should be emphasized in disease research, not minimized as the literature generally indicates. Parola and Raoult's study suggested that mechanical transmission of a pathogen from any vector, proven competent or not, could allow for successful pathogenesis, and resulting human disease. The literature indicates the risks associated with improper removal of *Dermacentor* ticks, or with slapping an *Aedes* mosquito in Manitoba, have not been studied. Given the robust populations of these species in Manitoba, these significant exclusions might be needed to accurately assess risk in the province. Research has been slim in Canada, and investigations into the ecology, pathology and population biology of host-parasite systems need to be approached from a multidisciplinary perspective, and involve both classical and cutting edge technologies and methodologies (Daszak, Cunningham and Hyatt, 2000). Qualitative community model analysis may provide a meaningful alternative to standard population-based models of vector-borne disease, and coupled with biomathematical models of vector-borne disease transmission, a foundation for future disease risk analysis would be in place (Zavaleta and Rossignol, 2004). Mathematical models according to Sonenshine are the most significant development to understanding tick biology, and his group at Dominion

University is currently in the process of creating one such model integrating tick populations with their disease risk (2009). In Canada, much further research is necessary, but a recently published paper on the emergence of Lyme in Canada shows promise of that (Ogden et al., 2009).

## Lyme Disease Hosts and Reservoirs

The vertebrate hosts and reservoirs of Lyme disease include all vertebrates that vectoring arthropods feed on in nature. Non-reservoir hosts may have contact with infected ticks, but are unable to transmit the infection to ticks, whereas reservoir hosts are a proven natural source of infection for ticks or insects (Gray et al., 2002).

Recent studies suggest that the genospecies of *B. burgdorferi* s.l. are propagated by a vastly different spectra of vertebrate hosts, mainly birds and rodents, contributing to the complex zoonotic transmission cycles of the disease (Hanincova, Taragelova, Koci, Schaffer, Hails, Ullmann, Piesman, Labuda and Kurtenbach, 2003). Koci, Derdakova, Peterkova, Kazimirova, Selyemova and Lubida (2006) reported *B. burgdorferi* sensu lato spirochetes have evolved remarkable ability to survive in diverse ecological niches during transmission cycles between ticks and vertebrate hosts by variable gene expression. This success is seen as infected *I. scapularis* have been found on over 50 species of mammals and over 55 species of birds in North America (Morshed, Scott, Banerjee, Fernando, Mann and Isaac-Renton, 2000), and on 14 lizard species (Keirans, Hutcheson, Durden and Klompen, 1996). Each host animal has its own unique properties, from food sources to reproductive habits to environmental sensitivities, which together determine their roles in influencing Lyme disease risk to people (Ostfeld, 2005). The primary vector of *B. burgdorferi* in North America, *Ixodes scapularis,* feeds on a variety of mammalian, avian, and reptilian hosts. *Peromyscus leucopus, Tamias striatus, Microtus pennsylvanicus, and Blarina* are small mammal species which can serve as reservoirs in an enzootic cycle of Lyme disease (Anderson, Swanson, Schwartz, Glass and Norris, 2006). It is very difficult in most geographical areas to determine the whole spectrum of hosts and reservoir hosts however, and further transmission experiments are

required. In the western U.S., tick vectors and mammalian hosts for *B. burgdorferi* s.l. are distinct from those in the eastern U.S. and considerably more variable (Foley, Foley, Brown, Lane, Dumlers and Madigan, 2004). In the southern U.S., where there is controversy regarding the presence of "true LD", two enzootic tick vectors *Ixodes affinis* and *Ixodes minor* that rarely bite humans, are more important than the human biting *I. scapularis* in maintaining the enzootic spirochete in nature, and maintaining the disease reservoir (Oliver, Lin, Gao, Clark, Banks, Durden, James and Chandler, 2003). Clearly the diverse ecology in different regions, and extensive variety of hosts makes risk assessment a very local challenge.

A 1983 Science report (Bosler, Coleman, Benach, Massey, Hanrahan, Burgdorfer and Barbour) indicated that spirochetes believed to be the cause of Lyme disease were isolated for the first time from white-footed mice and white-tailed deer, the preferred natural hosts of *Ixodes dammini* (*I. scapularis*), the tick vector. Evidence at the time suggested that deer act as a reservoir of the disease, and provide an overwintering mechanism for both spirochetes and adult ticks. Before the LD pathogen itself was identified, four deer management zones were identified to have high *I. dammini (I. scapularis)* tick densities in New Jersey during 1981 (Schulze, Bowen, Lakat, Parker and Shisler, 1984). Geographical distribution and density data indicated that elevation was the most important factor in explaining variability in both *I. scapularis* distribution and density, since deer range coincided with this. This vital link between vector and adult host distribution was the first important piece of the LD puzzle, made prior to any suspicion of its impending medical significance.

In 1984, the early patterns of spirochete prevalence on Long Island strongly suggested that white-tailed deer (*Odocoileus virginianus*), and white-footed mice (*Peromyscus leucopus)* were reservoirs of the pathogen, and thus fundamental to the ecology of Lyme disease on Long Island (Bosler, Ormiston, Coleman, Hanrahan and Benach, 1984). Infectivity lasts life-long in many small mammal species when infected with *B. burgdorferi s.s., B. garinii* or *B. afzelii,* and they are therefore deemed "reservoir hosts" (Gray et al., 2002). Although apparently "non-reservoir hosts" incapable of transmitting

the infection to ticks (since they may or may not develop a long-lasting infection themselves) white-tailed deer function as "maintenance hosts" of vector ticks, such as *I. scapularis* (Gray et al., 2002), helping to keep tick populations healthy. The adult ticks spend the winter, and mate on the body of this host (Edlow, 2003). Where host populations are strong, tick populations follow suit. Difficulties in assessing the true range of the pathogen reservoir exist however, as depicted in a 1997 Ontario study that yielded conclusions that infected *I. scapularis* nymphs, rather than persistently infected vertebrate hosts, likely served as the overwintering "reservoir" for *B. burgdorferi* on endemic Long Point (Lindsay, Barker, Surgeoner, McEwen, Campbell, 1997). Despite this information, studies have not been conducted to explore the nymphal population as a reservoir of disease in Canada.

Research on mammal diversity associated with LD began two decades ago when 14 species of small to large mammals from eastern coastal areas of the U.S. were surveyed for *Ixodid* ticks and serum antibodies to *B. burgdorferi* from 1987 to 1989: opossums (*Didelphis virginiana*), least shrews (*Cryptotis parva*), gray foxes (*Urocyron cinereoargenteus*), red foxes (*Vulpes vulpes*), raccoons (*Procyon lotor*), feral cats (*Felis sylvestris*), feral horses (*Equus caballus*), meadow voles (*Microtus pennsylvanicus*), house mice (*Mus musculus*), norway rats (*Rattus norvegicus*) and jumping mice (*Zapus hudsonius*) (Oliver, Magnarelli, Hutcheson and Anderson, 1999). Antibodies to *B. burgdorferi* were found in all species tested from each locale, indicating the necessity to research mammals in Canada for assessing LD risk. To date however, research has been minimal, and limited to high profile endemic sites.

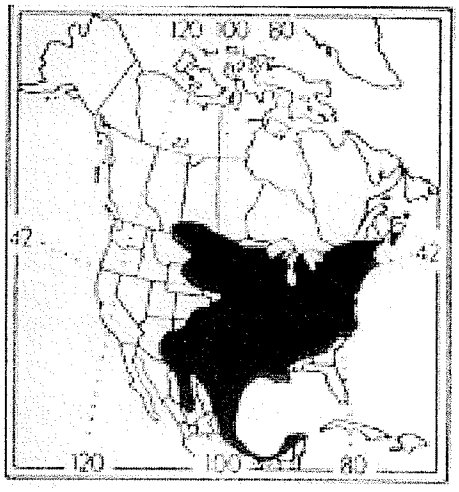
Small Mammal Hosts and Reservoirs

Small mammal hosts and reservoirs in which larvae and nymphal ticks feed, include a variety of rodents, but white-footed mice and deer mice (*Peromyscus maniculatis)* are confirmed as the most important (Gray et al, 2002). Spirochetes isolated from *P. leucopus, T. striatus* (chipmunks), and *D. variabilis* larvae were serologically and genetically indistinguishable from reference *B. burgdorferi* isolates. They concluded that

isolation of spirochetes from rodents is a method for identifying endemic areas of Lyme disease (Anderson, Johnson, Magnarelli and Hyde, 1985). Small rodents play an important role in almost any forest ecosystem, as linking species between producers and higher trophic levels. The diversity of species, and their populations varies from year to year as influenced by both biotic and abiotic factors such as the number and physiologic condition of the population, weather, habitat, food sources, natural predators and disease (Margaletic, 2003). Sinski, Pawelczyk, Bajer and Behnke (2006) indicated that any factor that reduces the density of a region's important small mammal hosts will also reduce the risk of human exposure to Lyme borreliosis spirochetes. It is clear that there are a significant number of factors determining human risk, the small mammal population being only one, and since risk is a fluctuating phenomenon complex modeling becomes all the more attractive.

The ticks that transmit Lyme disease in the American Northeast and upper Midwest feed on a number of animals, but the host most likely to pass the Lyme bacteria to young tick larvae in these regions is the white-footed mouse, which does not become ill from the bacterium, thus serving as "incubators for the disease," explained Rick Ostfeld, an animal ecologist at the Institute of Ecosystem Studies in Millbrook, N.Y. (Pleasant, 2004). When Ostfeld and his team surveyed Lyme infection rates in numerous small animals and birds in Dutchess County, N.Y., they found that 90 percent of white-footed mice were carriers, compared to only 10 percent of squirrels (infection rates for other small animals were similarly low).

Figure 8. Density of white-footed mice in North America




http://wildlife.wisc.edu/courses/301/mammals/wisconsin_mammals.htm, 04/04

Much evidence to support the importance of the white-footed mouse is documented. 75% of serum samples from 514 white-footed mice were positive for antibodies to *B. burgdorferi* s.l. in a 2004 study of mice collected in Connecticut (Bunikis, Tsao, Luke, Luna, Fish and Barbour, 2003), while Thompson, Spielman and Krause (2001) reported that 93% of white-footed mice captured in Connecticut had antibodies against *B. burgdorferi.* The density of *I. scapularis* ticks in the northeastern United States is positively correlated with that of white-footed mice (Gray et al., 2002).

After monitoring the population density of white-footed mice, burdens of *I. scapularis* on mice, and infection prevalence of host-seeking ticks in New York State from 1995 to 1999, Goodwin, Ostfeld, Schauber determined that fluctuations in population density of mice influence risk of human exposure to Lyme disease (2001). Furthermore, it was observed that there was a steady increase in the infection prevalence of nymphal and adult ticks over the four year time period, correlated with white-footed mouse populations.

An early 1983 study in Connecticut yielded spirochetes from the blood of white-footed mice, one woodland jumping mouse (*Napaeozapus insignis),* one northern mockingbird *(Mimus polyglottos)*, one gray catbird *(Dumetella carolinensis),* two prairie warblers

*(Dendroica discolor),* one orchard oriole *(Icterus spurious),* one common yellowthroat *(Geothlypis trichas),* and one American robin *(Turdus migratorius)* (Anderson and Magnarelli, 1984). One might expect that this early evidence of extensive biodiversity responsible in Lyme disease pathogenesis would have set the stage for extensive research in this area. Surprisingly, quite the contrary trend has been seen during the past 3 decades of Lyme disease research.

A 1984 study on white-footed mice and raccoons in Connecticut in 1982–1983 yielded comparable spirochetal infection rates in raccoons (*Procyon lotor*)(9%), as in white-footed mice (9%) (Magnarelli, Anderson and Chappell, 1984). Positive ticks for *B. burgdorferi* were removed from chipmunks (*T. striatus*), raccoons, white-footed mice (*P. leucopus*), and a red squirrel (*Tamiasciurus hudsonicus*) in Lyme, Connecticut in 1983 (Anderson, Magnarelli, Burgdorfer and Barbour, 1983). Serologically and morphologically indistinguishable spirochetes from all animals suggested that closely related serotypes are commonly present in wild mammals commonly parisitized by *I. dammini* (*I. scapularis),* and further supported the spirochete in the etiology of the disease. In the Midwest, chipmunks and shrews as well as white-footed mice serve as incubators or reservoir hosts (Pleasant, 2004). 71 rodents tested positive when examined for spirochetes in South Carolina in 2002, including eastern woodrats, cotton mice *Sigmodon gossypinus)* and hispid cotton rats (*Sigmodon hispidus)*, with 69%, 53% and 25% infection prevalence respectively (Clark, Oliver, James, Durden, and Banks, 2002), allowing for effective enzootic transmission in this region contributing to its endemicity.

Additionally, other small mammal hosts have been identified in North America. The western gray squirrel (*Sciurus griseus)* was recently shown to be a potential reservoir of *B. burgdorferi* in a 2004 California study (Eisen, Eisen and Lane, 2004). Researchers say that chipmunks (*Tamias striatus)* and shrews (*Cryptotis parva*) may play a larger role in the spread of Lyme disease than was previously thought (National Institutes of Health (NIH), 2004), and North America supports a healthy population of all of these species.

Raccoons were live-trapped and examined for ticks from 1990 to 1993 in North Carolina, yielding 5 species of Ixodid ticks on 351 (78%) of 449 raccoons (Ouellette, Apperson, Howard, Evans and Levine, 1997). *Amblyomma americanum, Dermacentor variabilis, Ixodes texanus* and *Ixodes scapularis* were frequently collected, and *Ixodes cookei* in rare numbers. 23 (26%) of 87 raccoons had *B. burgdorferi* spirochetes in their blood.

The eastern cottontail rabbit, *Sylvilagus floridanus* was identified in 1995 as a competent host for the rabbit spirochete strain *Borrelia andersonii*, a member of the sensu lato complex (Scott et al., 2001), as was the dusky-footed woodrat (*N. floridana*) since it remains infectious for ticks for at least 13 – 15 months in California. Dusky –footed woodrats, along with deer mice have proven to be capable experimental reservoir hosts of *B. bissettii*, while cotton rats (*S. hispidus*) remain infected and serve as reservoir hosts for >19 – 30 months (Eisen, Dolan, Piesman and Lane, 2003).

In Canada, small mammals were trapped and collected for testing from the Lunenberg, Nova Scotia site in May 2003 (Artsob, 2003). Of 36 small mammals captured, 5 deer mice had positive sera against *B. burgorferi* antigen, and one red squirrel was also positive by PCR utilizing flagellin primers. These studies in Lunenberg County, Nova Scotia provide the first evidence for established populations of *I. scapularis* in Atlantic Canada in association with their hosts. Another Canadian study revealed groundhogs (*Marmota monax*) as potential wildlife reservoirs of *B. burgdorferi* in southern Ontario in 1993, based on their observed capacity to transmit infection to *I. scapularis* (Barker, Lindsay, Campbell, Surgeoner, McEwen, 1993), while a roadkill snowshoe hare, *Lepus americanus* had *B. burgdorferi*-infected ticks removed from it at Grand Prairie, Alberta in 1995 (Scott et al., 2001).

The white-footed mouse is the most important reservoir for *B. burgdorferi* s.l. in North America, but the potential of so many other species has been overlooked in LD risk assessment. In Europe, the white-footed mouse is also the most important, but a variety of other small mammals including the dormouse (*Muscardinus arvellanarius*) and vole

(*Clethrionomys glareolus*), and some birds are also important (Nadelman and Wormser, 1998), reflecting the complex ecology of the *Ixodes* ticks.

The biodiversity of small mammal hosts in Europe differs from other geographical areas. The relative reservoir competence of European wood mice (*Apodermus flavicollis* and *Apodemus sylvaticus*) for degree and duration of infectivity was studied, with the conclusion that *Borrelia afzelii* is better adapted to these hosts than the more generalist genospecies *B. burgdorferi* sensu stricto (Richter, Schlee, Allgower and Matuschka, 2004). Home ranging of small rodents (*Apodemus flavicollis* and *Clethrionomys glareolus*) inhabiting the forest seems to be responsible for the spatial pattern of *Borreliae* infection according to a Czech Republic investigation of *I. ricinus* nymphs (Zeman and Daniel, 1999). Small wood mice in Europe remain infected with spirochetes their whole lives, as do the most efficient host reservoirs of *Borreliae* (Oliver et al., 2003). In a western Slovakian study in 2003, it was determined that mice were more heavily infested with *I. ricinus* ticks than bank voles (*Clethrionomys glareolus*), and a higher proportion of mice were infected with spirochetes than voles, however the infectivity of voles was much higher than that of mice (Hanincova, Shcafer, Etti, Sewell, Taragelova, Ziak, Labuda and Kurtenbach, 2003). A 1998 Polish collection of 213 small rodents via trapping yielded 3 species: *Apodemus agrarius Pall., Clethrionomys glareolus Schreber,* and *flavicollis Melchior,* with each contributing to a different degree in the transmission of the pathogen to subadult stages (Michalik, Hofman, Buczek, Skoracki and Sikora, 2003). The latter contributes significantly less than the former two species, which the authors concluded are necessary for maintenance of the pathogen in *I. ricinus* populations in disturbed urban forests. The role of bank voles, along with yellow-necked mice (*Apodemus flavicollis*) were studied for maintenance of both *I. ricinus* and *B. burgdorferi* s.l. in a 2004 Polish study (Pawelczyk, Ogrzewalska, Zadrozna and Sinski, 2004), with conclusions that each played some role in maintaining the genospecies *B. garinii* and *B. afzelii*. A two-year Czech study revealed wood mice were 58.8% positive for antibodies to *B. burgdorferi* s.l., bank voles 45.5%, and yellow-necked mouse 44.3%, indicating that spirochetes were widespread in the Czech Republic (Vostal and Zakovska, 2003). Further south, rodents *A. flavicollis* and *C. glareolus* had 16.6%

and12.7% *B. burgdorferi* s.l. infection prevalence when 1212 *I. ricinus* ticks were collected and studied in Italy during 2002 (Rizzoli, Rosa, Mantelli, Pecchioli, Hauffe, Tagliapietra, Beninati, Neteler and Genchi, 2004). The importance and distribution of small mammals in Lyme disease ecology was evident in Slovakia in a 2000 – 2003 study of rodents. The total seropositivity of all rodents was 18.78%, with *Apodemus flavicolis*, *Apodemus agrarius* (striped field mouse), and *C. glareolus* playing significant roles with high infection rates (Stefancikova, Bhide, Pet'ko, Stanko, Mosansky, Fricova, Derdakova and Travnicek, 2004).

Asian small mammal diversity is seen through a study in northwestern China revealing that rodents, including *Apodemus uralensis* (pygmy wood mouse) and *Cricetulus longicaudatus* (long-tailed hamster) positive for *B. burgdorferi* spirochetes contributed to the maintenance of *I. persulcatus* infection rates, and human risk in the arid region (Takada, Masuzawa, Ishiguro, Fujita, Kudeken, Mitani, Fukunaga, Tsuchiya, Yano and Ma, 2001).

Recent reports of the potential reduction in disease transmission through large-scale vaccination of white-footed mice in the U.S. may be a promising ecological strategy to help prevent the spread of the disease to humans (NIH, 2004). Immunization experiments were recently conducted in Connecticut, yielding promising results (Tsao, Wootton, Bunikis, Luna, Fish and Barbour, 2004). No such efforts have been considered in Canada to date.

Several studies have been conducted in an attempt to find out why mammals are effective hosts of *Borrelia,* while other animals, such as reptiles are not. Many previous studies support the notion that *B. burgdorferi* differentially expresses numerous genes and proteins to help it adapt to growth in the mammalian host (Brooks, Hefty, Jolliff and Akins, 2003), the TROSPA receptor site mentioned earlier is one. Dr. Robert Lane reports that California has a low incidence of Lyme disease because young ticks feed primarily on the Western Fence lizard. When the lizard's blood travels through the tick's body, it reportedly rids the tick of the disease. Missouri is also home to the Eastern

Fence lizard, and the incidence for Lyme disease is also minimal in Missouri (Pleasant, 2003). Experimentally, diseased ticks allowed to feed on both lizards and mice transmitted Lyme spirochetes to their host, however lizard blood seemingly destroyed or interrupted Lyme transmission, while the spirochetes survived in the mice (Pleasant, 2003). Pleasant (2003) further discussed tick larvae and nymphal preference in the western U.S. for feeding on lizards such as Western Fence lizards, commonly called "bluebellies," and southern alligator lizards, while choosing to feed on small mammals such as wood rats and kangaroo rats, to a much lesser extent. Robert Lane, professor of insect biology at the University of California at Berkeley, has found that a complex of enzymatic proteins in the lizards' blood actually kills Lyme bacteria (Pleasant, 2003). More recently however, Swanson and Norris (2007) reported a study that suggests that some lizards indigenous to the mid-Atlantic region may serve as alternative reservoirs for *B. burgdorferi*, and may affect the enzootic cycle of this tick-borne pathogen. Richter and Matuschka (2006) also presented evidence from a central European study site that lizards which were previously considered zooprophylactic for the agent of Lyme disease, appear to perpetuate *B. lusitaniae*. Interesting research like this to expand the LD knowledge base, and contribute to an improved understanding of human risk has not even been considered to date in Canada.

Large Mammal Hosts

Large mammal hosts and reservoirs that adult ticks usually feed on and infect, include primarily white-tailed deer (*Odocoileus virginianus*), but potentially mule deer, bears and domestic animals including dogs, cats, horses, sheep, cattle, goats, pigs, boars, chickens (Gray et al., 2002), and also people. The white-tailed deer is the most abundant species of large herbivores found in North America (Ecozones, 2004), and a significant 50% of white-tailed deer in Connecticut have antibodies against *B. burgdorferi* (Thompson et al., 2001). Gray et al. (2002) reported that local abundances of *I. scapularis* ticks are typically positively correlated with those of deer, which serve as important hosts for the adult tick stages (Nadelman and Wormser, 1998). Surveys for ticks on 5449 hunter-killed white-tailed deer were conducted from 1988 to 1990 in Michigan, and found *I.*

*scapularis* presenting as the majority of the tick population on deer from some regions, and a lower abundance of the ticks from other regions (Walker, 1998). Conservation efforts that have resulted in a population explosion among deer herds have contributed to a corresponding increase in the number of deer ticks (*I. scapularis)* (Nadakavukaren, 2000), and these populations remain strong.

In Connecticut, near the town of Lyme, the deer herd grew from 4,000 in 1936, to 12,000 in 1956, 30,000 in 1976, and in 1996 it exceeded 52,000 (Edlow, 2003). This correlates strongly with increased reforestation of lands that were originally stripped in the quest for fuel and building materials early in the twentieth century. Rand, Lubelczyk, Lavigne, Elias, Holman, Lacombe and Smith (2003) reported on a study measuring deer and tick densities, noting that ticks on deer increase with deer density, however decrease with elevation.

Studies exploring deer and tick density have led to different conclusions. A study of white-tailed deer that were introduced to Monhegan Island off the coast of Maine in 1955, showed that by the mid-1990s they had reached a density of 37/km$^2$ (Rand et al., 2004). *I. scapularis* was first noticed here in the late 1980s and flourished thereafter, with Norway rats (*Rattus norvegicus*) serving as competent reservoir hosts. From November 1996 to March 1999, all deer were removed from the island, and monitoring of ticks and infection prevalence was followed through 2003, with a gradual decline expected to continue. In another study, Stafford, Denicola and Kilpatrick (2003) again observed that tick densities decline with sustained reductions in white-tailed deer populations in Connecticut, citing densities of deer exceeding 90/ km$^2$ in 1992 and dropping to 10-17/ km$^2$ in 1994. An early study however saw the removal of 70% of deer from Great Island, Cape Cod, Massachusetts in 1984, which failed to markedly reduce the abundance of *Ixodes dammini (I. scapularis)* ticks in the area (Wilson, Levine and Spielman, 1984). The authors questioned whether other mammals substituted as suitable hosts in the absence of large deer numbers. Given the pathogenic success of the disease agent and the tick vector, coupled with resulting cases of human illness, other suitable hosts must have been available in the region.

In Canada, the white-tailed deer population is strong (Gallivan, Barker, Artsob, Magnarelli, Robinson and Voigt, 1998), as Snetsinger indicated "we already have a problem with white-tailed deer, and ongoing urban sprawl does not discourage the growth of the deer population, and if the host is spreading, the tick is more likely to spread too" (Flegg, 2007). The mean intensity of infestation of *I. scapularis* on 623 white-tailed deer in southern Ontario from 1985 to 1989 was180, with a significant 60% of the adult ticks infected with *B. burgdorferi* (Gallivan et al.,1998). Clearly the deer and the tick populations in this region had created a significant LD risk already two decades ago.

In Manitoba, the white-tailed deer population was a healthy 180,000 across the province in 2003 (Rebizant, 2004). "There was no shortage of white-tailed deer during the 2003 fall season, and hunters young and old had plenty of opportunity to bag an animal" drawing 4000 Americans into purchasing MB licenses (Bell, 2004). This large population is most concentrated in southern Manitoba, with "lots of deer in Bird's Hill, from Stonewall northward into the interlake area, along the Red River southward, in the LaSalle country, and in and along the Assiniboine River. Many connecting pathways for deer found in these regions increase the local populations" (Rebizant, 2004). The range of most deer is approximately 30 km (Lindsay, 2004). Rebizant estimated approximately 400 deer regularly reside within the City of Winnipeg, with the Assiniboine Forest, the Fort Whyte / Charleswood area west of Wilkes Avenue, the Sturgeon Road area, and The Down's region north of Headingley carrying the highest numbers (2004). He indicated that ticks from deer within the city limits have been identified to carry Lyme disease, however the deer themselves within the city and throughout the province had never been tested for Lyme disease. Manitoba Conservation examined the urban deer population due to a reported increase of deer collisions, and reports from property owners of deer feeding on shrubs, and generally causing an increased residential nuisance in 2004. The goal was to reduce numbers of deer within the city for these reasons, not to attempt to reduce the risk of Lyme disease transmission to urban residents (Lindsay, 2004). Clearly this measurable urban population of deer, if carrying diseased ticks, increases LD risk for a large population of Winnipeg residents.

Figure 9. Range of White-tailed Deer in Manitoba

Manitoba Conservation, 2004

Only a few *I. scapularis* ticks have been found on Manitoba's couple hundred white-tailed deer that have been collected as "road kill" (Lindsay, 2004). Instead of passive surveillance always being the method of choice to look for ticks, it was suggested that perhaps we should turn to other sources such as road kill, and educate those people exposed to wildlife to look for ticks (Lindsay, 2004). This might generate substantial information to enhance our ability to determine risk.

In Europe, seroprevalence to anti-*Borrelia* antibodies in deer, mouflons and hunting dogs were found to be 44.9%, 29.41% and 30.43% respectively in a 2004 Slovakian study (Bhide, Curlik, Travnicek and Lazar, 2004), indicating the host and reservoir capacity in Europe is significant. Closer to home, a serum study of 78 wild-trapped gray wolves *(Canis lupus)* from Wisconsin and Minnesota revealed one positive, and another suspect for *B. burgdorferi* infection based on presence of antibody to the spirochete, with the researchers concluding that the wolf is susceptible to infection by *B. burgdorferi,* and that wolves are being infected in the wild (Kazmierczek, Burgess and Amundson, 1988). Soon after, Thieking, Goyal, Bey, Loken, Mech, Thiel and O'Connor (1992) sought to determine the seroprevalence of Lyme disease in gray wolves from various counties of Minnesota and Wisconsin (USA). 589 serum samples were collected and tested, with 15 (3%) positive for *B. burgdorferi.* Three of the positive samples were collected from Douglas County in Wisconsin and twelve were from Minnesota counties. This study indicated that wolves are exposed to *B. burgdorferi* and are susceptible to Lyme disease.

This important data tells us that supporting ecology for *B. burgdorferi* exists just next door, and should be considered significant in assessing Canadian exposure and risk.

Some research has been conducted on the capacities of various domestic animals as hosts and/or reservoirs of disease. Domestic sheep *(Ovis aries)* and goats *(Capra hircus)* were screened for anti-*Borrelia* antibodies in 1999 and 2000 in Slovakia, with significant seroprevalence in both of 15 - 20% indicating great possibility of transmission via co-feeding to humans (Travnicek, Stefancikova, Nadzamova, Stanko, Cislakova, Pet'ko, Mardzinova and Bhide, 2002).

The sera of 80 healthy Connecticut dairy and beef cattle *(Bos)* living in tick-infested regions were recently tested for antibodies to *B. burgdorferi s.s.* with 57 (71%) positive (Magnarelli, Bushmich, Sherman and Fikrig, 2004). Cattle are not reservoir hosts of *B. burgdorferi* s.l., and the association of infected ticks with cattle pastures is likely due to the presence of other hosts in the same habitat (Gray et al., 2002). Serosurveys have also shown that equine *(Equus)* infection is widespread, and seroprevalence may be as high as 45% in some areas (Gray et al., 2002).

Domestic animals also can carry infected ticks into areas where humans live, "but whether pet owners are more likely than others to get Lyme disease is unknown" (CDC, 1999). Snydman (1989) reported however that during the initial investigation in Lyme, Connecticut, more pet owners were infected than non-pet owners. This information is important, yet has not been considered, or communicated in Canada.

6 of 15 sick dogs tested in a *B. burgdorferi* s.l. endemic region of northwestern Poland were shown to be infected with the pathogen, suggesting that dog exposure to the spirochete is common and should be important to local vets (Skotarczak and Wodecka, 2003). Of 299 canine sera tested for antibodies to *B. burgdorferi* in 1992 and 1993 from Menominee County, Michigan, 25 (8%) were positive (Walker, 1998). In another Wisconsin/Illinois study, 0 – 40% of healthy canines sampled by area, tested positive to *Borreliae* antibodies (Guerra et al., 2001). 143 of 277 dogs tested in Rhode Island in 2001

were seropositive for *B. burgdorferi* strongly suggesting that the disease agent poses a risk to dogs and humans in the area (Hinrichsen, Whitworth, Breitschwerdt, Hegarty and Mather, 2001). *B. burgdorferi* was isolated from an *I. scapularis* tick from both a dog in Thunder Bay on October 31, 1995, and a cat on November 24, 1994 (Spika and Ashton, 1996). Both animals had never traveled outside the city, showing that LD infection is possible within urban Canadian settings. In a 2004 report of data collected in Rhode Island from 1991 to 2000, it was determined that dog seropositivity was significantly correlated with human cases by county, and this could serve as a useful tool in assessing the geographical distribution of Lyme disease risk (Johnson and Stricker, 2004).

Serosurveys in Europe suggest widespread canine Lyme, and suggest that domestic cats may also be affected where they are exposed to a heavy tick challenge (Gray et al., 2002). They draw the conclusion that there is no doubt that Lyme disease occurs in domestic animals globally, however, there is little evidence to support any significant morbidity in any domestic species, with the possible exception of the dog, or that any of these species function as important disease reservoirs. Hiraoka, Shimada, Sakata, Watanabe, Itamoto, Okudo, Masazuwa and Inokuma (2007) recently indicated infected ticks carried by companion animals can be introduced into the human environment. Their study focused on 1136 dogs and 134 cats collected all over Japan, which were examined for *Borrelia* infection. The PCR analysis utilized revealed a new species, *B. tanukii*, and yet another novel *Borrelia* species closely related to *B. valaisiana.*

Although mice and deer and other species play a large role in the ecology of Lyme disease, their limited geographic ranges cannot account for the rapid spread of disease (Lang and Territo, 1997; Edlow, 2003), and researchers now agree that infected ticks have been hitching rides on ninety-nine different species of migrating birds. Comstedt, Bergstrom, Olsen, Garpmo, Marjavaara, Mejlon, Barbour and Bunikis (2006) examined the role of migratory passerine birds as reservoirs and disseminators of *Borrelia*, in Sweden. 1,120 immature *Ixodes ricinus* ticks were removed from 13,260 birds and Lyme borreliosis spirochetes were detected in 160 (1.4%) ticks. *Borrelia garinii* was identified as the most common species. The authors concluded "migratory passerine birds host

epidemiologically important vector ticks and *Borrelia* species, and vary in effectiveness as reservoirs on the basis of their feeding behavior".

Passerine birds disperse several species of *Ixodid* ticks in Canada, and during spring migration translocate ticks from the U.S., and both Central and South America, some of which are infected with *B. burgdorferi* (Scott et al., 2001). The first infected tick identified on a Canadian songbird (a common yellowthroat, *Geothlypis trichas*) was collected in Nova Scotia on May 28, 1999 (CDC, 1999; Artsob, 2004). Of epidemiologic significance is the recognition that the common yellowthroat acts as a competent reservoir for *B. burgdorferi,* and migratory birds have been firmly identified as important reservoir hosts (Artsob, 2004). Songbirds from Minnesota and Wisconsin were found carrying spirochetes northward into Canada, and 9 of 14 cases of LD in Thunder Bay between 1984 and 1995 were associated with these birds since those infected had no travel history (Regush, 2000). "The old theory was you had to go to an endemic area for Lyme disease. Now people (could get) Lyme disease who've never traveled" said John Scott, president of the Lyme Disease Association of Ontario and lead author of a study published in the *Journal of Medical Entomology* in July 2001. The study showed that songbirds can carry ticks for long distances, and widely distribute infected ticks. Researchers identified 9 species of ticks from 33 species of migrating birds from 14 locations within southern Canada from 1996 to 2000. The isolation of the bacteria and the continued presence of the carrier suggest that transmission of the disease to humans is possible (MB Health, 2003). Manitoba Health recently reported that most blacklegged ticks found in the province are believed to have dropped off migratory birds from the south (MB Health, 2006). The role of birds as hosts for *B. burgdorferi* was "long discounted" according to Dr. Bjorn Olsen and colleagues in Sweden (Reuters Medical News, 2000), and requires more attention.

The research on the role of birds in the pathogenesis of Lyme disease is quite sparse. In the U.S. during 1996, sixty larval and nymphal *B. burgdorferi*-infected ticks were removed from eight species of birds in northwestern Wisconsin. Across the continent in New York during 2001-2002, it is interesting to note that researchers observed no

correlation between the effect of bird feeders on the density of *I. scapularis* ticks, and on the prevalence of Lyme disease (Townsend, Ostfeld and Geher, 2003). A 2006 California study examining birds of 45 species revealed an overall bird *B. burgdorferi* infection rate of 6.4%, with notable differences between the species (Wright, Lemenager, Tucker, Armijos and Yamamoto, 2006). The researchers suggested that birds play a role in the distribution and maintenance of *I. pacificus*, and possibly of *B. burgdorferi* in California.

In Europe, a 3-year study period of passerine birds in northeast Poland was recently reported (Gryczynska, Zgodka, Ploski and Siemiatkowski, 2004). 1254 birds of 42 species were captured, and blood was drawn for PCR testing, with 4.2% of all birds seropositive for *B. burgdorferi* s.l. More specifically, 21.2% of all tree pipit (*Anthus erivialis*), 15.8% of dunnock (*Prunella modularis*), 12.7% of chaffinch (*Fringilla coelebs*) and 9.3% of song thrush (*Turdus philometos*) tested positive. Birds were cited as a major reservoir for *B. burgdorferi s.l.* following an Italian study in 2003 yielding *B. garinii* and *B. valaisiana* as the most common genospecies at the study site (Mannelli, Boggiatto, Grego, Cinco, Murgia, Stefanelli, DeMeneghi and Rosati, 2003). A 2006 Swiss study (Marie-Angele, Lomano, Humair, Douet, Rais, Schaad, Jenni and Gern) of 1,270 birds identified *Ixodes ricinus* as the dominant tick species. *B. valaisiana* was the species detected most frequently in these ticks, followed by *B. garinii* and *B. lusitaniae*. 23% (6/26) of the birds infested by ticks, were infested by *B. lusitaniae*-infected larvae, with the researchers concluding migratory birds appear to be reservoir hosts for *B. lusitaniae*. A 2006 German study (Pichon, Kahl, Hammer and Gray) identified *B. valaisiana* and *B. garinii*, and *B. afzelii* in ticks that also contained bird DNA, suggestive of the ticks' prior hosts.

A particular seabird tick, *Carios capensis*, infests the nests of brown pelicans and other ground nesting birds along the coast of South Carolina (Reeves, Loftis, Sanders, Spinks, Wills, Denison and Dasch, 2006). The researchers indicated this tick is "associated with pelican nest abandonment and could pose a threat to humans visiting pelican rookeries if

visitors are exposed to ticks harboring infectious agents". Ticks were collected, and *B. lonestari* was identified by PCR.

Since *I. uriae,* the seabird tick, has been shown to be a vector of *B. burgdorferi* s.l. in both hemispheres, the large-scale movements of birds and the subsequent dispersal of ticks will have important consequences for the dynamics and coevolutionary interactions of the pathogen with its different vertebrate and invertebrate hosts (McCoy, Boulinier, Tirard and Michalakis, 2003). A study to determine whether *B. garinii* is present in seabird ticks on the Atlantic Coast of North America was conducted by Smith, Muzaffar, Lavers, Lacombe, Cahill, Lubelczyk, Kinsler, Mathers and Rand (2006). 61 ticks from Gull Island, Newfoundland, were collected, and ten were positive for *B. garinii*, with the researchers concluding "the potential for introduction of this agent into the North American Lyme disease enzootic is unknown". Comstedt, et al. indicate that *B. garinii* is a causative agent of Lyme in Europe and Asia, and variants of the spirochete have been recently identified and are likely the result of distinctive selective pressures from their bird hosts (2009). The fact that migratory birds can carry ticks and infection long distances, clearly forces us to recognize that the potential for human infection exists outside endemic tick areas. An interesting study recently cited demonstrated that infection in redwing thrushes was reactivated in response to migration, making birds more infectious to ticks during their migration and therefore important long-range disseminators of the pathogen (Gray et al., 2002). Clearly, further examination into the role birds play in Lyme disease transmission is necessary. The risk of acquiring Lyme disease in Canada is clearly elevated with recent information collected on birds, and the European *Borrelia* species *B. garinii* carried most frequently by birds, is currently not recognized as a species capable of causing Lyme disease in Canada.

Other Lyme Disease Risk Factors

As previously indicated, LD risk in Manitoba and Canada is determined strictly by established *Ixodes scapularis* and *pacificus* populations in endemic sites, and most factors used in evaluating LD risk elsewhere around the globe have not been considered in

Canada to date. A closer look at these extensive LD risk factors allows us to distinguish whether or not they should be incorporated into our local risk assessment protocols, and if our perception of risk might be altered with a greater understanding of these risk factors.

The extent to which the biodiversity and community composition of ecosystems affect their function in disease transmission is an issue that grows ever more compelling as human impacts on ecosystems increase (LoGiudice, Ostfeld, Schmidt and Keesing, 2003). Ecoepidemiology focuses on land use and activity patterns that bring people into areas where vectors and hosts are present, and plays an intricate role in understanding the pathogenesis of Lyme disease, and evaluating risk. Changes in human demographics and behaviour greatly influence infectious disease emergence and transmission, and since 75% of existing and emerging human diseases are zoonotic in origin (Mavris and Halos, 2005), the number of interactions and relationships yet to be explored is vast. Ecological risk factors and human behaviour for example, have been cited in association with a doubling of Lyme disease in the Netherlands between 1994 and 2001 (den Boon, Schellekens, Schouls, Suijkerbuijk, Van Leeuwen and Van Pelt, 2004), yet are not studied here. The proliferation of deer, abandonment of farmland that reverts to thick secondary vegetation, and increased use of coastal sites for human recreation or habitation have contributed greatly to increased deer-associated zoonoses such as LD (Thompson et al., 2001), but again, have not been studied here. Terrestrial latitudinal, along with altitudinal gradients of biodiversity play a significant role in Lyme disease transmission (Gray et al., 2002), influencing global disease patterns. This predictive tool has also not been considered in Canada.

*Borrelia* species were originally named because of their discovery in the boreal forest (Artsob, 2002). To date, the northern boreal forest has been largely undisturbed by industrial activities north of 51°, however threats to biodiversity in the boreal include forestry, industrial logging, oil and gas extraction, mining, hydro-electric dams, roads and climate warming (Pither, 2005). These, along with impacts of burning to produce yards,

corridors and mosaics might be influential in increasing LD risk, yet have yet to be studied in Canada.

We read that

> over the last century, changes in land-use, modification of agriculture-livestock production systems, disruption of wildlife habitats, increase of human activities, higher frequency of international and intercontinental travels, wider circulation of animals and animal products have contributed to alter the distribution, presence and density of hosts and vectors. As a result, the number of emerging and reemerging diseases, including zoonoses, have greatly increased

(DeMeneghi, 2006), and Lyme disease is one of these. As indicated earlier by one scientist, the rise in Lyme disease incidence has been associated with fragmented forests (Walters, 2003) that have been dissected by roads and separated by developments, can no longer support large natural predators, but since they remain ideal habitats for deer and mice, the likelihood of human exposure is increased. Fourteen patches of forest in a hot spot for Lyme disease in New York State were studied, with results indicating that the number of disease-carrying ticks increased dramatically as the patches of forest shrank (Jones, 2003). A recent study in New York State exploring Lyme disease risk and human-induced changes in the landscape showed fragmentation seemed to increase risk (Estrada-Pena, 2009). Researchers have long speculated that interfering with environments increases infectious disease prevalence. Breaking up forests into smaller patches is known to alter populations of animals living there, and mice thrive because most of their predators and competitors leave (Jones, 2003). "Lyme disease soared in the late 1990s as Americans built more and more homes in the woods, bringing people into contact with disease-carrying ticks" reports the U.S. CDC (2001). According to Wodecka (2003) the highest probability of encountering *I. ricinus* ticks is at the edge of forest paths, both in spring, and in late summer. Edlow (2003) added that reforested areas and desirable parklands, coupled with an enormous jump in the deer population have added to this elevated incidence of disease. Mawby and Lovett (1998) cited reforestation in Europe and North America as one example of anthropogenic change, which tended to produce suitable tick habitat, and has been identified as a factor contributing to current levels of *B. burgdorferi* infection in the environment, and LD

incidence in humans. In Canada, Ogden, Trudel, Artsob, Barker, Beauchamp, Charron, Drebot, Galloway, O'Handley, Thompson and Lindsay (2006) indicated risk for tick occurrence in southern Quebec is highest in lower latitudes with woodland cover. The literature does not indicate how the environmental landscape within Manitoba, and within the country influences Lyme disease risk, because it has not been studied.

Once environmental conditions associated with a high incidence of disease are identified and mapped, comparisons to other geographical areas can be extremely useful (Cromley and McLafferty, 2002). The authors cited elevation, vegetation and soils as 3 of the 127 environmental variables associated with tick distribution. This type of analysis might produce suggested areas where disease may be underreported, where disease incidence might increase if people move in, or where disease may spread to in the future (Cromley and McLafferty, 2002). These authors stressed that studying the ecology of the disease is important due to important regional variations in the disease cycle. Since *B. burgdorferi* s.l. shows a great ability to adapt to different environments, including the arthropod vector, and the mammalian host (Anguita, Hedrick and Fikrig, 2003), risk might vary significantly from region to region, and transform quickly.

Qualitative community model analysis is a useful tool in predicting Lyme disease risk in oak forest communities, and positive correlation between deer abundance and risk of disease confirmed by this method are consistent with other published observations (Zavaleta and Rossignol, 2004). Interestingly, U.S. researchers have shown that people who live in regions where most of the wild animals have disappeared may be at greater risk of getting Lyme disease, since a wide variety of animal hosts usually results in ticks feeding mainly on those that do not carry or transmit disease effectively (McIlroy, 2003). This Dilution Effect Model predicts that high species diversity in the community of tick hosts reduces vector infection prevalence by diluting the effects of the most competent disease reservoir, the white-footed mouse (LoGiudice et al., 2003). So preservation of vertebrate biodiversity and community composition and resulting "dilution hosts", can reduce the incidence of Lyme disease. Sparagano (2007) in support stated "epidemiological studies on vectors and the pathogens they can carry are showing some

correlations between infection rates and biodiversity highlighting the "dilution" effects on potential vectors". The literature completely lacks any studies of this nature in Canada.

Given acorns are a critical food for white-footed mice, an abundance of acorns draws white-footed mice into oak forests, and mice and deer are the primary hosts of *I. scapularis*, Jones, Ostfeld, Richard, Schauber and Wolff (1998) suggested that acorn production in forests is linked to Lyme disease risk. Ecologist Rick Ostfeld (2005) reported that mouse populations can be one-hundred times larger when acorn numbers are high, and this significantly affects populations of infected nymphal *Ixodes* ticks responsible for human disease. Large crops are produced every two to five years, with few or no acorns produced during the intervening years (Pleasant, 2000), which enables a fairly accurate prediction of annual mice populations, and potential disease monitoring. Ostfeld's 13 year assessment of the determinants of Lyme disease risk in the epicenter of U.S. Lyme disease, New York led to the conclusion that the strongest predictors of any current year's risk, were the prior year's abundance of mice and chipmunks, and abundance of acorns two years previously (Ostfeld, Canham, Oggenfuss, Winchcombe and Keesing, 2006). Manitoba forests support these species, yet studies have not been documented to determine their influence on local Lyme disease risk.

Remote sensing approaches, and spatial information technologies have been used widely for investigations into arthropod pests and vectors of human diseases over the past decade, with *Ixodes* and other tick vectors of Lyme disease investigated by such methods (Thomson and Connor, 2000). GIS and environmental data have been utilized to create habitat profiles and grid maps identifying soil order and land cover conducive to tick vector presence (Guerra, Walker, Jones, Paskewitz, Cortinas, Stancil, Beck, Bobo and Kitron, 2002). *Ixodes neotomae* has also been implicated for its involvement in California in maintaining Lyme disease in the woodrat population, but not as a bridging vector to humans. Cromley and McLafferty (2002) point out that monitoring infectious disease patterns through vector-host surveillance has been difficult. The use of host animals to determine vector distribution such as collecting ticks from hunted deer has been useful, but is obviously limited to locations where deer are killed. The use of

remote sensing and GIS analysis to develop a model for tick habitat was employed in a Rhode Island study. However, without comparisons to human cases and vector distribution, environmental modeling is difficult. Nevertheless, this study revealed a strong correlation between human cases and entomologic risk. Risk maps, and predictive risk analysis have emerged from this technology. Opportunities for human exposure to vectors depend on sufficient numbers of encounters between the two, which surveillance data, field and laboratory experimental data, and statistical and mathematical modeling can assess. Applying new biological tools from molecular biology, digitized databases, environmental characterization, and appropriate analytical methods helped Cortinas, Guerra, Jones and Kitron (2002) to evaluate vector geography, and predict invasions of new territories. Assessing the territory of known vectors, evaluating the vector potential of yet unconfirmed vectors, and predicting the range of disease with the use of all available methods will offer enormous insight into Lyme disease risk. Recently, a study examining the geographical distribution patterns of *Ixodid* ticks engaging in host-seeking behavior in dense woodland habitats of a climatically and ecologically diverse county in north coastal California reflected risk of human exposure to host-seeking ticks rather than the true distribution of the ticks (Eisen, Eisen and Lane, 2006). In another study in this county, Eisen et al. (2006) explored risk of human contact to *I. pacificus* vectors, and showed that "11.9% of the county was classified as habitat posing at least moderate risk of human exposure to nymphs (> 6.4 nymphs per 100 $m^2$), and high-risk areas (> 10.5 nymphs per 100 $m^2$; 1.7% of the county) tended to cluster in the central interior and most heavily populated region of Mendocino County, but were rare in the proximity of coastal population centers". No studies of this nature have been documented in Canada.

Linard, Lamarque, Heyman, Ducoffre, Luyasu, Tersago, Vanwambeke and Lambin's 2007 study concluded "vector-borne and zoonotic diseases generally display clear spatial patterns due to different space-dependent factors". They indicated that land cover and land use influence disease transmission by controlling both the spatial distribution of vectors or hosts, and the probability of contact with susceptible human populations. Their study concluded that Lyme disease transmission risk was higher in mixed landscapes with forests and spatially dispersed houses, and greater risk was also

associated with wealthy 'peri-urban' areas. Is the large population of urban suburb residents sprawling into rural landscapes within Manitoba at higher risk for LD? Do they have an elevated exposure to the pathogen(s), vectors and/or hosts involved in LD transmission, and are they being infected in Manitoba?

## Lyme Disease Symptoms and Diagnosis

Complicating public health surveillance, in order to meet the U.S. Lyme disease CDC case definition criteria, exposure to an endemic geographic region supporting both the pathogen, and the vector must be met. In Canada, the requirements are similar, but vary slightly between the provinces. The Manitoba provincial protocol indicated in 2001 that the case definition of Lyme disease or diagnosis requirements were based on one of three specific scenarios, of which exposure to an endemic area was included, along with an erythema migrans rash, and/or laboratory confirmation for *Borrelia burgdorferi* (Manitoba Health, 2001). A current, revised Manitoba Health 2003 update for physicians indicates that the diagnosis of Lyme disease is based on the clinical picture, together with serological and epidemiological data, particularly history of exposure to ticks. This has been problematic in certain areas, such as in the southwestern United States, where individuals with multiple Lyme disease symptoms do not live in areas with identified vectors of disease (Cromley and McLafferty, 2002). Burkot, Maupin, Schneider, Denatale, Happ, Rutherford and Zeidner (2001) presented the concern that unrecognized enzootic cycles of *B. burgdorferi* sensu lato that may be infective to humans, might exist in geographical areas not yet acknowledged for Lyme disease risk. Specifically, they focused on a western U.S. tick species, *Ixodes spinipalpis,* known as a nidicolous tick (not hosting on humans) due to its close association with its rodent hosts (wood rats and mice) and nests, and thus its insignificance in vectoring disease to humans. Coupled with this, it is noted that an examination of the geographical distribution of the recognized North American Lyme disease vectors *I. scapularis* and *I. pacificus* leaves areas of U.S. CDC case defined Lyme patients geographically positioned where there are no known vectors, yet where *I. spinipalpis* resides (Gray et al., 2002). Minimal research and literature is devoted to these important gaps, yet an extensive examination exists in the

literature on disease correlation with accepted tick vector species. An examination of the research on *I. spinipalpis* provides convincing support of some potential to vector *Borrelia burgdorferi* s.l. Furthermore, it is an excellent example of one species remaining outside the realm of recognized competent vectors.

Despite *I. spinipalpis'* description as a nidicolous tick, the authors revealed it has been collected on humans in Oregon, New Mexico, and Canada (but do not indicate where in Canada) by dragging, and on rabbits and birds. *I. spinipalpis* has been shown to maintain an endemic cycle of *B. bissettii,* a *Borreliae* species associated with clinical Lyme disease in Europe, and is currently under investigation for the depth of its role in Lyme disease (Gray et al., 2002). The question regarding the questing potential of *I. spinipalpis* for hosts, and potential health risk to human hosts was investigated in 1999. A sentinel host and trapping system was utilized from March through October, and this method of studying the questing behaviour of *I. spinipalpis* ticks was successful in demonstrating the tick's ability to transmit *Borreliae* pathogens. Results of the study indicated that of 155 animals of 6 different wild rodent species trapped, deer mice (*Peromyscus maniculatis)*, Mexican wood rat (*Neotoma mexicana)* and prairie voles (*Microtus orchogaster)* were the most prevalent (Burkot et al., 2001). The rock mouse (*P. difficilis),* mountain cottontail *(Sylvilagus nuttallii)* and the western harvest mouse *(Reithrodontomys megalotis)* were also collected. All species except for the latter were positive on ear culture for *B. bissettii.* 392 *I. spinipalpis* ticks (346 larvae and 46 nymphs) were removed from five of the six species captured (excluding the mountain cottontail), with infestation rates varying amongst the species. In addition to the *I. spinipalpis* collected from the rodents, *Haemaphyslis leporis-palustris,* and 301 fleas (*Orchopeas neotomae, Orchopeas leucopus, Aetheca sagneri, Neopsylla inopina,* and *Malaraeus telchinus)* were also found. The data on the sentinel mice indicated that 19% of the mice became infested with *I. spinipalpis* throughout the study (unexpected to the researchers), with monthly variations in infestation rates. 2 of 46 sentinel mice were infected with *B. bissettii,* fed on by an *I. spinipalpis nymph.* In order for any arthropod to transmit a pathogen, Burkot et al. (2001) pointed out the two governing variables: The infection rate in the pathogen, and contact with a susceptible host. They concluded that

given high infection rates of *B. burgdorferi s.l.* in *I. spinipalpis*, coupled with the tick's identification on the variety of rodent species discussed and also the yellow-breasted chat, it may quest outside rodent nests for a host. Recent evidence suggested that *I. spinipalpis* may even be more significant than *I. scapularis* in maintaining *B. burgdorferi s.l.* because of the much higher infection rate discovered in this tick. Evidence also indicated that *I. spinipalpis* transmits the human pathogen *B. microti* among rodents. Cumulatively, the authors concern that *I. spinipalpis* may play a role in vectoring Lyme disease seems well grounded. Furthermore, since *I. spinipalpis* plays an important role in maintaining *B. burgdorferi* s.l., including *B. bissettii* in enzootic cycles, increasing human populations in shrubby habitats could increase contact between humans and *I. spinipalpis,* and increase Lyme disease risk. This is clearly important research from a public health perspective. Importantly, in 1997 Dolan, Maupin, Panella, Golde and Piesman demonstrated in the lab that *I. spinipalpis* is a competent vector of *B. burgdorferi* s.l. with a 75% transstadial passage success rate. *I. spinipalpis* at the very least should be recognized as a potential Lyme disease vector, and in light of the research cited by Burkot et al. (2001) and Dolan et al. (1997), questions as to why it is not should be raised. Furthermore, questions regarding other potential vectors and supporting ecology in other geographical areas where Lyme disease symptoms and patients surface, should be raised, such as Manitoba. Human risk is clearly ambiguous if these questions are not addressed. Do other potential vectors transmit LD in Manitoba, are LD hosts and reservoirs established in Manitoba, and has enough research been done to determine whether patients in Manitoba have been exposed to LD through unrecognized pathways?

A number of nontraditional potential pathways of exposure to the Lyme disease spirochete have been documented. Doctors recognize that spirochetes can pass through the placenta from infected mothers to their children (Lang and Territo, 1997). Although Lyme disease in pregnancy has produced severe birth defects, fortunately the risks to the fetus are minimized if the disease is adequately treated in pregnancy (Pekkanen, 1989). If *Borrelia* infected mothers are not treated, there is definitely a higher chance of adverse pregnancy outcome (Lakos, 2009). Dos Santos and Kain (1999) reported that *B. burgdorferi* can be transmitted by blood transfusion, since spirochetes can persist in

blood. Blood screening in Canada is not practiced, however the Red Cross will not allow anyone who has had an active Lyme disease infection over the past year to be a blood donor (Lang and Territo, 1997). The Public Health Agency of Canada has indicated that Lyme disease may pose a small threat to the safety of people receiving blood transfusion (2003). Mushahwar (2007) reported Lyme amongst other infectious diseases is "very rarely transmitted by transfusion in industrialized countries. However, an awareness of their possible transmission is essential for the control of spread of these diseases among the public by human-to-human transmission via blood transfusion". Transfusion transmitted Lyme disease has never been reported in the literature, even though there is a practical risk that *B. burgdorferi* may be transmitted through blood transfusion. If the risk of acquiring Lyme disease through blood transfusion exists, it is very low. Blood donors are not routinely screened for antibodies to *B. burgdorferi*" (Edlow, 2003). Continuing studies have isolated spirochetes from breast milk, and it seems probable that transmission can occur through this pathway, but further studies are necessary (Lang and Territo, 1997). Additionally, sexual transmission from male to female is probable, given that enough fluid is transmitted from male to female to potentially pass spirochetes (and is highly recognized in a similar spirochete, syphilis), however this has yet to be proven. A number of cases of husband/wife infection have been documented to date (Lang and Territo, 1997). Collectively, these potential pathways of disease transmission are not factored into risk assessment, however minimal their contribution.

As previously mentioned, LD is a multi-symptom disease. The Canadian Lyme Disease Foundation reports a list of 75 symptoms beginning with a rash (often a typical erythema migrans (EM) rash), and other neurological (optic and auditory included), psychological, digestive, musculoskeletal, respiratory and circulatory problems, along with other symptoms associated with general well-being (2009). The website indicates that attention should be paid if a patient experiences 20 or more of the symptoms, and that "symptoms may come and go in varying degrees with fluctuation from one symptom to another. There may be a period of what feels like remission only to be followed by another onset of symptoms." The American Lyme Disease Foundation reports a similar

list of symptoms, and classifies symptoms as we do in Manitoba and Canada into early, disseminated, and late stage disease (ALDF, 2007; Manitoba Health 2001).

American LD doctor Dr. Kenneth Leigner suggested there are "so many different manifestations of Lyme disease, that we are going to find that it has a role in a lot of things that we do not yet accept or realize" (Jones, 1999). The demand for answers to pathogenic complexity in the medical arena, uniquely coupled with the outcry for answers from the victims is driving current research. We read throughout the literature that Lyme disease incidence is increasing (Hoppa and Bachur, 2007). Millar, Xu and Moore (2007) reported that in developed countries, the emergence of infectious diseases, such as Lyme disease have "stimulated public interest and inspired commitments to surveillance and control". They indicated the control of infectious disease is extremely important, and "the ability to control such bacterial infections is largely dependent on the ability to detect these etiological agents in the clinical microbiology laboratory".

The diagnosis and treatment of Lyme disease on a global scale has been fraught with difficulties, and is largely controversial for numerous reasons (Stricker, 2007; Artsob, 2004), one example being the difficulties surrounding laboratory detection of disease. Lyme disease has posed difficulty to practicing physicians in both diagnostics and treatment, following the identification of the disease agent in 1981 (Burrascano, 2001). Political dissent has been around almost from the 'beginning of Lyme', and quickly moved into the arena of clinical practice guidelines (Ronn, 2009). Currently a re-thinking of conflicting diagnostic and treatment guidelines through a legal agreement is underway in Connecticut, in an attempt to unite all policy makers (Ronn, 2009). Evidence of conflict and controversy elsewhere has surfaced in Canadian LD diagnostics.

Taking a closer look at issues in testing, Burgdorfer himself reported that this relatively large *Borrelia* is not readily detectable in blood smears or thick drops of Lyme disease patients and susceptible host animals, yet engorgement on infected hosts results in up to 100% infected ticks (1999). Culturing of *B. burgdorferi* from treated Lyme disease patients, suggesting the existence of seronegative chronic Lyme disease (Scythes and

Jones, 1999) further increases the difficulties in diagnosis. Lab findings confirming the development of membrane-derived cysts, blebs, spherules and vesicles, with the potential to transform to motile, helical spirochetes as a bacterial "survival mechanism" in the face of unfavourable environmental conditions (Burgdorfer, 1999), cause much difficulty in disease diagnosis – the cysts are hard to find. Burgdorfer speculated that this survival mechanism of the spirochetes is responsible for the diverse pathology of these organisms, and "their ability to survive as cystic forms thereby producing prolonged, chronic and periodically recurrent disease" (1999). Furthermore, his belief that up to 90% of Lyme disease is cystic in nature, and being unresponsive to conventional antibiotic treatment, might facilitate chronic illness. Microbiologists working on spirochetes in their labs have seen these unique prokaryotes in action, and the "curling up" of membranes to protect themselves is apparently fascinating to watch. Dr. David Dorward's work on spirochetes has shown *B. burgdorferi* (the common North American laboratory strain B31) to have an affinity for B cells, has seen them enter B cells, and seen them emerge with a "new set of clothes" - a B cell membrane as a cloak around their cell wall (Grier, 2004). Dr. Lynn Margulis of the University of Massachusetts works on spirochetes found in the natural environment. Her research indicated spirochetes can survive in a resistant cystic form for a few years in dry mud, becoming active when introduced into a favourable medium, and suggested its likely for *Borrelia* to burrow into human tissue, making similar resistant bodies, and come out when conditions are suitable twenty years later (Jones, 1999). Zajkowska and Hermanowska-Szpakowicz reported that cysts, spherical forms and "blebs" (gemmae) of *B. burgdorferi* are probably connected with MS and Alzheimer's disease (2002). A medical hypothesis put forth in May 2003 focused on the likelihood of two distinct but connected forms of human *B. burgdorferi* infection, with the yet-unrecognized form appearing to have wider geographic distribution, and vastly greater prevalence (Harvey and Salvato, 2003). The authors concluded that Lyme disease currently acknowledges only its zoonosis arm, and is a limited conceptualization of a far more pervasive and unrecognized infection state that must be considered a global epidemic. There is much literature to indicate that diagnostics and surveillance in LD have been extremely challenging.

In the United States, the case definition for surveillance purposes differs from the clinical case definition (CDC MMWR Weekly, 2007). For surveillance purposes, the CDC defines a reportable case of Lyme disease as "a physician-diagnosed erythema migrans at least 5 centimetres in diameter, or at least one objective late manifestation (i.e. musculoskeletal, cardiovascular, or neurologic) with laboratory evidence of infection with *B. burgdorferi* in a person with possible exposure to infected ticks" (2007). Lyme disease is diagnosed clinically in the U.S., according to Dr. Richard Sadovsky of the American Academy of Family Physicians (CityNews, 2007), based on considerations by the attending physician. He also indicated that "infection is often accompanied by false-negative serologic tests, and the positive predictive value of serologic testing is low in patients with vague symptoms unaccompanied by any objective signs". This is one example of the multitude of challenges the Americans have reported in diagnosing LD.

Health Minister Tony Clement (2007) indicated that The Public Health Agency of Canada (PHAC) recognizes the emerging threat of Lyme disease, and the need to work with stakeholders to fight this serious illness in Canada. He indicated that the 1991 National Guidelines on Lyme disease were under revision with Health Canada scientists and laboratories of the Canadian Public Health Laboratory Network (CPHLN) (2007), and the "PHAC has an ongoing program to examine the current distribution of Lyme disease and its vectors in Canada to assess the potential impact of a changing climate on distribution. Underlying this activity is the fact that the passive surveillance of ticks has resulted in the recognition that there is a more geographically widespread risk of Lyme disease in Canada" (Wilson, 2007). This is contrary to what the CPHLN March 2007 Guidelines for diagnosing and treating Lyme disease in Canada seem to indicate however (CPHLN, 2007), and to what practicing clinicians build their perceptions from.

In Canada, Lyme disease can be diagnosed clinically as indicated, or by laboratory test results according to the Canadian Public Health Guidelines (CPHLN, 2007). For a clinical diagnosis, a patient must have had a typical erythema migrans (EM) rash, and have been exposed to an environment where blacklegged ticks are endemic. If the rash is atypical, or "occurs in circumstances in which exposure to the appropriate tick vector

species was unlikely, diagnosis is based on a serological response to *B. burgdorferi* (CPHLN, 2007). Since many patients do not develop an EM rash, nor do they live/travel to vector endemic areas, it is clear that a clinical diagnosis may not be possible for many patients. In the laboratory, currently a two-step approach is used for serological testing, where a borderline or positive enzyme immunoassay (EIA), is confirmed by a second Western blot test, which the CPHLN (2007) stated "should assure that the vast majority of Lyme disease cases are recognized". These guidelines have been criticized heavily for their limits in both considering other paths of exposure, and also with respect to the reliability of serological testing. The CPHLN themselves indicated "the available serological screening tests have limitations to their specificity" (2007). They further add "serological testing should not be undertaken without a thorough appreciation of the geographic and seasonal setting in which the diagnosis is being considered, as well as an assessment of the likelihood that a specific symptom or symptoms complex is due to Lyme disease". Serious concerns arise for the potential to 'miss' patients with Lyme disease in light of these guidelines, and for the ongoing perception in the medical community that Lyme disease patients are rare.

A July 2007 Toronto news report indicated detection and testing is challenging at best. According to the Canadian Public Health Laboratory Network, "Lyme disease is most often recognized by the development of a characteristic skin rash called erythema migrans (EM) at the site of a tick bite" (2007). Health Canada indicated about 70% of individuals bitten by a tick carrying the Lyme bacteria will develop the characteristic "bull's eye" rash, or erythema migrans, however other sources indicate it is only 30% of cases that display a rash (CityNews, 2007). To display the value of a rash in obtaining an accurate diagnosis, Aucott et al. (2009) present a recent study indicating 54% of Lyme disease patients without a rash were initially misdiagnosed, and even 23% of those with a rash were misdiagnosed; evidence that the diagnosis of Lyme disease continues to remain a challenge today. Dr. Kenneth Singleton, a Lyme disease specialist in Baltimore pointed out that "without a rash, diagnosis can be challenging. I believe if we had a good test that was really as reliable for diagnosing Lyme as the HIV test is for diagnosing HIV, many

of the questions we have today would be cleared up because there would not be such a controversy" (Parks, 2007).

The literature has strongly suggested that both testing and diagnostics in Lyme disease have been attacked for a number of reasons (Lang and Territo, 1997). Decades of information regarding testing reliability and sensitivity have forced many patients to question their diagnoses, and to seek further opinions. "Despite published guidelines, controversy persists about the diagnosis and management in patients who do not meet strict diagnostic criteria" (Hoppa and Bachur, 2007). We read "in the absence of erythema migrans, the basis for diagnosis of Lyme disease is the demonstration of an antibody response against *Borrelia burgdorferi* in an appropriate clinical setting" (Gomes-Solecki, Meirelles, Glass and Dattwyler, 2007). Petrovic (1998) discussed difficulties in diagnosis of late stages of Lyme disease based on low sensitivity of serological testing in a 1998 Belgian report. Much evidence for inaccuracy in Lyme disease testing is cited in the literature, due to sensitivity and specificity issues. Numerous concerns regarding the potential for the misdiagnosis of Lyme disease using commercial assays have been voiced by the U.S. Food and Drug Administration (FDA) (Brown, Hansen and Langone, 1999). In their study of the American and European widely used C6 enzyme-linked immunosorbent assay (ELISA) based on an IR6 region, researchers indicated that "contrary to prior reports, the assay sensitivity is greater when the IR6 peptide is derived from the sequence of the same infecting *Borrelia* genospecies" (Gomes-Solecki et al., 2007). This C6 ELISA is the same initial screening test relied upon in Manitoba and Canada. This recent report might be critical in pinpointing a factor behind why such low numbers of diagnosed Lyme disease occur in Canada, if different *Borrelia* genospecies are present. Further indication of questionable diagnostic approach is seen through Harrer, Geissdorfer, Schoerner, Lang and Helm's 2007 demonstration of a clinical case of seronegative Lyme borreliosis, and suggestion that "additional diagnostic approaches may be needed to demonstrate *Borrelial* infection".

New strategies for the diagnosis, treatment and prevention of Lyme disease are urgently needed according to Stricker, Lautin and Burrascano (2006). Stricker et al. (2006) further

indicated the affinity for multiple cell types and the presence of non-replicating forms of *B. burgdorferi* contribute to persistent infection and antibiotic treatment failure. "The controversial clinical science of Lyme disease has impeded reliable diagnosis and effective treatment of this illness. Two major clinical hurdles are the absence of a therapeutic endpoint in treating Lyme disease and the presence of tick-borne coinfections that may complicate the course of the illness" (Stricker et al., 2006). The controversy surrounding the absence of a therapeutic endpoint is splashed throughout the literature, and we read headlines such as "Clinicians clash over new Lyme disease guidelines" (HealthDay News, 2007). Numerous reports paralleling this July 2007 Forbes article about physicians being locked in a fierce debate over how long the course of antibiotics required to kill the infection are documented. As of November 2006, the Infectious Disease Society of America (IDSA) stated "95% of LD cases are cured within 10 to 28 days of oral antibiotics", while a substantial number of doctors and patients argue against the guidelines (HealthDay News, 2007). To exemplify coinfections as a second hurdle in treatment, Hovius, Ramamoorthi, Van Dam, Barthold, Van Der Poll, Speelman and Fikrig (2007) reported a simultaneous infection of *B. garinii* and *B. burgdorferi sensu stricto* results in more severe Lyme borreliosis. Swanson, Neitzel, Reed and Belongia, 2006 indicated the risk of human coinfection differs by geographic location, with the true prevalence of coinfecting pathogens remaining largely unknown for the majority of geographic locations. Furthermore, they cite North America and Europe to present the greatest number of coinfections among people with diagnosed LD, and that abnormal laboratory test results are frequently observed as a result. It was troubling to read that "no prospective studies to assess the immunologic effects of coinfection among humans have been conducted" (Swanson et al., 2006). Owen (2006) also reported difficulties in the diagnosis of Lyme disease by indicating that accumulating evidence suggests that Lyme disease is a far more complex condition than borreliosis alone, in that it may be more appropriate to consider it a tick borne disease complex. The variety of tick microbes which might coinfect a human host, might synergistically act in complex ways and present in a variety of clinical syndromes. Clinicians should consider the likelihood of coinfection when pursuing laboratory testing, or selecting therapy for LD patients

(Swanson et al., 2006). Owen (2006) suggested that "many pieces of the puzzle are missing, and our knowledge of how the pieces fit together is rudimentary".

Prompt diagnoses and antibiotic treatment for LD usually lead to complete recovery, but the literature indicates many cases diagnosed at later stages of illness do not respond successfully to treatment. Furthermore, patients who don't quickly respond to treatment are often denied long-term antibiotic treatment. Much controversy over this issue is woven through Lyme history, and can be summed up in Stricker's 2007 report noting "patients with persistent symptoms after standard (2-4 week) antibiotic therapy for this tickborne illness have been denied further antibiotic treatment as a result of the perception that long-term infection with *Borrelia burgdorferi*, and associated tickborne pathogens is rare or nonexistent". Is there a possibility for some Canadian Lyme patients to go undiagnosed and untreated with perceptions like these in place?

It is clear that the complexity of Lyme disease and its epidemiology present a significant challenge. Patterns identified through examining the geographic distribution of the disease from a global, North American and local perspective in the next chapter bring an additional challenge to determine the perception and potential of the local LD situation. Both a thorough understanding of LD, and an understanding of spatial patterns of the disease are important to assessing risk in Manitoba.

## Chapter IV: Spatial Distribution of Lyme Disease. A Case for Manitoba

Lyme disease is the most common arthropod-borne human disease widely distributed in temperate regions of the northern hemisphere and mild climate areas (Sambri, Marangoni, Storni, Cavrini, Moroni, Sparacino and Sevenini, 2004; Hubalek, 2009; Hildenbrand et al., 2009). Contrary to the global pattern, LD is not widely distributed in temperate Canada, and is considered to be relatively isolated in Manitoba. In this chapter I will examine the distribution of Lyme disease in Manitoba and across the globe, with emphasis on regions that are ecologically similar or are adjacent to the province. These distributions introduce the possibility that the disease is being overlooked in Manitoba.

The ecology and epidemiology of LD presented in chapter two prefaces our discussion of the distribution of disease in the province. As indicated, Lyme disease prevalence is geographically associated with established populations of two preferred tick hosts, the white-footed mouse (*Peromyscus leucopus*) and white-tailed deer (*Odocoileus virginianus*), coupled with their proximity to humans (ALDF, 1999). The widespread geographic distribution of the pathogen in reservoir animal hosts and several tick species suggests that current disease incidence results from the dynamic interplay of tick-host interactions, and natural and anthropogenic environmental changes (Korch, 1994). Lyme disease has emerged as a dynamic and complicated global disease, yet is considered only to be recently emerging in Manitoba.

Global and European Patterns

Lyme borreliosis is the most frequent tick-transmitted disease in the northern hemisphere and especially in Europe and North America (Wilske, 2005; Wormser, 2005). Lyme disease has been reported globally on almost every continent. In Asia, it has been found in China, Korea, and Japan. In Africa, cases have been reported in Morocco. It has appeared in Australia; and South America (Brazil). While LD is endemic in large parts of North America. It is also well established in Europe, where there are an estimated 85,000 new cases of Lyme borreliosis annually (The Commission of European Communities, 2005). These cases are not evenly distributed, however, and some countries experience a much higher rate than others. The recorded incidence in Europe by country is given below (Table 6).

Table 6. Incidence of Lyme disease in European Countries

| Country | 2002 | | 2003 | | 2004 | | 2005 | |
|---|---|---|---|---|---|---|---|---|
| | Incidence | Cases | Incidence | Cases | Incidence | Cases | Incidence | Cases |
| Slovenia | 169 | 3359 | 177 | 3524 | 193 | 3849 | 206 | 4123 |
| Austria (estimate based on physician survey) | - | - | - | - | - | - | 135 | - |
| Netherlands (estimated number of EM cases) | - | - | - | - | - | - | 103 | 17000 |
| Czech Republic | 36 | 3658 | 36 | 3677 | 32 | 3243 | 36 | 3640 |
| Lithuania | 26 | 894 | 106 | 3688 | 50 | 1740 | 34 | 1161 |
| Lander of former East Germany | 18 | 3029 | 24 | 3991 | 26 | 4497 | - | - |
| Finland | 17 | 884 | 14 | 753 | 22 | 1135 | 24 | 1236 |
| Latvia | 14 | 328 | 31 | 714 | 31 | 710 | 21 | 493 |
| Estonia | 23 | 319 | 42 | 562 | 36 | 480 | 21 | 281 |
| Slovakia | 11 | 568 | 14 | 726 | 13 | 677 | 16 | 843 |
| Belgium | 12 | 1269 | 11 | 1118 | 16 | 1607 | 16 | 1644 |
| Bulgaria | 6.5 | 514 | 7 | 550 | 12 | 949 | 13 | 979 |
| Poland | 5.3 | 2034 | 9.4 | 3575 | 10 | 3822 | 12 | 4406 |
| Norway | 2.4 | 111 | 3.2 | 144 | 5.5 | 251 | 6 | 280 |
| Hungary | 12 | 1238 | 12 | 1208 | 12 | 1208 | - | - |
| England and Wales (voluntary reporting | 0.6 | 340 | 0.6 | 335 | 0.9 | 500 | 1.1 | 595 |
| Scotland | 1.7 | 8.5 | 1.6 | 81 | 1.7 | 86 | 1.9 | 9.6 |
| Italy | 0.05 | 29 | 0 | 0 | 0.02 | 10 | 0.001 | 4 |
| Portugal | 0.02 | 2 | 0.01 | 1 | 0.01 | 1 | 0.04 | 4 |

Eurosurveillance, 2005

The incidence of LD in Europe presented from 2002 to 2005 generally increased annually, and clearly there is a significant range of disease incidence among these

European countries. Many of the countries geographically situated nearby one another share similar LD incidence, which might be expected considering the ecology of the disease. The highest number of Lyme disease cases has been recorded among forestry workers or inhabitants of wooded areas in Europe (Niscigorska, Skotarczak and Wodecka, 2003). Lyme disease risk and prevalence are largely unknown in several other countries, including France, however estimates put the incidence in that country at between 86 and 210 cases per 100,000 inhabitants (Blanc, 2009). Given a population of 64 million and a mean incidence (150/100,000) based on that range, nearly 100,000 individuals in France might be infected with Lyme disease. Nearby, LD is very common in Germany, and one of the most frequent bacterial infections (Huppertz, Bohme, Standaert, Karch and Plotkin, 1999). Conversely, the incidence rates in Great Britain are consistently very low (Table 6). Lyme disease is firmly established throughout most of Europe, and although with varying incidence, considerably higher than incidence in either Manitoba or Canada.

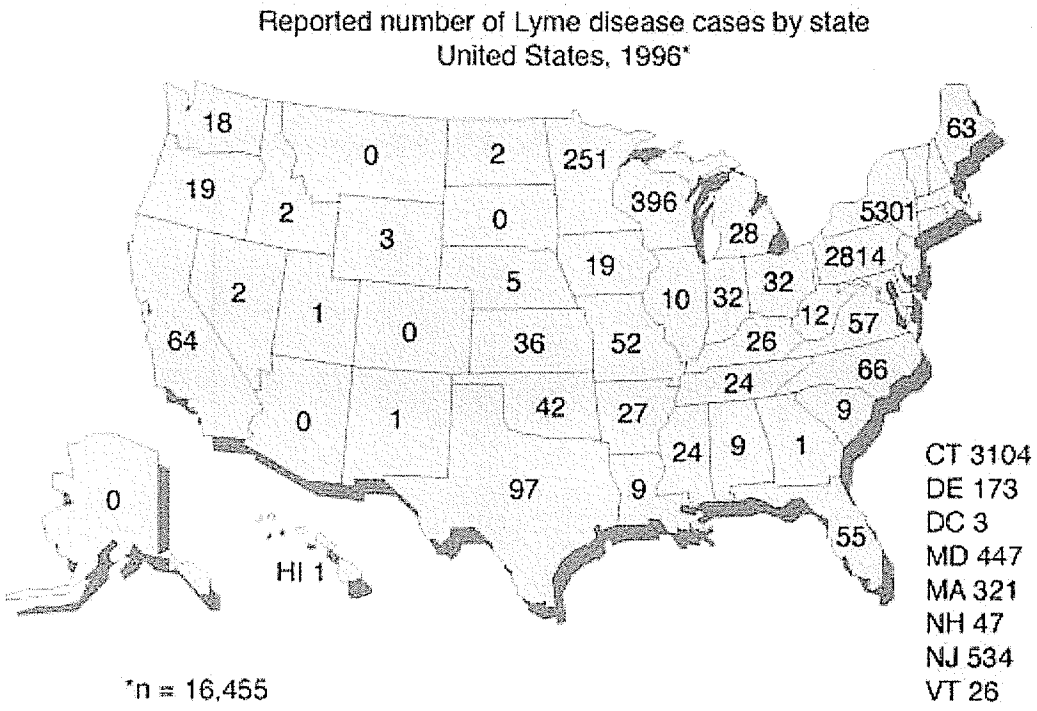
North American Patterns

In North America official recorded incidence tends to take on a broadly binary distribution; that is, there are far more new cases in the United States than in Canada each year. Lyme disease is the most common vector-borne disease in the United States (Hildenbrand et al., 2009), and at least 20,000 Americans have contracted LD every year since 2002 (CDC, 2009). It is not uniformly distributed throughout the country, however. More than 90 percent of the 1999 Lyme cases came from nine states: Connecticut, Maryland, Massachusetts, New York, New Jersey, Pennsylvania, Rhode Island, Wisconsin and Minnesota, all states in which the deer tick is relatively common (CDC, 2007). Ongoing since 1996, the states with the highest number of cases are found in the northeast, with a band extending westward as far as Minnesota. As of 2008, the highest rates were found in the wooded states of the Northeast, with Connecticut (78.2 confirmed cases per 100,000 population), Delaware (88.4), New Hampshire (92.0) and Massachusetts (60.9) leading the way. These were followed by the Atlantic coastal states, farther south (e.g. Pennsylvania 30.7, New York 29.5), and parts of the Midwest (Minnesota 20.0 and Wisconsin 26.5, but compare to Michigan at 0.8). At the other end of the spectrum, the South (e.g. Georgia and Florida both at 0.04), Northwest (Washington 0.03, Oregon 0.05) and Southwest (New Mexico 0.02, Utah 0.01) were largely unaffected by the disease.

Lyme disease accounts for more than 95% of all vector-borne illness in the United States (Edlow, 2003), and ranked ninth on the list of most commonly reported nationally notifiable infectious diseases in U.S. adults in the 1990s, with 21,176 cases reported 1992 – 1994, and a rate of 3.85/100,000 (Johns Hopkins University, 1999). Thorne (1999) indicated that 11,700 U.S. cases were reported in 1995, and figures were climbing steadily. By 1998, there were 16,455 reported cases of Lyme disease in the United States according to David Weld, executive director of the American Lyme Disease Foundation, Inc. (Thorne, 1999). From 1992 – 1999, with more than 100,000 reported cases, Lyme disease ranked as the most common arthropod-borne illness in the U.S. Lyme disease occurrence in many states such as New York for example, which has increased over time and spread throughout the state, yet minimal investigation into the epidemic and spatial

dynamics in this particular state, or others has occurred (Chen et al., 2005). Incidence of disease in the U.S. is largely concentrated in urban centers, yet any acceptance that LD might be contracted within urban landscapes in Canada is largely unrecognized.

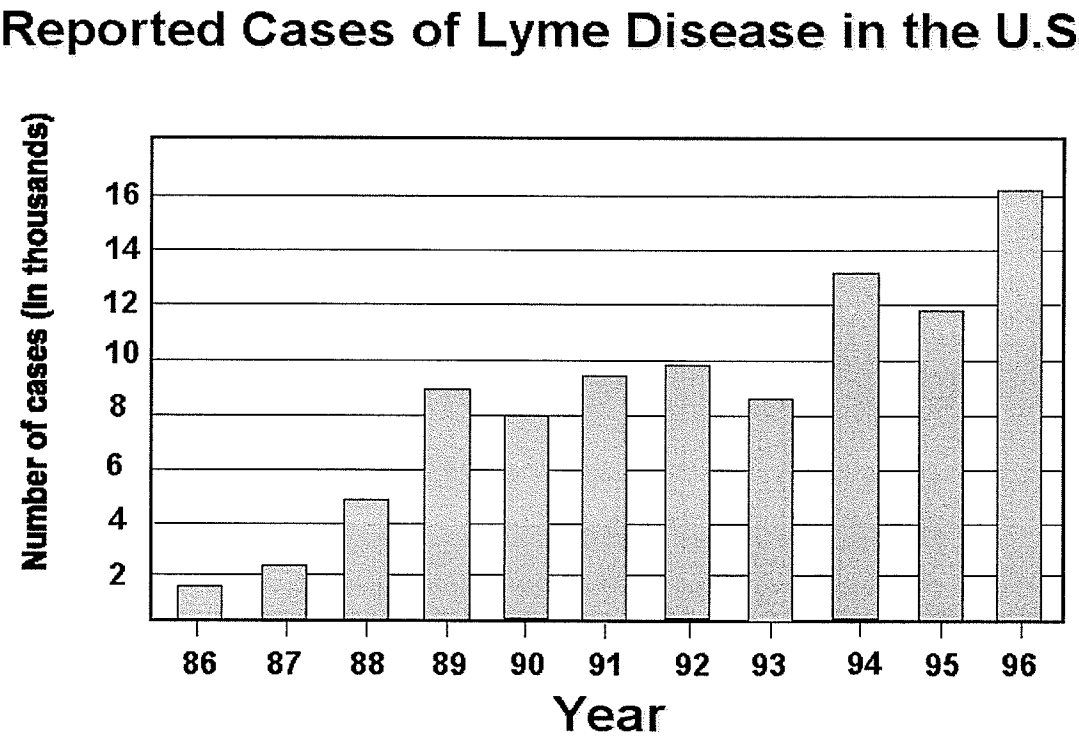
Figure 10. Reported Number of Lyme Disease cases by state United States, 1996




http://www.healthatoz.com/healthatoz/Atoz/images/ency/00042843.jpg, accessed 01/04/04

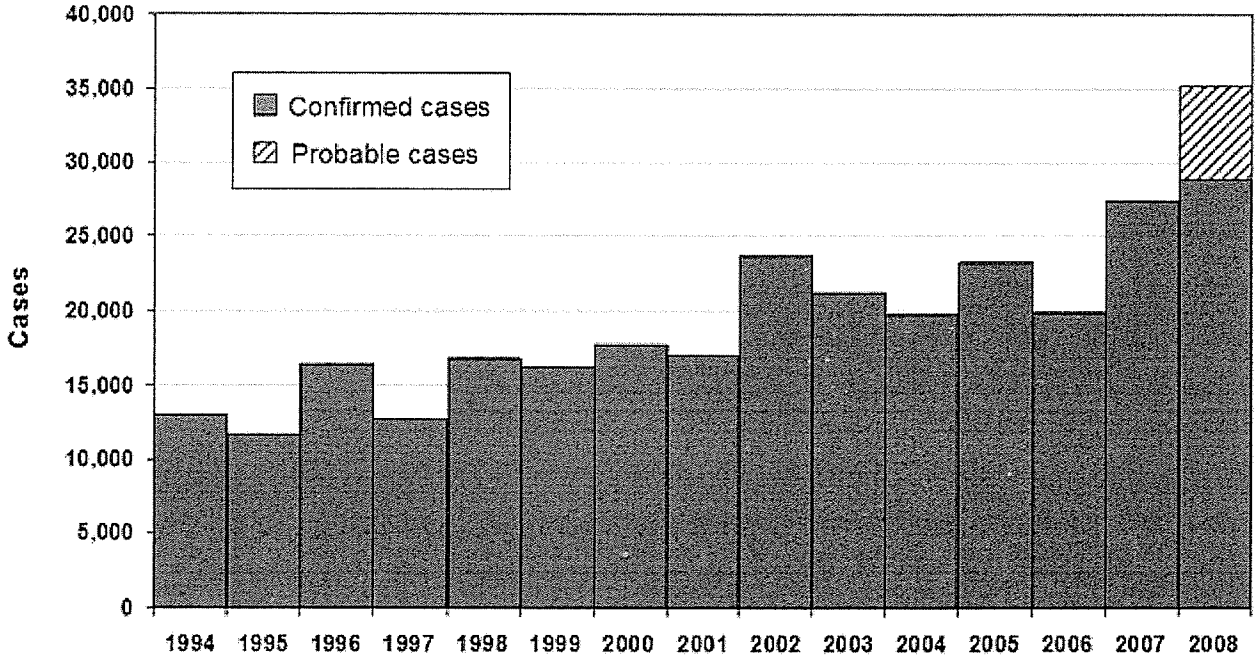
Lyme disease incidence was increasing in the U.S. since it was first recognized in the 1980s, largely due to increased contact between humans and the tick vectors as Americans increasingly build homes in wooded areas, the ideal habitat for ticks (CDC, 2001). Prevalence in the U.S. is primarily associated with established populations of preferred tick hosts – white-footed mice and deer, coupled with their proximity to humans (ALDF, 1999). Edlow (2003) further indicated that reforested areas and desirable parklands, coupled with an enormous jump in the deer population are the main contributors to the climbing incidence in the U.S. The steady increase can be seen in the overall number of annual reported cases between 1986 and 1996 (Figure 11) and 1994 and 2008 (Figure 12).

Figure 11. Reported Cases of Lyme Disease in the U.S. 1986 - 1996




http://biosun.bio.tu-darmstadt.de/infekt/Spirochetes/sld016.htm, accessed 06/23/07

Figure 12. Reported Cases of Lyme Disease by Year, United States, 1994-2008

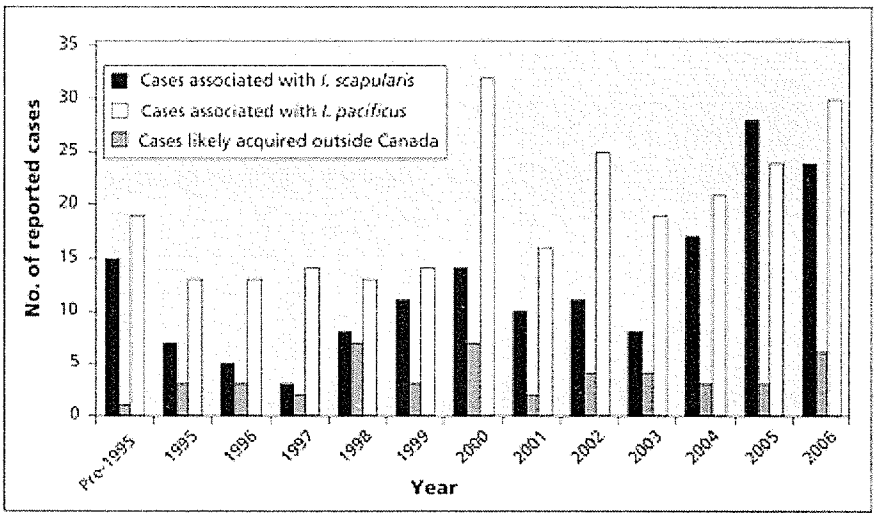



(CDC, 2009)

The number of new cases in the U.S. surpassed the 20,000 mark in 2002 and 2003 as seen above, during which time Manitoba was documenting its very first cases. A significant stretch of Manitoba's border is shared with Minnesota, and the number of Lyme disease cases in 2002 in Minnesota alone was 867 (U.S. CDC, 2001).

During the decade 1984 to 1994, 205 cases of Lyme disease were reported in Canada, with the recognized tick vector being found in several Canadian provinces (dos Santos and Kain, 1999). Between 1987 and 1996, Regush (2000) indicated 278 cases were reported, with the conclusion that *B. burgdorferi* is an accomplished traveler, yet these case numbers in Canada are incredibly low compared to our neighbours in the U.S. Many of the highest rates in the U.S. are situated adjacent, or near to the Canadian border, with presumably similarly environmental conditions and fauna. Given the current levels of travel between Canada and the United States, "we must anticipate an increase in the number of imported cases of these pathogens in Canadians" (dos Santos and Kain, 1999). The U.S. Centres for Disease Control (CDC) reported 23,305 cases of Lyme disease in 2005, while the Public Health Agency of Canada indicated there were 30 to 50 cases of Lyme disease diagnosed that year in Canada (CTV 2007), with Ogden, et al., 2009 graphically indicating 20 to 50 cases have been diagnosed annually from 1995 to 2006.

Figure 13. Annual number of cases of Lyme disease cases reported voluntarily by the provinces and territories since the late 1980s.




Cases of Lyme disease in British Columbia were probably transmitted by *Ixodes pacificus*, whereas cases from all other provinces with cases that were potentially locally acquired (i.e., Manitoba, Ontario, Quebec, New Brunswick, Nova Scotia and Newfoundland and Labrador) were probably associated with *Ixodes scapularis*. Cases affecting patients with a history of travel to an endemic area outside Canada during the period when they likely acquired the infection are considered travel-related or nonendemic.

(Ogden et al., 2009)

Reproduced with permission from the CMAJ 01/09/09

Jackson, Hilborn, and Thomas reported in January 2006 that the incidence of Lyme disease in the U.S. continues to grow, while Ogden, Barker, Beauchamp, Brazeau, Charron, Maarouf, Morshed, O'Callaghan, Thompson, Waltner-Toews, D., Waltner-Toews, M., and Lindsay added in 2006 that the potential for range expansion of Lyme vectors into Canada is an important reality.

Patterns in Canada and Manitoba

Lyme disease is a relatively "new" disease to Manitobans, only recently being added to the list of reportable illnesses in January 1999 (MB Health, 2000). Lyme disease was first tracked nationally in the United States in 1991 (CDC, 2001), a couple of years later in Canada (Artsob, 2004), and only within Manitoba since 1996 (Paul and Zeilig, 2000).

In 2009 LD became a nationally reportable disease in Canada (Ogden et al., 2009). The relatively recent arrival of information into Manitoba's medical arena was evident when a search of the Manitoba Health website by the researcher in April of 1999 turned up no documents related to the following terms: Lyme, tick, borreliosis, or burgdorferi. However, a recent search in August 2009 returned 67 relevant hits based on the same terms, a clear increase in available literature. Looking back, from 1977 through May 1989, 30 cases of LD were reported to Canada's Laboratory Centre for Disease Control (LCDC, 1999), and 5 of the 30 cases were reported in Manitoba. Furthermore, they cited "the areas in Canada with the highest number of cases border on the American states with the highest reported incidence of LD". Other earlier data from the Canadian CDC (1991) reported that 17 cases of the 140 reported from 1984 to 1990, were from Manitoba, and that "Lyme disease is generally rare in Canada".

Moving into this decade, as of the year 2000 Manitoba Health had reported only one lab-confirmed case of Lyme disease ever, in 1999 (2000). They also indicated to Manitoba physicians that the expected number of cases in Manitoba annually, as a result of this statistic, was zero (2001). Certainly the message among the health care providers and the media seems to be that the disease is of little significance in Manitoba. Some provincial physicians believed that the disease was not contractible in Manitoba due to the lack of a significant vector (Fallding, 2003; Peschken, 2000). Studies have shown however, that Lyme disease can be contracted even without the presence of an established vector (Artsob, 2004; Leong, 2001; Rollason, 2001).

In May 2000 we read "Dr. Galloway said he has heard of only one case of Lyme disease in Manitoba" (Saari, 2000), an entomologist studying *Ixodes* ticks in Manitoba. Following the 2001 passive surveillance study (Lindsay, 2001), a summer 2001 Winnipeg *Free Press* article "Parents must prevent bug bites" informed readers that "mosquitoes and ticks can carry debilitating and sometimes life-threatening illnesses." The article also prompted individuals to send ticks to a laboratory to find out if the tick carried Lyme disease. During the same summer week in 2001, an article also appeared in the community newspaper *The Lance*, indicating the same information. Manitobans developing a rash or other symptoms were encouraged to see their doctor, and anyone

finding "suspected deer ticks" were asked to submit them to Dr. Terry Galloway, Department of Entomology, University of Manitoba for identification. This 2001 study apparently made physicians more aware of Lyme disease, and they apparently tested more for it as a result (Dawood, 2004).

The veterinary community was apparently versed in canine Lyme however, since a Woodlands, Manitoba Veterinary Clinic bill dated August 16, 2002 indicated "Lyme disease is in our area...ask about vaccine for dogs". Clearly the presence of the bacterium, and a potential vector route to pets had already been recognized as an issue prior to this time. Sharing of information between the veterinary community and those involved with public health wasn't taking place in 2002, and has yet to take place in Canada.

A November 2003 *Free Press* article (Fallding) informed us that the Canadian Lyme Disease Foundation had received 20 to 30 calls, an unusual number, from Manitobans who suspected they might be infected with LD. There had been only a couple of confirmed cases in Manitoba since the disease became reportable in 1999, and there was some controversy over whether tests miss some cases (Fallding, 2003). CLDF President Jim Wilson indicated that the majority of the calls they received were from individuals facing difficulties with diagnosis, or treatment (Fallding, 2003). Several people apparently sought testing in U.S. labs, where a more sophisticated test was used over the Canadian initial screening test, and were diagnosed. "A couple of dozen cases of Lyme disease are reported every year in Canada, mainly in B.C., the southern tip of Ontario, and now Nova Scotia" he added, but these are clinical diagnoses, not test-positive diagnoses. In 2005, Manitoba Health reported three cases of Lyme disease identified after they had visited the southeastern corner of Manitoba (News Release, 2006). By 2006, a total of 8 reported cases of Lyme disease ever were documented in Manitoba (Manitoba Legislative Assembly, 2006). The Public Health Agency of Canada, in conjunction with Manitoba Health and Health Canada undertook a field survey in southeastern Manitoba during spring 2006 (Manitoba Health, 2006). The results confirmed the presence of the first established population of *I. scapularis* in Manitoba, in

areas around Buffalo Bay. Manitoba has since been a confirmed endemic site for *I. scapularis.*

Comparing Canadian LD incidence to that of adjacent New York and Pennsylvania states, the U.S. CDC reported 28.9 cases of Lyme disease per 100,000 people in 2005, and 34.5 cases per 100,000 respectively. Across the international border, Ontario's associate chief medical officer of health Dr. David Williams indicated "the number of cases in Ontario is consistent to what we've seen in the last five years", with 38 confirmed cases in 2006, and "only 11 came from ticks in Ontario" (Talaga, 2007). Kingston's Public Health medical officer Dr. Ian Gemmill says Lyme disease risk is "something everyone should be aware of. We don't think there's a big risk, an everyday risk for people to be worried about all the time", but if left untreated, the effects of Lyme disease can be serious (Flegg, 2007).

So much attention regarding risk is directed toward geographical areas where *Ixodes* ticks are endemic in Canada. Lyme disease cases however have been diagnosed in areas of Canada where these ticks are not endemic. 26 years ago, in 1983, an 8-year-old boy vacationed at Pigeon Lake south of Edmonton, Alberta, where tick vectors of LD do not reside, and returned with a diagnosed EM rash (Lycka, 1986). This patient's presentation "suggested a new geographic location for Lyme disease, northern Alberta", and Dr. Lycka stressed the need for physicians across the country to be aware of the manifestations and treatment of Lyme disease (1986).

Geographical variations in disease incidence seem to correlate with the prevalence of infected ticks, which are mainly associated with varied deciduous forest (O'Connell, Granstrom, Gray and Stanek, 1998). More specifically, Lyme disease incidence is geographically correlated to the global distribution of ticks in the *I. ricinus* species complex in the northern hemisphere, where boreal forest dominates (Gray et al., 2002). Canada is "draped" with a green scarf of boreal forest, as Canada's largest biome occupies 35% of total Canadian land area, and 77% of Canada's total forestland (Natural Resources Canada, 2004). However, as previously indicated, important tick vector populations are reportedly not established throughout most of our boreal landscape. Yet

as presented earlier, local tick populations can exist over a widespread area via migratory birds. Nature is dynamic, and microbes like *Borrelia* can take advantage of new landscapes when presented with opportunity, reminding us not to be closed minded when it comes to epidemiology.

We read that the "geography of infectious disease is being driven by shifting weather, and the microbes can be here a lot faster than we recognize and respond to them" according to one microbiologist Stephen Morse (Regush, 2000). Zoonoses such as Lyme can emerge in previously nonendemic areas when circumstances favorable to their maintenance and transmission arise (Fritz, 2009). The Canadian Public Health Laboratory Network (CPHLN) reports that "few cases are seen", with most occurring in "close proximity to small geographical areas where infected ticks have become established" (2007). Small geographical areas can emerge annually when supportive conditions arise, but without yearly investigation they might not be identified. Furthermore, they indicated that "Lyme disease incidence in Canada is very low or zero in most parts of central Canada, and in certain parts in western Canada". An incidence of zero seems unlikely given the potential for disease through migratory birds alone. Do messages of zero incidence set the stage for a nationwide risk belief system? This message is communicated to Canadian physicians, along with a note to consider the possibility of false-positive test results in light of this expected low incidence. Is this message of very low risk accumulated or adopted into the perception base of practicing Canadian physicians, when it is entirely possible that several hundred new cases of Lyme disease surface per year in Canada? This could be projected into several thousand cases of Lyme disease that have to date accumulated in Canada. The logic for this can be seen in exploring several factors. First, doctors versed in Lyme have diagnosed several thousand Lyme cases globally over the last 10 years (Wilson, 2009). If the rate of disease in endemic areas of Canada is anywhere near the rate seen in the U.S., then 1000 new cases per year might be diagnosed. In 2005, 13,000 new cases of Lyme disease were diagnosed in the 56 million people who live in the U.S. states Maine, New Hampshire, Vermont, New York, Michigan, Ohio, Pennsylvania, Wisconsin and Minnesota, bordering eastern Canadian provinces (CDC, 2007; Ogden et al., 2009). Also in 2005, approximately 23 million Canadians lived in these eastern Canadian provinces with

endemic foci, including Prince Edward Island, Nova Scotia, New Brunswick, Quebec, Ontario and Manitoba (Statistics Canada, 2008), and seventy-five percent of these Canadians, approximately 17 million people, lived within 150 kilometres of the U.S. border. Comparing to the U.S. statistic of 13,000 cases per 56 million (0.000232), then we might expect roughly 4000 cases for the 17 million Canadians near the eastern borders, if our ticks are infected at the same rate. If the rates of tick infection vary, then it would follow that case numbers would vary accordingly. Given that we have a number of established Lyme endemic areas in eastern Canada, at the very least several hundred new cases of Lyme per year is not an unreasonable estimate. Several hundred is at least ten times the 20 to 50 annually reported number of Canadian cases (Ogden et al., 2009). Furthermore, the number of cases in British Columbia account for at least half of the total number of Canadian cases (Ogden et al., 2009), and have not even been entered into this calculation. Physicians who hear and read that there are 20 to 50 new cases of Lyme disease a year are left with the perception that Lyme disease risk is extremely minimal in Canada, and does their perception of seriousness influence their practice? Furthermore, is Canada an isolated nation in a global sea of higher-risk countries, or is the distribution broader than assumed? Finally, if the disease is more widespread, are there individuals with LD who have not been diagnosed?

## Lyme Disease Mimicry

If, perhaps, LD incidence is being underreported in Manitoba the question arises as to what has happened to these cases. One possibility may be that they are being misidentified. An examination of the literature indicates several diseases that have been diagnosed in sick individuals when in fact the underlying disease was later identified as Lyme disease. The original epidemiological study in Lyme, Connecticut resulted from a suspected record number of cases of Juvenile Rheumatoid Arthritis (JVA). Only upon investigation over some period of time was the true etiology of this mysteriously concentrated "JVA" identified: *Borrelia burgdorferi.* As previously pointed out, Lyme disease has posed difficulty to practicing physicians in both diagnostics and treatment since etiology was identified in 1981 (Burrascano, 2001). There is a growing awareness of the role infections play in a variety of different diseases says Garth Nicolson,

Scientific Director at the Institute for Molecular Medicine in Huntington Beach, California. He believes that the new technologies of our time will allow further detection of infections as causalities, and this awareness to increase (Jones, 1999). Looking back on the last decade to the identification of *Helicobacter pylori* as the causal bacterial agent of peptic ulcers teaches us that we must continuously be open to new and "surprising" information research offers, and be accepting of exciting breakthroughs that defy conventional medicine. Dr. Kenneth Leigner (1993) indicated "Lyme disease will continue to pose difficult problems for us, for our patients and for our society as human intelligence strives to fathom and checkmate *Borrelia burgdorferi*, a biological evil genius". With a label like this, one can't argue with both the need for further LD research, and our need to be open to new and perhaps surprising information.

Around the globe, Lyme disease has been highly misdiagnosed as a number of other illnesses due to the multitude of symptoms victims might display (Edlow, 2003). Dr. Kenneth Leigner suggested there are "so many different manifestations of Lyme disease, that we are going to find that it has a role in a lot of things that we do not yet accept or realize" (Jones, 1999). Dattwyler reported that overlap of Lyme disease symptoms with numerous other infections, and non-infectious diseases makes a definitive diagnosis of Lyme a complicated one (1997). As a result, it has earned the nickname "The Second Great Imitator" (second to syphilis), and been misdiagnosed as multiple sclerosis (MS), chronic fatigue syndrome, lupus, fibromyalgia, ALS, depression, schizophrenia, and Parkinson's among others (Lang and Territo, 1997), before a correct diagnosis of Lyme disease. The Canadian Lyme Disease Foundation reports a list of up to 75 Lyme disease symptoms beginning with a rash, and covering neurological (optic and auditory included), psychological, digestive, musculoskeletal, respiratory and circulatory problems, along with symptoms associated with general well-being (2007), surpassing Grey et al.'s list of 40 symptoms (2002). An August 2007 report out of Lyme endemic Baltimore identified lupus, chronic fatigue syndrome and schizophrenia as a few of Lyme's important imitators (Parks, 2007). Toronto's CityNews staff (2007) reported that in addition to the diseases mentioned above, Lyme has been misdiagnosed as juvenile arthritis, rheumatoid arthritis, infectious arthritis, osteoarthritis, Raynaud's syndrome, scleroderma, Alzheimer's disease, and Crohn's disease. This report asked Canadians to

"imagine that medical doctors can't agree on who has it, how long to treat it, and if in fact the disease is really a problem in Ontario". The Canadian Lyme Disease Foundation website added that individuals have also received misdiagnoses for colitis, Parkinson's, gastroesophageal reflux disease, Fifth disease, Ménières, Sjogren's syndrome, irritable bowel syndrome, prostatitis, encephalitis, sleep disorders, and thyroid disease before receiving an accurate diagnosis of Lyme disease (2007).

The incidence of MS in Manitoba (MS Society, 2000), fibromyalgia (Free Press, 2001), lupus (Free Press, 1999), and Crohn's disease (Paul, 2001), all with an unknown etiology, conjured curiosity and questions several years ago about an environmental link. An examination of the literature on these diseases revealed many connections to the genus *Borrelia* (Edlow, 2003; Lang and Territo, 1997) – the genus responsible for Lyme disease.

Dr. John Bleiweiss, an American physician known for treating many Lyme patients reported "Prior to proper diagnosis, patients habitually report that they were assigned the following diagnoses most often: CFS, MS, fibromyalgia, lupus, candidiasis, chronic mononucleosis, hypoglycaemia and stress-related illness". He wrote "If these appear in a differential diagnosis, then LD should also be considered" (1994). A 1997 news report indicated that "unless a physician has accurate, up-to-date information, misdiagnosis is very likely" (The State, 1997). Is it possible that some LD patients are misdiagnosed in Manitoba, and assigned "conventional" disease diagnoses that best fit the patient's array of symptoms?

Although speculative, if a small proportion of MS, ALS, Parkinson's, Chronic Fatigue Syndrome and fibromyalgia cases are actually misdiagnosed Lyme disease, then clearly our annually reported Canadian Lyme cases would climb. In Canada, approximately 1000 cases of MS, 500 cases of ALS, 5000 cases of Parkinson's, and 340,000 cases of Chronic Fatigue Syndrome and fibromyalgia are diagnosed annually (MS Society, 2000; Brain Research Centre, 2009; AHFMR, 2003; FM-CFS Canada, 2007). These diseases are difficult to diagnose in their early stages, and if only 1% of MS, ALS and Parkinson's are misdiagnosed Lyme, this would represent 65 cases per year. Additionally, if 1% of

new Chronic Fatigue and fibromyalgia cases are actually Lyme, then this number increases dramatically.

In a study of 103 U.S. patients exhibiting clinical evidence of multiple body system involvement, 94 were identified as positive for *Borrelia burgdorferi* by the Mattman Blood Culture test (a highly reliable and valid test which supports the cell-wall deficient forms – cysts, blebs, L-forms) (Whitaker and Mattman, 2000). The original diagnoses, before Lyme disease, and numbers of patients follow:

Table 7. Original Diagnosis of Patients testing positive for *Borrelia burgdorferi*

| Original Diagnosis: | Number of Patients Diagnosed: |
|---|---|
| Multiple Sclerosis | 40 |
| Fibromyalgia | 30 |
| Amyotrophic Lateral Sclerosis (ALS) | 17 |
| Chronic Fatigue Syndrome | 8 |
| Osteoarthritis | 1 |
| Mixed Connective Tissue Disease | 3 |
| Polymyalgia Rheumatica | 1 |
| Lupus Erythematosus | 1 |
| Ankylosing Spondylitis | 1 |
| TOTAL: | 103 |

Whitaker and Mattman, 2000

A review of these results allowed Whitaker to conclude "it is paramount to accept the fact that Lyme disease is the most common and rampant vector-borne infectious disease in the U.S."(2000). Given Whitaker's results show that misdiagnoses were in fact made, and eventual correct diagnoses of Lyme disease followed after serological testing, why did the original diagnoses come in so many varieties? It seems reasonable to assume that patients presented with a plethora of symptoms which varied in type, intensity and body systems affected. Since human beings differ in their predisposition to infectious diseases and resulting clinical manifestations, factors including genetics, age, sex, nutritional status, previous exposure, and immune competence contributing to a greater or lesser

susceptibility to a particular infectious disease require attention (Snydman, 1989). The clinical presentation of *Borrelia* in such varieties as displayed in Whitaker's report may be a result of wide-ranging individual differences among patients, in addition to possibilities of coinfections. The Fox Chase Cancer Center's Department of Pathology reported that Lyme disease is capable of producing a wide variety of clinical pathologic conditions and lesions having common histologic features of collagen-vascular disease (Duray and Steere, 1988). Furthermore, they reported that much of the histologic derangements suggest immunologic damage in response to persistence of the Lyme spirochete. The combined outcome for clinical presentation is often so varied, that it has been widely accepted that misdiagnoses do occur (Lang and Territo, 1997).

There is a growing awareness of the role infections play in a variety of different diseases stated Garth Nicolson, Scientific Director at the Institute for Molecular Medicine in Huntington Beach, California. His belief is that the new technologies of our time will allow further detection of infections as causalities, and this awareness to increase (Jones, 2000). "Diagnosis (of Lyme disease) is based on the clinical picture, coupled with serologic data and epidemiologic findings, particularly history of tick exposure. Late stages of Lyme disease usually are associated with extensive nervous, cardiac or joint involvement, and tend to mimic many of the diseases misdiagnosed in Whitaker's study (Lang and Territo, 1997). Differential diagnoses between Lyme arthritis, encephalopathy or polyneuropathy and other syndromes such as chronic fatigue or fibromyalgia is difficult, and the management differs significantly" (MB Health, 2000).

Exploring a few of the most common Lyme misdiagnoses, particularly MS, uncovers some intriguing and useful information for both physicians and patients, and is relevant to this study.

Multiple Sclerosis

Multiple sclerosis (MS) is a diagnosis of exclusion, and accurate diagnosis is increasingly important with available disease modifying therapy (Trojano and Paolicelli, 2001). Unfortunately the rate of misdiagnosis remains around 5%-10%, indicating that 1 in 20

patients thought to have MS has, instead, a condition resembling MS. Furthermore, Trojano and Paolicelli (2001) described conditions often confused with MS that may be inflammatory, genetic, or infectious, such as Lyme disease, and "the key to the accurate diagnosis of MS is vigilance for atypical features, suggesting the possibility of an alternative diagnosis".

Lyme disease has been misdiagnosed as MS around the globe (Lang and Territo, 1997). In Canada, nine cases of Lyme disease were identified in Saskatchewan, and "some people had been misdiagnosed and told they had multiple sclerosis" (MacBean, 1998). The epidemiology of MS, and the geographic distribution parallels that of LD (Bleiweiss, 1994), and should be considered in a province reporting high incidence of MS. In personal correspondence from a Manitoba rheumatologist, it was indicated "please keep in mind that while MS is, unfortunately, relatively common in Manitoba, Lyme disease to date is quite uncommon because of the vector distribution". (Peschken, 2000).

According to the literature, environmental studies in multiple sclerosis had been very much neglected, until it was noted by Steiner that there is a definite geographic difference in the occurrence of this disease (1952). He also indicated that intrafamilial cases in the state of Michigan were common, but noteworthy was that in all of the familial cases, afflicted persons shared the same household. Furthermore, of interest in Steiner's study was the case of a girl with MS, who lived with a boarder nurse who took care of another woman across the city with MS. The girl with MS had cats, and at the time of the MS patient's death across town, it was found that this patient's cat had to be removed because it was flea-ridden. It was suggested by Steiner that it was possible for the nurse to transfer fleas from clothing, etc. to the home of the girl on her frequent trips. The conclusions of this study were that a particular environmental extrahuman reservoir of the disease agent is highly probable, and its accumulation was found more so in rural areas. Furthermore, insect vectors were reported to be questioningly significant, with the evaluation of this factor a difficult one due to the chronicity of the disease (Steiner, 1952). Other examples of familial cases of MS are evident in the literature. "Tom Norris, dependent on a wheelchair, was recently diagnosed with Lyme disease after nine years of being told he had multiple sclerosis" reported Rebecca Merritt in the Oregon

paper the *The Bulletin* (1999). Norris' brother was also diagnosed with MS. Johns Hopkins reported that physicians must rely on their clinical judgment in diagnosing someone with Lyme disease, and a "clustering of Lyme disease symptoms among family members" should be looked at suspiciously (1999).

Half a century ago, in a 1954 study of four MS patients, findings of abundant numbers of spirochetes in their central nervous system prompted further questions (Steiner, 1954). The genera of spirochetal bacteria recognized in the CNS of these patients seemed to be *Borrelia.* A fatal case of multiple sclerosis was reported two years prior by Steiner (1952), and spirochetes were discovered in the CNS of the patient post-mortem. The spirochetes "resembled the *Borrelia*-type of spirochete, with minimal thread angle at 60°, maximum at 130°, and average being 97°, which is totally different from the *Treponema*-type spirochetes". In conclusion, Steiner wrote "it is well known to the pathologist that the microscopic search for the agent in chronic infections such as syphilis is often troublesome, and does not succeed. Why should it differ in multiple sclerosis?" This 1952 report also detailed findings of spirochetes in other MS patients up to 1936: Among 48 examined cases of MS, 12 (25%) were spirochete-positive. Two of the 12 cases had numerous spirochetes. At the time of this study, Lyme disease had not been identified. There were no diseases related to *Borrelia* infections in the United States to date, however these MS patients were found to likely have the spirochetes in their tissues.

"Lyme disease, caused by *Borrelia burgdorferi* in fact may be one of the major causes of Multiple Sclerosis," wrote Kurtz in a 1986 medical hypothesis. Kurtz reported that little was known about the physiology of the Lyme spirochete at the time, and that relatively little had been done on *Borrelia* physiology, but that both ticks and lice spread the spirochetes to vertebrate hosts, including humans, birds and rodents. The literature prompting her investigation included up to fifty references citing a connection between MS and spirochetes. Most interesting to note is before World War II, no cases of Multiple Sclerosis were reported on the Faroe Islands, until a cluster of 24 cases arose between 1943 and 1960 after British troops were stationed there (Kurtz, 1986). A strict control on importing dogs had been in place until the troops arrived, and brought their dogs along. Also, in Iceland, MS appeared as an epidemic from 1945 – 1954, and also in

1923 – 1944, after WWII and WWI respectively. Dogs or cats may be suspected as reservoirs of the disease "agent", since many MS patients had close contact with them (Kurtz, 1986). Her report also indicated in 1986 that the lipid content of the human central nervous system myelin provides an excellent growth medium for *Borrelia.* Kurtz also indicated Ross Ichelson was able to cultivate organisms from spinal fluids of persons with MS, which were similar in morphology to those described by Steiner. Additionally, Kurtz indicated a Dr. Sibley in Tucson, Arizona tested a group of 8 MS patients and found that 2 of the patients had elevated antibody titers to both *Borrelia burgdorferi,* and *B. hermsii.* This report further detailed that *"Borrelia* infections, when left untreated, are now known to be capable of causing neurological problems, relapses as much as ten years later, and increased IgG/albumin ration and increased lymphocytes. These same things hold true for Multiple Sclerosis patients" (Kurtz, 1986). The fact that there are a variety of types of MS: relapsing-remitting, primary-progressive, secondary-progressive, progressive-relapsing, benign MS and malignant MS, which have wide-ranging impacts on affected individuals (MSSC, 2000) obviously invites the questions "are these a group of different diseases with different etiologies" and "could Lyme disease be one of them"? Some MS patients present relapsing-remitting disease while Lyme disease and its pathology presents in the same clinical fashion.

In 1987, Weder, Wiedersheim, Matter, Steck and Otto reported five patients with chronic meningitis who were hospitalized several times for progressive neurological symptoms, where "from the clinical point of view it was repeatedly difficult to exclude multiple sclerosis". Lyme disease was not considered initially because there was no history of tick-bite or rash. The latency between the first symptoms and diagnosis had varied from 3 months to 5 years, and with penicillin treatment, all patients significantly improved. The case of a 45 year-old female with the diagnosis and 15 year history of relapsing/remitting MS was documented by Lana-Peixoto (1994). Positive serology on both ELISA and Western blotting from two different labs confirmed *B. burgdorferi* infection. The author suggested that as Lyme neuroborreliosis may clinically mimic multiple sclerosis, the presence of antibodies to *B. burgdorferi* in serum of patients with MS-like disease in non-endemic areas for Lyme disease may be troublesome.

The U.S. CDC indicated Lyme disease is often difficult to diagnose because its symptoms and signs mimic those of many other diseases, and "neurologic signs can mimic those caused by other conditions, such as multiple sclerosis" (1991). In 1999, the NINDS indicated "recent reports suggest that the neurological problems associated with Lyme disease may present a clinical picture much like MS". Dr. Ronald Murray replied to a Colorado HealthNet question "someone who suffers symptomatology for multiple sclerosis should have a check for the possibility of Lyme disease" (1999), and "the differential diagnosis in MS considers Lyme disease" (Weinreb, 1999).

The literature is rich with evidence that Lyme and multiple sclerosis are often difficult to distinguish. Brinar and Pozer reported the importance of laboratory methods for MS diagnosis is often overestimated now, and that several conditions may mimic the appearance of MS, one of which is Lyme disease (2002). "Lyme disease is known to cause intermittent neurologic events. CSF findings may resemble those found in MS, and MRI may show a white matter disease" (Spencer S. Eccles, 1999). "Lyme disease comes with a variety of symptoms. It's hard to diagnose because it can mimic so many other diseases, including, in later stages, Alzheimer's disease and multiple sclerosis" (Albany County Health, 1999). "Sometimes the signs of Lyme disease can mimic those of other neurological illnesses, such as multiple sclerosis, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease)" (Pekkanen, 1989). "Diagnosis of Lyme neuroborreliosis is a major challenge because neurological signs and symptoms may imitate those of multiple sclerosis, and many other diseases of the nervous system" (NIH Guide, 1993). Kubova (2006) reported "neuroborreliosis is a form of borreliosis that affects the central and/or peripheral nervous system", and can "mimic neurologic and ophthalmologic disorders such as multiple sclerosis and optic neuritis".

"So many people ask how we can be "sure" they have MS. Other rarer illnesses can have a somewhat similar clinical picture, including Lyme disease which is a common concern" (Yanofsky, 1999). Agosta, et al. (2006) reported that neuroborreliosis is frequently indistinguishable from multiple sclerosis (MS) on both clinical and radiologic grounds. Dr. Bleiweiss reported that MRI does not reliably distinguish between MS and LD because there is too much overlap in their supposedly distinct appearance and location of plaques (1994). Furthermore, Bleiweiss reported that a case of LD which fulfilled all

criteria for MS was documented, and patients with LD, previously diagnosed with MS, responded to antibiotic therapy. "Diseases that may mimic MS include Lyme disease, so MRI and a careful review of symptoms and history are important in making the diagnosis" (Scott, 1991).

Batinac, et al. (2007) indicated that studies suggest that bacterial infections, especially *Borrelia burgdorferi* infection, play a role in etiology of MS. Furthermore, Batinac et al. described the distribution of the Lyme pathogen to be parallel with MS prevalence, and concluded that "late stage Lyme disease can cause demyelinating involvement of the central nervous system, and MS can be erroneously diagnosed".

It is important to note that "if Lyme disease is left untreated, in its later stages it can cause symptoms similar to MS. It even causes MS-like plaques to show up in MRI scans. For this reason, a test for the absence of Lyme disease antibodies is now a standard diagnostic test for confirming a diagnosis of MS in the United States (Kansas Dept. of Health, 1999). Triulzi and Scotti reported "it is widely accepted that magnetic resonance imaging (MRI) findings are not totally specific for the diagnosis of multiple sclerosis. White matter lesions that mimic those of multiple sclerosis may be detected in both normal volunteers and patients harbouring different diseases. Different conditions such as Lyme disease...can be virtually indistinguishable from multiple sclerosis on conventional MR images" (1998). Dr. Bleiweiss reported that MRI does not reliably distinguish between MS and LD because there is too much overlap in their supposedly distinct appearance and location of plaques (1994). Furthermore, Bleiweiss reported a documented case of LD which fulfilled all criteria for MS, and that other patients with LD, previously diagnosed with MS, responded to antibiotic therapy.

In 2002, Fritzsche indicated that in temperate climates infection rates of *Borrelia garinii* in ticks collected from seabirds matched the global geographic distribution of MS. This was the pathogen species recently collected from seabirds in Newfoundland (Smith et al., 2006). Curiosity regarding *Borrelia garinii* arises due to its reputation as the most neurotropic of the genospecies of *B. burgdorferi* sensu lato that cause Lyme disease in Europe (Smith et al., 2006). Given *B. garinii* is in Canada, and the fact that we do not

test for this species in Canada nor the variety of other *Borrelia* species that have been associated with neurological disease presentations, we might suspect that some individuals are infected, and being misdiagnosed.

In 2005, Fritzsche described the necessity for a scientific, sound approach and an antibiotic trial in patients from *B. burgdorferi* endemic areas with established MS and/or *Borrelia* L-forms in their spinal fluid. Fritsche's basis for this recommendation is in recognition of the devastating impact MS has on individuals and their families, and that no curative treatment exists. The fact that "worldwide, MS prevalence parallels the distribution of the Lyme disease pathogen *B. burgdorferi,* and in addition to known acute infections, no other disease exhibits equally marked epidemiological clusters by season and locality" (Fritzshe, 2005) cannot be overlooked.

Lida Mattman, Professor Emeritus of Microbiology at Wayne State University has studied bacteria over decades (Jones, 2000), and pounds her fist to the table when stating:

> I would like a neurologist to tell me why he can't believe that multiple sclerosis (MS) is due to a spirochete when all you have to do is take a drop of spinal fluid and look at it – you can see them. Look at the people having trouble with MS, and no research being done on this – it's criminal.

Dr. Patrica Coyle M.D. Ph. D. presented a study at the 1996 San Francisco International Lyme Conference titled "MS vs. LD a diagnostic dilemma" (Grier, 2004). In this study, 47 patients were tested for Lyme disease, of which 15 (31%) had laboratory findings consistent for LD, and 13 of the 15 positive patients responded to antibiotic therapy. The conclusion was that Lyme patients are being misdiagnosed with MS.

> The Canadian Public Health Laboratory Network Guidelines (2007) indicate: a patient with typical findings of multiple sclerosis or chronic fatigue without objective findings is highly unlikely to have Lyme disease, and both the physician and patient should be dissuaded from serological testing. In such settings, the low pretest likelihood of Lyme disease greatly increases the chance of a false-positive result; such false-positive results are often difficult to discount by either the ordering physician or the patient, and these results often lead to unnecessary treatment.

Clearly, the Canadian Guidelines present a picture of risk that is not in line with the rest of the northern temperate world. What impact does this have on an acting physician when a potential Lyme disease patient walks through the door?

Reports of elevated prevalence of MS are common in the literature. Warren and Warren (1993) reported prevalence rates of clinically definite MS for Westlock, Alberta to be 200/100,000 in 1991, compared to 1.91/100,000 from the first recorded incidence 1950-1959. A prevalence study was also carried out in the Barrhead area of Alberta, where the prevalence rate for clinically probable/definite multiple sclerosis on January 1, 1990 was 196/100,000 (Warren and Warren, 1992). "The average annual incidence rates for patients living in the area at onset were 1.31/100,000 for 1950-59, 4.97/100,000 for 1960-69, 3.77/100,000 for 1970-79, and 4.22/100,000 for 1980-89." It is interesting to note that the majority of patients (40%) experienced multiple symptom onset, and that 50% were of single ethnic origin (either British or German), while the rest were predominantly of North European heritage. Additionally, 40% of patients reported another MS relative. A 1984 – 1989 study in Alberta identified the overall crude prevalence of MS at 216.7/100,000, with the researchers indicating the prevalence to be among the highest in the world, and the province appeared to be "an excess risk area relative to other global locations" (Svenson, Woodhead and Platt, 1992). Furthermore, they indicated that because of the uneven distribution of MS throughout the province, the involvement of environmental factors related to illness onset was supported. At home, we read "Manitoba is a hot spot for multiple sclerosis" (CBC News, 2004). MS prevalence and incidence in Manitoba are among the highest in the world, with 110 new cases diagnosed annually in the province as of 2004 (CBC News, 2004). Furthermore, it was reported that the Manitoba data parallels Alberta and Saskatchewan, as well as northern European areas. With Canadian Guidelines in place, the people living with MS in these communities are restricted from testing access to investigate the possibility that they are living with a treatable Lyme disease infection.

Lupus

A radio promotion for the Lupus Society of Manitoba (Garland, 2007) indicated approximately 50,000 Canadians live with Lupus. In a Winnipeg *Free Press* article published in 1999 (Strachan) we read "Lupus is an autoimmune disease, the cause and the cure for which is unknown". Vice-president and director for the Lupus Society of Manitoba at the time reported that Lupus is very different for everyone who has it, which is why it's called the disease of a thousand faces (Dohan, 1999). Lyme disease has been misdiagnosed as lupus (Lang and Territo, 1997). Lois Ravotti, a Williamstown, New Jersey resident had been diagnosed with lupus. She eventually received a diagnosis, and treatment for Lyme disease, along with her 10 year-old daughter, Dana (Stanton, 1997).

Federlin and Becker (1989) reported a 39-year-old woman with a tick bite in June 1987, followed by local erythema. She had developed pain in various joints with Raynaud's phenomenon at the fingers, swelling of her knee joints, and shoulder pain. Demonstration of antibodies against *B. burgdorferi* antigen was shown, but she was ultimately diagnosed with SLE (lupus). The authors indicated interference of both diseases and their similarity in symptoms may impede correct diagnosis.

Dr. Paul Lavoie, a San Francisco rheumatologist recovered *B. burgdorferi* DNA in the blood of patients diagnosed with systemic lupus erythematosis (SLE) (1992). The CDC also accepted three of Dr. Lavoie's case studies suggesting that a spirochete might contribute to the immunopathogenesis of SLE (lupus). Furthermore, they reported that the disease captures the imagination of physicians, especially in endemic areas such as Wisconsin. We read in the Wisconsin Medical Journal "Lyme arthritis is FREQUENTLY mistaken clinically for rheumatoid arthritis, or systemic lupus erythematosis" (Hejka, 1989).

Manzeniuk, Vorob'eva, Kozarenko and Andreichuk (2004) studied sera test-systems for their sensitivity and specificity to detect *Borrelia* antibodies in patients with syphilis, Epstein-Barr infection and SLE. Their results indicated that the simultaneous use of the ELISA test-systems for detecting IgG and IgM "significantly increased the efficacy of

diagnosis of early borreliosis". It is interesting to note that some physicians in the U.S. are finding success treating lupus, along with rheumatoid arthritis, with antibiotics (Lavoie, 1992).

Arthritis

Seidel, Domene and Vetter (2007) reported "the symptoms of Lyme borreliosis are similar to those of a variety of autoimmune musculoskeletal diseases". We read in the Wisconsin Medical Journal that "Lyme arthritis is FREQUENTLY mistaken clinically for systemic lupus erythematosis or rheumatoid arthritis" (Hejka, 1989).

Arthritis was one of the first symptoms identified in the Lyme, Connecticut community during the 1970s before the Lyme disease pathogen was identified (Lang and Territo, 1997). In a survey of one hundred and twenty-four doctors treating Lyme disease in an endemic area, arthritis was the presenting sign in 16% (Eppes, Klein, Caputo and Rose, 1994). Lyme arthritis has plagued many patients, and generally arises apparently spontaneously, predominantly affects the knee, and has an intermittent course (Pourel, 2007). "Patients with Lyme arthritis usually present with a mildly painful swollen knee" (Feder, Abeles, Bernstein, Whitaker-Worth and Grant-Kels, 2006). Corapi, White, Phillips, Daltroy, Shadick and Liang (2007) pointed out that since many patients with Lyme disease develop arthritis and are referred to rheumatologists, it is important that these health-care providers are well versed in the disease.

Fibromyalgia

An article released in the *Journal of Rheumatology* on the London Fibromyalgia Epidemiology Study in 2001, and reported by the Winnipeg *Free Press* (2001) revealed that fibromyalgia affected 700,000 Canadians at the time. One in 20 women were reported to be affected, costing taxpayers $350 million each year. "It's not a fake illness, there is something real here" says Kevin White, a London, Ontario rheumatologist and epidemiologist described fibromyalgia as the "ache and fatigue you have with the worst flu you have ever had". Lyme disease has been misdiagnosed as fibromyalgia (Lang and

Territo, 1997). In a study of 287 patients seen with Lyme disease during a 3.5 year period, 22% (85) had fibromyalgia associated with this illness (Dinerman and Steere, 1992). Three infectious diseases have been linked to the development of fibromyalgia, and all may develop clinical and serological features suggesting a diagnosis of lupus – Lyme disease is one of these (Bennett, 1999).

Crohn's disease

Manitoba has the highest rate of Crohn's disease in the world (Paul, 2000). In the same front page *Free Press* article, provincial epidemiologist Dr. Jamie Blanchard stated "Many of us think there is an environmental component, whether it is dietary, environmental or infectious". The prevalence rate for Crohn's in Manitoba is 200 per 100,000 individuals – more than double the highest rate reported in countries where the disease is most common. Epidemiologic theories mentioned currently revolve around MAP, a mycobacteria related microbe which Manitoba's terrain and high water table could support, a potential link to large-scale farming practices, or an environmental association of some kind to temperate climates (Paul, 2000). It is definitely interesting to note that Manitoba is home to the highest global infection rates for *both* Crohn's disease and multiple sclerosis, and according to Dr. Jamie Blanchard, that the geographical distribution of the two diseases are strikingly similar in Manitoba (Paul, 2000). Certain individuals at greater risk for contracting zoonotic diseases, include those occupationally exposed, such as farmers, or those recreationally exposed, such as hunters (Artsob, 2000). Incidentally, the farming community is documented to have higher than average Lyme infection rates.

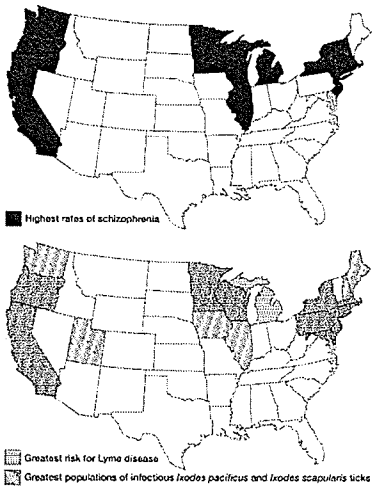
Two intriguing reports worthy of some attention follow. First, a New Jersey doctor, Martin Freid found Lyme infections in at least two patients with Crohn's disease, which affects some 50,000 Canadians, and whose cause has eluded medical researchers (Jones, 1999). Dr. Freid saw an 8-year-old girl with blood in her stool, a typical symptom of ulcerative colitis and Crohn's. Once prescribing an anti-inflammatory, he sent a tissue biopsy for analysis, which returned an active Lyme infection. The girl made a complete recovery after one month of antibiotic treatment (Jones, 1999). Second, Dr. Gordon

Greenberg, Professor of Medicine at the University of Toronto cited evidence from his own clinic with success in treating Crohn's patients with specific antibiotics (Jones, 1999).

Other Diseases

Fritzsche (2002) wrote about seasonal fluctuations of Lyme borreliosis in Europe in association with both MS and schizophrenia. Psychiatric manifestations of Lyme disease are apparent in the literature, and Fritzsche noted the periodicity of *Ixodes* along the west and east coast in the U.S. with MS, schizophrenia, and a similar trend in amyotrophic lateral sclerosis (ALS). The conclusion emphasized the seasonal and geographical overlap between schizophrenia, MS, and neuroborreliosis, and a causal relationship from exposure to a flagellar virulence factor at conception and delivery. Dr. Kenneth Singleton recently reported "It's thought that fifty percent of all Lyme patients have severe neurologic and psychiatric illness associated with their Lyme" (Parks, 2007). Dr Brian Fallon further indicated in 1997 "Anytime you see a young patient with memory problems, then you have to start wondering, could this be Lyme disease?" (Doctor's Guide, 1997).

Figure 14. Schizophrenia and Lyme disease in the United States:




Red = Highest risk of schizophrenia

Gray = Highest risk of Lyme disease

http://www.ij-healthgeographics.com/content/figures/1476-072X-1-2-2.jpg, accessed 06/23/07

In a Minnesota study, 9 of 27 patients with Lyme encephalopathy displayed depression, with fatigue being a common symptom as well (Kaplan, Meadows, Vincent, Logigian and Steer, 1992). A more recent study surveyed 88 patients at a Lyme disease clinic, and reported significantly higher depression scores in the seropositive patients (Kaplan et al., 1992). Depression as a Lyme disease symptom is well documented, and would likely be identified as Lyme only if coincident with other symptoms. Dr. Singleton indicated many Lyme patients suffer with depression, and are often suicidal as a result (Parks, 2007).

Tsirmpas and Tsirmpas (2006) indicated Lyme disease is a multi-system illness, and because it mimics other pathologic conditions, goes frequently undiagnosed and consequently untreated. The extremely low incidence of Lyme patients in Manitoba when compared to other northern temperate regions might be cause to look further into other diseases such as MS that mimic Lyme, and have higher than average incidence in the province. The possibility exists that perhaps Lyme disease is more widespread than currently recognized, and might correspond to the population distribution of disease mimicry victims.

Given the limited presence of Lyme disease in the province of Manitoba according to official statistics, and the possibility of misidentification with other diseases, questions may be raised about the actual distribution of the disease. Of critical importance, then, is whether the critical science behind Lyme disease is reaching all of the front-line health workers, those who are responsible for initiating the process of disease identification, and the public. Moreover, a crucial related question concerns the degree to which those frontline people may have been influenced in their diagnosis by the apparent rarity of the disease. In the chapter that follows I will explore the knowledge and attitudes of four select groups: scientists, policy/decision makers, clinicians, and disease-specific advocates to determine their level of LD knowledge, and the application of this knowledge to their perceptions and practices. Of additional concern is whether or not there is effective dissemination of the critical LD information between the groups.

## Chapter V: Risk Perception of Lyme Disease in Manitoba

Given the complexity of the science behind the epidemiology and distribution of LD, it would seem likely that there would be differences in the perception of its risk between different groups in Manitoba. In order to assess those differences, twenty-three detailed interviews were carried out and then analyzed for content. A grounded theory approach was utilized for analysis, which produced three themes, and, eventually, a summative theory. In this chapter I will present the results of these interviews and grounded theory analysis, while referring back to the literature for support. The analyzed data from this study will be organized around the research questions posed to the key informants.

### Research Question One: What is your level of expertise/knowledge regarding Lyme disease?

This broad and open question was delivered first to provide the researcher with a baseline to build the rest of the interview, and was designed to draw out details of the informants' education and expertise, or lack thereof prior to further questions. The key informant responses to this particular question were extremely varied, both between the four groups of participants, and within the groups themselves. Within the group of regulatory and academic scientists, very few individuals indicated a high level of knowledge, with most reporting above average, moderate "with holes", fairly familiar, informed, or knowledgeable of certain disease details within the context of their particular field, such as entomology. It was noted that there are very few scientists with significant self-identified expertise in Lyme disease. This important finding sets the stage for further exploration of this small group's contribution to the understanding of Lyme in Manitoba. Logically, this group's expertise should peak the hierarchy of information flowing to all other groups. As the interviews developed, it became apparent that certain barriers seemed to be in place, preventing the full and unfettered communication of this expertise to the other groups.

Amongst the group of policy and decision makers, there was a slight range of responses, but collectively this group seemed to have a fairly high level of knowledge. Those policy makers with clinical backgrounds held slightly varying levels of knowledge. The practicing clinicians not involved as decision makers on the other hand, surprisingly, did not. As a group, the infectious disease specialists professed an above average level of expertise, whereas those from outside the field of infectious disease (I.D.) indicated that their knowledge was minimal. It appeared that the rarity of the disease played a factor in limiting their expertise, as all physicians reported that they had yet to diagnose a patient with Lyme disease, or they rarely see one. Our Manitoba clinicians simply cannot acquire experience with LD when they don't have the opportunity to see such patients. As a general response from this group, the lack of LD knowledge they expressed was accompanied by a certain level of discomfort, and perhaps recognition that they should know more, but might be lacking resources.

Finally, amongst the disease-specific advocacy groups, the response was wide-ranging. Specifically, Lyme-affiliated advocates considered themselves to be very knowledgeable both through personal experience, and/or extensive long-term reading and research, while non-Lyme advocacy groups admittedly knew very little or nothing about the disease. It was noted that the latter group might reflect the knowledge level of the general public, and an uninformed public places greater trust and reliance on the medical system serving them.

After noting each participant's estimation of their knowledge level, this research sought specifically to determine what the participants did know. It became apparent early in the interview process that the participants' LD knowledge base was linked to their perception of risk – a point we will revisit later.

**Research Question Two: What do you know about the pathogen that causes Lyme disease?**

The Lyme disease pathogen, *Borrelia burgdorferi* was identified as a bacterium by all of the scientists, with those in the field of entomology or wildlife biology limited to understanding its basic biology, and that it is vectored by ticks. Scientific experts in Lyme disease were aware of the varieties of genospecies, and some of the recent science and research on the bacterium, although no researcher in Manitoba currently conducts specific research with the LD pathogen. One expert indicated "we know that *Borrelia* is changing to some extent [...] bit by bit it is becoming more diverse in northeastern North America" and that local research needs to be done. Another reported that "environmental pressures cause mutations so I think things could happen and heck we see in Europe there are three different genospecies. We see some variation [...] we know there is some genetic variation that is occurring so the bacteria does change a bit." As discussed at length earlier, this basic scientific information is critical for formulating basic policy, however it has not been communicated to the policy makers, who are educated on the bacterium and its basic biology, but not on the relevance of the variety of genospecies.

The genetic variation that is occurring has the potential to limit the tests used in Manitoba to diagnose LD, and this information is crucial for the policy makers. Yet, this group did not present any significant knowledge on the spirochete, or the variety of genospecies as the expert scientists did, and collectively seemed to minimize the importance of knowing such things. They emphasized their reliance on the scientific experts to develop tests that incorporated any and all variations of disease within the province, yet were unaware that tests have not been developed to capture various genospecies that may have emerged in Manitoba to date. The resulting consequence to public health could be significant, with infected individuals limited to the current range of tests designed to capture the traditional LD bacterial species *Borrelia burgdorferi* sensu stricto.

Within the group of clinicians interviewed, those working in infectious disease seemed to be fairly knowledgeable about the bacterium, mainly "from reading", however those

clinicians outside of I.D. knew very little about the bacterium itself. The implications of this to public health are considerable, since a lack of understanding of how any specific pathogen operates in a human host will limit effective treatment. For example, doctors effectively treating a *Streptococcus* throat infection are fully versed about the bacterium they are dealing with, and follow their patients' recovery usually without complication. The medical history associated with treating syphilis, caused by another spirochetal bacterium *Treponema pallidum*, which is very similar to *Borrelia burgdorferi,* was initially fraught with difficulties due to the nature of the spirochetal bacterium itself, until significant medical expertise was established to successfully treat infected patients. Recognition of the need to understand the LD pathogen is crucial to an effective medical community dealing with Lyme disease patients. Throughout subsequent interview questions regarding the other ecological components of LD transmission, we frequently see a lack of knowledge as expressed here with the pathogen.

Amongst the disease-specific advocacy groups, the non-Lyme advocacy participants knew nothing at all about the pathogen, with the exception of one knowing that it was a bacterium. It could be suggested that this might reflect the average knowledge level of the general public, again leaving them at the mercy and trust of well-versed clinicians. The Lyme disease advocates on the other hand, expressed extensive knowledge and understanding of the bacterium, the genospecies, and variants of the bacterium including the existence of cysts, and certain difficulties in diagnostics that stemmed from this. This level of understanding seemed to prompt these individuals to a higher level of concern regarding this disease provincially and nationally, which seemed to correspond with thoughts of the expert scientists. Furthermore, the knowledge shared by those informed in this group, is from the current global Lyme research, which is of tremendous value in determining the complexity of this disease, and a realistic picture of the human risks. *Borreliae* spirochetes have only been under the microscope for a quarter of a century, and it is important for us to recognize how much of this extensive research is useful to us in assessing our risk in Manitoba. Unfortunately, our policy makers and clinicians have generally indicated that they operate on a limited understanding of the pathogen itself,

and do not recognize the adaptive potential of this bacterium, and resulting complications in public health that might follow.

This second question about the pathogen allowed the researcher to determine the extent of a portion of the participants' LD knowledge base, before moving onto a question intended to draw out their perception on how common the disease is.

**Research Question Three: How common is Lyme disease in Manitoba? In Canada?**

Amongst the scientists interviewed, those with generally lower levels of LD knowledge, such as one wildlife biologist indicated that the disease was "rare or very rare". While those with varying higher degrees of knowledge, including the Lyme experts, all indicated that they thought the disease was not particularly or overly common, or "reasonably rare", they also expressed certain concerns that the disease might either be more common than recognized, or could become more common in time. It was noted by one expert scientist that "although it is a relatively rare disease in Manitoba, it is a growing problem", and "we know we are missing a number of cases in the numbers we capture, we certainly don't think they are in the thousands, [...] I don't know, but there are cases that don't get captured in our figures". Given the serious consequences of this disease if not captured, it is crucial to investigate why some cases of LD are reported as "missed" in Manitoba. With significant expertise in the ecology and epidemiology of LD, the expert group of scientists approached this question thoughtfully, spending more time expressing their opinion, and ultimately relayed a higher level of concern over other groups.

The decision makers produced quite variable responses to this question including "generally uncommon", "very uncommon", "I thought that Manitoba was still to have its first home grown case" through "it is difficult to determine [...] we don't capture all of them". During the interview process it was noted by respondents that this group ought to be receiving accurate and up to date scientific information before working toward provincial policy, and the individuals in this group should present a similar viewpoint;

this was not the case. This is concerning, as is the notable difference between the expert scientists' response to this question and the decision makers. The question of why their responses differ is important: is it a lack of in-depth and accurate information being delivered, is it a lack of understanding or acceptance of the science being delivered, or is their understanding of how common LD is in Manitoba based on their personal perception influenced by other factors such as the number of reported cases? Since this group is collectively responsible for developing provincial policy to manage LD, their general response of how uncommon the disease is might influence the direction given to the Manitoba medical community. It is vital to public health that a shared perspective is held between the expert scientists and the policy makers on how common Lyme disease might actually be in Manitoba, and overall, there needs to be common and reasonably complete knowledge passed down the chain. It is possible that an effect on diagnoses might result with inconsistencies between these two groups.

The clinicians responded similarly to the policy makers, and indicated that Lyme was relatively uncommon, although one clinician did indicate "I think more common than people give it credit for". Amongst the I.D. clinicians the general qualitative responses were that in Manitoba and in Canada it is very uncommon, and one indicated "I know what I read, and I think in Manitoba we had six cases, one proved and five clinical". The repeated message from the I.D. doctors on how common LD is in Manitoba was that they based their response on the number of reported cases in Manitoba. Does this in itself have a feedback effect - do doctors operating with the assumption that LD is very uncommon because of the numbers approach their patients differently than if they held the same general perception of the expert scientists, and are less cases diagnosed as a result? And if fewer cases are diagnosed as a result, does this re-supply the original operating assumption that the disease is relatively uncommon? If this feedback loop does exist, it may be difficult to escape the pattern without increased education of this group, and enhanced communication from the expert scientists.

The non-Lyme advocate participants indicated that Lyme disease was "not common, but that's based on the media", "I know it isn't common in Manitoba", or "I don't know how

common it is". Their general responses likely reflect those of the general public, telling us that most people have little awareness of this disease in Manitoba. The Lyme advocate participant response was informed and quantitative however, including "for the year 2006 the Canadian Government confirmed around 50 cases Canada wide". One member of this group asked however "how uncommon is uncommon? People that suffer with the disease don't care how common or uncommon; they want treatment and deserve treatment". Their obvious passion was heard in their responses, and the latter point that appropriate diagnosis and treatment of all affected individuals is a responsibility of the public health system should be addressed. This group seemed to generally express their concern that Lyme is not taken seriously in the province, which is in part due to the general belief that it is not a very common disease, but also in part due to personal difficulties faced by the select few interviewed.

Observing the participants' responses to this question was helpful at this point in data collection, since it allowed for the interviewees knowledge base and understanding of LD to further surface within the context of their perspective of disease prevalence.

**Research Question Four: How familiar are you with the ecology and vectors involved in Lyme disease transmission?**

As previously described, the research behind the vectors responsible for Lyme disease transmission is vast. Studies on the key vector species, the competency and potential of these and other possible vectors, and the importance of vector life stages in transmission dominate the scientific literature on LD, and are vital to analyzing this qualitative research. Attention to this extensive and far-reaching vector research allows us to more effectively analyze the depth of our participants' knowledge, so that we might draw a more precise picture of the depth of vector knowledge in Canada, and ultimately see how this projects to local risk assessment and perception.

When responding to this question about vector biology and ecology, it was expected that the entomologists would be most informed, and it was unknown how the other

participants would respond. Much time and attention was devoted to the critical nature of this particular question, since it sweeps across many vector issues and associated risks, and carries tremendous value to this study.

The response of the scientists in general to this question was that they know of the two principal vectors of concern in Canada, *I. scapularis* and *I. pacificus*, with the former established in the southeastern corner of Manitoba. Those scientists not identified as Lyme experts or entomologists, were somewhat aware of certain vector details such as life cycles or general ecology, but beyond that were limited in their knowledge. The entomologists and Lyme experts on the other hand had variable expertise that could be classified overall as broad. Much discussion time during the interviews was devoted to the issues surrounding the vectors including the life stages and competency of the known vectors, other possible disease vectors, and other potential transmission pathways such as mechanical transmission. It was clear from the responses of this group that there is significant recognition on their part that other elements of LD risk associated with the vectors exists, however the 'summarized' science communicated is that risk in Manitoba and Canada is assessed solely with the presence of established *I. scapularis* or *I. pacificus* populations.

The Lyme experts and entomologists interviewed were well aware that the nymphal *Ixodes* ticks transmit LD more effectively than any other life stage. One scientist noted that:

> The risk in an endemic area is going to be greatest during May, June and July when the nymphal populations are going to be at their peak. There are many more nymphs on the ground than there are adults, and there is a 90% mortality between the stages so however many adults you have, you have 90% more nymphs on the ground, so the potential for exposure to the nymphs is much greater. The risk is most tied to "where are the nymphs?" which people overlook, and will miss.

The scientists with less expertise were not aware of this, with one admitting "Oh, me being like the rest of the public thought the risk was mainly in the fall time", and this focus on the adult tick is in itself a concern. If certain scientists associate LD risk with only the adult ticks, it seems likely that the general public does as well. Regrettably, the

understanding of the risk associated with the nymphal stage of *Ixodes* seems to end with select scientists. If this important information is being communicated, the message is not being received.

The competency of the known vectors, and the potential for other tick or insect vectors was discussed at great length with the Lyme experts and entomologists. After inspecting the wide scope of literature on the topic, it was clear that our scientists did indeed share risk concerns like those indicated in the literature. Our scientists indicated that although *I. scapularis* and *I. pacificus* are the major or primary vectors, another tick species, *Ixodes angustus* was PCR positive for *Borrelia burgdorferi* in British Columbia, and this species along with *Ixodes muris* may have roles as maintenance vectors. One scientist pointed out that aside from *I. scapularis* and *I. pacificus,* "there are other vectors; *Ixodes dentatis* I think is one for example and *dentatis* is relatively rare in Canada. There is another one but the names is not coming to me that is found on small mammals in the western United States and again extremely rare in Canada, and seldom comes into contact with people so it wouldn't be much of a risk for humans." Another scientist responded "Absolutely we are convinced of that" when asked if there might be other Lyme vectors in Canada. The general perception of this group regarding the element of risk other potential vectors introduced however, was that it was largely unexplored and unknown.

*Dermacentor variabilis* is of particular concern in Manitoba given our abundant population, and one entomologist pointed out "If a *Dermacentor* tick was feeding on a host where the bacteria was present in the blood they would certainly acquire the bacteria, and people have found and isolated the bacteria from *Dermacentor* ticks elsewhere in North America. To elaborate on why little attention was paid to the risk of LD infection from these ticks, this scientist indicated that "*Dermacentor* does not very effectively carry the infection through a moult", and "the probability of them being important in transmission of Lyme disease is not that great I don't think". The general consensus among the Lyme experts and entomologists was that if an infected *Dermacentor* tick present in an endemic area, did end up on a human host, it was entirely possible to become infected. Although the vector competency of *Dermacentor variabilis*

is poor, the entomologists disagreed on the potential for mechanical LD transmission. One entomologist stated:

> Slapping, people doing it the wrong way and leaving mouth parts in there, and it could stay under the skin and you would have a chance for it to actually replicate and move on, why wouldn't it? Just like West Nile, *Aedes vexans* is out there in huge numbers, it is known to have West Nile in it but it is not a competent vector but it is still on the list. We know it is not going to get passed easily but we know it can carry it.

Another expressed "I know the literature suggests it, but mechanical transmission...this is a little sketchy". Other insects were mentioned during the interviews. Thus, one scientist noted that "Horseflies have been implicated as accidental mechanical vectors but again I think it would be a very low likelihood of transmisson". Following a comment that *Aedes vexans* had been implicated as a potential vector in Europe one replied that "If it was Aedes vexans then that would be a huge concern if it was able to transmit the disease here because it is our most numerous mosquito". When asked if it would be worthwhile to test our mosquitoes in areas like southeastern Manitoba for infection, another entomologist replied "As potential vectors, yes but again it is a dollar issue, it is not cheap doing all this testing. I'd like to see it done; I think it could be a potential vector." During data collection with constant comparison analysis, saturation on this topic was reached early on, and repetitive data was further collected. One expert indicated "I just wanted to make a point that there are some other species that we know could have a minor role", while another indicated "Yes, there has to be, I really do believe that [...] what's there and what other vectors that we don't know about."

Despite the potential for other LD vectors in Manitoba, one expert scientist explained that none of this knowledge, or these scientific perceptions of other potential vectors enters into our risk determination system for LD since "in terms of our messaging to the public and physicians there really are the two principal ones", referring to *Ixodes scapularis* and *Ixodes pacificus* alone. Furthermore, one expert expressed that the evidence for other potential vectors is

> not that compelling and I guess...I am just thinking in terms of public health, would it hurt or would it confuse? I mean we tell people about which ticks there are, but is it even important to know which ticks are important? [...] Is it

> absolutely important that they know that it's one or two or ten or any tick? I think we are giving them the proper message right now [...] I am just not sure that classifying them that way (for their importance as vectors) is going to produce something at the end of the day that is going to be useful or help physicians or help the public and it may actually create...if you were to say or even suggest that *Dermacentor variabilis* has a role to play even if it is a minor one in Lyme disease transmission I think you would see much anxiety in the public.

Does this personal perception influence the resulting flow of information to the province, physicians and the public? This expert further noted that "almost exclusively risk is assessed by *I. scapularis* and *pacificus* and this information is passed to the public and physicians, but risk extends outside of endemic areas. There are forty other tick species in Canada and doctors need to look at tick exposure in general." Unfortunately, this important train of thought, that other vectors might transmit LD in Canada does not appear to be communicated to those involved in the creation of policy.

The decision/policy makers were aware of the two known vectors of LD in Canada, and as such their role of establishing risk in the province would be based strictly upon exposure to these known vectors. The lack of knowledge amongst this group regarding specifics of the vectors themselves was obvious. They expressed no awareness of vectoring research, or of other potential vectors to be able to comment. This was rather surprising to identify in the data considering the important role of this group, however the general consensus was that they were confident that the information communicated from the entomologists they relied upon, was complete. There was little understanding additionally within the general group of the different risk factors associated with various life stages of these ticks. In fact, one policy maker had the misperception that the adult ticks are more infective than the nymphs indicating "I haven't actually investigated the fact that the nymph would be more infectious than the adult. From my other information it is usually the reverse that the older they get, the more they have bitten things and would have a higher germ load". This was noted as concerning at the time, when indeed information that the nymphs vector 90% of the disease is important risk assessment data, and was known to the entomologists. Why and how had this important risk message not been communicated or received? Furthermore, when asked if *Dermacentor variabilis* was given any attention as a potential vector, a member of this group indicated that "we

have discussed this with entomologists and their information is that the studies on those show that the dog tick or wood tick does not carry this", "we asked [...] about whether or not we needed to worry about the wood tick", and that "the possibility of research would be an easy way to find out with the dog tick, you test a bunch of dog ticks and if they are all negative. Even dog ticks from the southeast corner would probably be a good way to test that theory out." Again, a lack of understanding was apparent, since it is known that wood ticks indeed carry the bacterium, but what is questioned in the literature and by our scientists, is if they are competent vectors in the traditional sense. We know some potential for mechanical transmission from an infected *Dermacentor* tick has been documented, and this risk, however slight, is important in light of the huge population of these ticks in our province and country. This information is not being received, or utilized by the policy makers, and the end result might be felt with our low numbers of reported cases.

The only information the non-I.D. clinicians had received was that the deer tick vectors Lyme disease. Their awareness was limited, and unfortunately did not include knowledge that the most important vector life stage, the very small nymph, was difficult to see, or to find on its host. Given clinical guidelines indicating that attention should be paid to history of a tick bite, an obvious problem in diagnosis exists when people might not know if a nymphal tick has fed off of them. Amongst the I.D. clinicians, there was an awareness of the specific *Ixodes* ticks involved in vectoring the disease, and a general awareness of the life stages, but no recognition that the nymphs were the largest concern. One I.D. clinician indicated "I have gone to many talks but I cannot tell you that, [what stage is associated with the highest disease transmission rates] just that there are some stages that are more risk than others, that is entomology not medicine". It could be argued that vector knowledge forms the core of LD epidemiology, and is crucial to medicine. One I.D. clinician was aware that an earlier stage of the tick was important, but incorrectly indicated "it is the larval form that is the most dangerous for people", and additionally "that is why I am a bit puzzled that when they say in the public health report that the biggest risk is in October and November when the adults are at their peak because my understanding is that it is the larval from that pose the greatest risk for

people". Clearly this clinician had identified an important problem in the communication of risk, and vital information on the nymph being the most important vectoring stage is completely overlooked in Manitoba, likely confusing other clinicians as it did this one. It seems entirely possible that this might have some influence on diagnosis in our province. Additionally, when asked if aware of other potential vectors, one I.D. clinician replied "nope". When asked if they were concerned about *Dermacentor variabilis* in our province in light of some intriguing research on its potential to vector LD mechanically, I.D. responses included "no, it doesn't concern me because it is entomology", "I suppose it's possible. It would be a theoretical concern but it is not a big issue in the causation of Lyme in people", and "Interesting, but the last thing we need is that information in amongst the public; the fear, panic and misperception that would arise is beyond description". If our common wood tick *Dermacentor variabilis* might play some role in LD transmission, shouldn't all parties be made aware? Effective public health can be enhanced when individuals are informed, and apply appropriate health practices in response. It was clearly noted in collecting the responses to this question that some clinicians' vigilance to capture human cases might be compromised, in light of their low-level of awareness of the current vector research. Furthermore, barriers in communication to this group were recognized, as the current research hadn't entered their knowledge base, and what had was generally dismissed as unimportant.

Members of the non-Lyme disease advocacy groups all indicated their knowledge was limited to knowing that "ticks are involved", an answer that might be expected from the general public. Unfortunately this general understanding leaves the tiny nymphal ticks out of the picture, and a very unrealistic idea that people only need to watch for large and visible ticks. This places the public at greater risk of LD exposure, and the simple message that tick vigilance is required at all times would likely minimize disease. The knowledge expressed by the Lyme advocates however was rather comprehensive. Discussing the known tick vectors was not the passion of this group, but rather other potential vectors such as *Ixodes angustus* in Alberta and B.C., *Dermacentor* ticks, mosquitoes, other biting insects, and research of these dominated the discussion. The potential for mechanical transmission from an infected insect was raised in discussion,

and one Lyme advocate indicated it is absolutely pertinent that we research this here because "if our doctors aren't told what ticks and biting insects carry, how are they going to know what to diagnose". Another indicated "I don't think any research has been done to exclude mosquitoes as a vector of Lyme disease, they know it carries the bacteria". Awareness and understanding of the literature was very evident in interviewing this group on LD vectors, and collectively their view that further research is necessary was heard.

**Research Question Five: How familiar are you with the ecology and life cycles of the hosts involved in Lyme disease transmission?**

As with the vectors, a certain amount of understanding from the literature presented earlier about LD hosts and reservoirs is important for analyzing the responses to this question, and the depth of understanding operating in the province.

The scientists best versed in the host and reservoir ecology associated with LD were the Lyme experts and wildlife biologists, while the entomologists were aware of the basic science. All the scientists acknowledged that the most important hosts and reservoirs, mice and deer, are widespread across the entire southern portion of Manitoba, with one expert noting that there is "No question. The hosts here are perfectly suitable" for LD. The mice were reported to have a small range, but white tailed deer can have up to a 100 square kilometer home range, and these might allow for risk to expand beyond recognized LD endemic borders. "There's no fence" at the Canadian/U.S. border explained one scientist, while another indicated "moving ticks large distances is very possible" with deer or bird migratory movement, and disease emerges as a result.

It was noted that there is very little knowledge, and very little interest in studying either the small or large mammals associated with LD in the province, but that it is likely a funding issue. Most of the scientists agree that research and increased knowledge of disease factors always has some benefit. The scientific community is simply aware that deer are involved in LD transmission, however it was interesting to learn from one

scientist that "hunters have noted large infections of deer ticks, we have taken note of that but that's basically it". If scientists are aware that large numbers of deer ticks exist on deer in the province, why hasn't this been explored? If these ticks are indeed found on deer in significant numbers, it would seem that deer tick populations in Manitoba are both higher and more widespread than is currently recognized. The role of other large mammals in LD has not been explored either. The small mammal hosts of the larval and nymphal ticks in Manitoba include deer mice, and potentially all terrestrial small mammals like squirrels, chipmunks, raccoons, foxes, lynx, bobcats, shrews, and members of the weasel family according to one scientist, "particularly since many of them eat deer mice as prey routinely". These small mammals populate the entire province, and would form a widespread reservoir for the bacterium in Manitoba, if they were to become infected. Vectoring insects then feeding on these small mammals in proximity to humans would clearly provide an opportunity for disease exposure. Surveillance of small mammals is key to identifying reservoirs of disease.

Concern regarding human exposure to the hosts and disease was discussed during the interviews. One scientist relayed that deer mice populations respond to disturbance either immediately or within a year, and then for up to 10 – 18 years, meaning more mice will be found where people are disturbing the habitat, like around roads or in cabin country. In other words, people have significant exposure to mice habitats. The deer populations respond similarly to human disruption according to one scientist, and with increasing population, in time more fringe areas will develop, bringing more people and deer together. This idea was repeated in talking with the scientists, and concerns for future exposure in Manitoba were evident.

The fact that birds can fly makes them a host of particular interest, given they have more potential than any other type of host to widely distribute ticks and disease. In discussing the important role of birds and their potential for seeding a seasonal endemic area, one scientist was very intrigued that this could "potentially be a mechanism for leap-frogging the disease over different areas", with all of the ecology to support infected ticks

abundant throughout southern Manitoba, urban areas included. According to one Lyme expert

> Ticks are introduced annually, we're bombarded with probably 100, 150 million nymphs annually, and can survive to moult, and adults can probably overwinter in Manitoba in a wide range, and obviously represent some level of risk to people out there because they do get on people. The bottom line is prevention, and to make sure that the physicians know the facts, the risks, and the symptoms.

It is crucial to note that people do not have to travel to have exposure to Lyme disease. Given the scientists have been saying this for some time. This expert continued with "The risk of Lyme disease in Canada due to ticks dispersed by migratory birds is very low, but geographically very much wider than posed by endemic populations". Another expert stressed "We absolutely have tried for the longest time to say we think people can get infected anywhere. We have been pushing that message for a long time. They can come in on birds; they don't necessarily have to be in established sites", and "I don't know how well the message is...I have to sort of laugh a bit because we have been giving them the same message for a long time [...] I said nothing to this reporter that I haven't been saying five to ten years ago". If the expert scientists have been giving the message that people can get infected anywhere for up to ten years, why has this not been heard in the medical community, or by the public? Where is the barrier in communication taking place, and why are most groups seemingly unaware of the potential for exposure anywhere in the province?

The policy makers were fluent with deer and mice serving as hosts in the province, and the potential role birds play, indicating up to date information has been received by this group. The scientists are clearly passing information to this group, who in turn have indicated the message has been funneled to clinicians. "There is information that's gone to physicians recently and in the past that say they have been found throughout the province and have been dropped off by birds" replied one member of this group. Other specific responses included "Yes, we sent that out again and the message seems to be taken up a little better, we made a bigger letter and so it seems to have got their attention more this time, but it was sent out in the past as well", and "I would have to go back and look at the letters that were written, but the message has always been that you can see

Lyme disease anywhere" as a result of hitchhiking ticks dropped from migratory birds. So the overall response from this group indicated that a concern for exposure anywhere in the province was being delivered to the clinicians via letter, and through medical postings. Unfortunately, this message however is not being received, or accepted down the line.

When asked if Lyme disease exposure can exist anywhere in the province as a result of migratory birds carrying hitchhiker ticks, only one clinician was aware and indicated "it is just a matter of time before they go from Buffalo Point to other parts of the province [...] I guess only time will tell to where these organisms will go". The other clinicians had not heard about any bird research, or given it much if any thought, and indicated in discussion they believed there was very little chance of exposure in any urban setting, even if birds played a role. A break in the communication chain between policy development and practicing clinicians is evident. With millions of hitchhiking ticks entering into Canada each year on birds, it would be beneficial if all clinicians were well aware of the potential exposure associated with birds. Essentially this extends potential LD exposure throughout the entire province, meaning anyone anywhere can contract LD, yet there are extremely few cases. This group in general were unaware of any details regarding any of the LD mammal hosts, however one clinician was informed about the white-footed mouse and deer involvement. Although it is likely unnecessary for clinicians to be well versed in LD host ecology, an awareness of the extensive availability of the hosts throughout Manitoba might set the foundation for understanding that the ecology in Manitoba is fully supportive of the disease.

The non-Lyme advocacy participants were unaware of hosts and reservoirs, or their role in the disease, whereas the Lyme advocates were well versed with the literature, expressing a couple of important points. One indicated that "there are a lot of host reservoirs that are being ignored and it can be regional", commenting on the large marmot and vole population in one Canadian region that had not been studied for their potential role as hosts. Another indicated their knowledge and concern with "deer mice all over the province and that is definitely a host", speaking of Manitoba. Collectively

this group expressed a need for further research into the hosts and reservoirs of Lyme, particularly as the disease was emerging in Canada.

**Research Question Six: What is the risk of contracting Lyme disease while living in Manitoba?**

Further to research question three, this question was an indirect approach to finding out whether the participants were aware of factors utilized in risk assessment, and was also intended to draw out personal perceptions of risk. This important research question was asked midway through the interview following substantial discussion of LD, with the goal that the participant would openly express their thoughts with the interviewer.

The scientists, according to their levels of expertise, had varying perceptions about LD risk. Scientists with less knowledge or understanding of LD responded with comments such as "it has been minimal to none because the disease is so rare, and the threat to humans is rare", and "Well I mean 7 cases is that a real, real risk?" Collectively, most of the scientists expressed their perception of risk to be very low, to rare. When we examine the responses of the scientific Lyme experts however, a somewhat heightened sense of risk was heard. "It depends on where you live, and where you go. There's a low level of risk in the populated areas of Canada, and zones of higher risk are based on endemicity", said one expert. Another indicated "I think the risk is low for the Canadian population as a whole, however there are clearly some areas where there is much greater risk and the number of areas where there is greater risk is increasing. In other words the number of endemic areas is increasing." If endemic areas are indeed expanding in Canada, risk will increase directly hand-in-hand. Another commented that there are "low levels of risk in most of the areas, and higher risk in some areas obviously". Clearly, these scientists are basing risk on the isolated and established endemic sites where the known *Ixodes* vectors are located. As indicated previously an established endemic site is located in the Buffalo Point region in the southeastern corner of Manitoba. The scientists all indicated that risk would be the greatest in this particular area. There was no scientific acknowledgement of other potential exposure pathways when this group provided their responses. One expert

pointed out however "How do you define risk? Does risk equal the number of, is it a sort of global Canadian estimate of what's the incidence of Lyme or do you quantify it as what are risk behaviours or risk places?" As introduced previously, LD risk is formally defined as the chance of acquiring an infection, as a result of exposure to the bacterium. Clearly people need to be in ecological proximity with the bacterium, and a vectoring agent must be present to transfer the infection. It was noted that the collective perception of the scientific experts was that varying degrees of risk exist, which are primarily hinged on the recognition of endemic areas, and human exposure within these areas. Yet, their recognition that birds drop off hitchhiker ticks and potentially create local pockets of endemicity anywhere needs to be incorporated into what the scientists have said here. In drawing from their combined responses, they agree that there is a risk of contracting LD anywhere throughout southern Manitoba, however this risk increases with known areas of *Ixodes* endemicity, and also with certain human behaviours.

Policy makers, taking their direction from the scientists, indicated that risk in the province as a whole is "rare to maybe low", "very low", or "it is difficult to quantify the exact risk and I think that is something that we are looking at. We certainly know from our human data that the risk is much higher in the southeast corner". These statements suggest that the policy makers associate risk quantitatively with the numbers of documented patients, which in turn is dependent upon the clinicians' ability to diagnose the disease effectively. Since this group clearly defines risk from the data on reported case numbers, their general response that the risk of LD in Manitoba is very low seems entirely reasonable. Given the nature of the risk assessment process, and what the scientists present however, a risk to all Manitobans exists in varying degrees throughout the province.

The clinicians generally believe the risk of LD in the province is low, and multiple I.D. clinicians agreed stating it is a "very small" risk. With this collective perception, are the clinicians approaching their patients with the understanding that Lyme disease is a possibility, or that it is unlikely in this province? It was becoming evident at this point in the interviews that their perception of risk was influencing their practice, and we will see

with further questions, the unfolding skepticism that their patients might have Lyme infections. One scientist had pointed out that when quoted to say there is "low risk", there was some anger expressed by an individual "because if you say low risk doctors consider that no risk, and won't even consider it as a possibility". The relationship expressed here between qualified risk and medical practice should be noted - does clinician understanding of risk influence medical practice, and have an influence on diagnoses? For example, is heightened awareness of H1N1 infection leading to increased vigilance amongst clinicians, and a greater number of cases reported, and does this principle apply to other diseases like LD? Additionally, might heightened awareness of LD in the public, lead to increased self-diagnoses, ultimately taxing the system?

The non-Lyme advocates as predicted, indicated that they thought the risk of contracting Lyme in Manitoba was low, and restricted to rural areas like "woods", mostly based on reports from the media. It seems likely that this perception might reflect that of the general public. Furthermore, since the media is being used as an official organ for transmitting health information, does the government need to take deficiencies in the media message more seriously?

The Lyme advocates collectively indicated that they did not think the risk "was huge", but stressed that without research, and without educating the physicians and public, the risk is greater for everyone. The implications of this point are important to highlight, since knowledge of LD plays a key role in reducing risk for everyone. Furthermore, one expressed "I believe we are really under-recognizing this issue in Canada", and research will "give us a much better picture of whether we have a problem with Lyme disease". This group repeatedly stressed that further research and knowledge is critical to minimizing LD risk. The frequency of references to the importance of LD knowledge identified during analysis allowed this category to be realized as the core category in this study.

The Lyme advocates presented information that they believed that there had been 435 cases of LD in Manitoba that hadn't been documented over three years time during April

1995 to March 1998. One indicated that "they were from all over the province from one end to the other, so I think it is very important that they don't limit contracting Lyme to Buffalo Point where it is supposedly endemic and the only place it has been properly researched", and further stated that in a meeting with Manitoba Health

> I have it documented that he said during that meeting that the public needs to be informed about this 435, and that Manitoba needs to definitely educate the physicians and the public so that they can prevent this disease, and what happened in that case was instead of going forward and doing something about it they went backward and just shut everything down.

What exactly happened here was unfamiliar to the individuals currently working with LD in the province, and unknown where this number actually came from. It was noted that it seemed unusual that this clearly important detail had apparently 'vanished' from information documented about LD in Manitoba, and if the information was valid, what does this mean for actual risk in Manitoba?

**Research Question Seven: Are you aware of any difficulties associated with diagnosing Lyme disease?**

The scientists' responses to this question naturally fell into two groups: those uninvolved with laboratory diagnostics, and those directly involved. Comments from those uninvolved with laboratory diagnostics included "I think the symptoms lack specificity so diagnosis can be difficult"; "Yes, to some level but I'm no physician"; "It is a great mimicker"; and that without the classic bull's eye rash the media portrays difficulties in diagnosis. It was determined from their responses that this group who is uninvolved with laboratory diagnostics, are generally aware that there are some difficulties with diagnosing Lyme disease. This differed greatly from the responses of the Lyme experts, who generally agreed there were important problems, but in reference to laboratory diagnostics specifically, one answered "Yes [...] there are controversies about the clinical diagnosis of Lyme and there are controversies about the lab testing". Continuing with this, it was mentioned that "I do believe that the tests for disseminated Lyme are very good but absolutely not perfect" and "the tests [ELISA and Western Blot] are no good for early Lyme, and we recommend a clinical diagnosis". Another expert concurred

indicating "It depends on when, what time they're tested. In the early stages people's response is quite variable. The EM is more useful than any serological assay." Examining these responses, we hear the repeated message that there are concerns about the LD tests currently in place, particularly when they are performed early on during the initial course of the infection. This groups' knowledge of testing sensitivity and reliability is expressed through the frequent references to selected testing times. The overall perspective drawn from these responses is that we cannot completely rely on lab tests, particularly in the early stages of the disease, which is when many patients might be visiting their doctors. The scientists' recommendation for a clinical diagnosis requires a certain level of expertise on the part of the acting physician, which is likely hindered due to the few reported cases in the province. The importance of getting the message regarding lab testing to clinicians, coupled with the message that a clinical diagnosis is critical, falls into the hands of the decision makers.

The consensus amongst the decision makers was that Lyme disease is a difficult diagnosis, and they are aware of the limitations of the lab tests in the early stages of the disease even through the first couple of months of infection. One member of this group indicated "There is some controversy around the testing" while another commented "Yeah it would be nice to have a gold standard test but we don't, so we have to rely on exposure, clinical impression, and put all that together with the lab tests and make decisions based on that. Do we need a better lab test for Lyme disease? Absolutely." Clearly, the general responses of this group matched the scientists, so information was being appropriately channeled to, and received by the decision makers. Given the understanding of difficulties in diagnosis, particularly in reference to troubles with the ELISA and Western Blot tests being administered too early to detect infection, it is of vital importance that clinicians are well informed of their increased responsibility to be vigilant when patients present with a potential Lyme infection.

The clinicians general responses to this question were varied and included "the symptoms are vague, it's not the first thing anybody thinks of", "Let's face it, the disease is sometimes difficult to diagnose", and "You have to be practical. It is reasonable to have

a low suspicion of diagnosis in areas of low exposure", with some personal perceptions surfacing. The collective response was clear to indicate that these Manitoba clinicians had little experience with LD, and did not expect they would see patients with the disease. Furthermore, they neglected to comment on laboratory testing, or on the difficulties deciphering LD symptoms from other diseases. Some clinicians had considered Lyme when diagnosing patients, but none had provided a clinical diagnosis if the test results came back negative, regardless of when they were administered. Recognizing that a clinical diagnosis is important, responses amongst those working in the I.D. field were surprisingly different. One specialist indicated "None of the serological assays used are in the area where we would say that they are highly reliable or specific, and that's the problem", going on to indicate the reason to test is to support a clinical diagnosis. Furthermore, they had only seen one patient with EM, and noted clinical identification of disease is imperative but problematic given "we are never really dealing with Lyme, and never get the experience to become really good at it, and our book of knowledge is going to be just that". Another specialist presented their perspective that diagnosis might be difficult in the early course of the disease, but not in later disease because we have excellent public health authorities, and "people with great expertise in this area are the ones who develop the tests and decide what is appropriate testing". Another clinician expressed that they had no concerns at all with diagnosing Lyme disease or with the serological assays currently in place, going on to say "Bottom line, we have an appropriate test", failing to acknowledge that the tests were considered unreliable in the early stages of disease, when patients might be visiting their doctor. Given one clinician's comment that "medicine is much an art as it is a science", the critical role of a physician to ensure their knowledge level is complete, and their vigilance in clinical diagnosing their patients is obvious. One clinician indicated that they may not even agree to see a patient if they didn't present with an EM rash, and that appropriate history was vital for them to consider Lyme disease. "It would be hard for me to consider Lyme disease in someone who lives in River Heights in Winnipeg if they haven't left River Heights" overlooks the message that exposure can be anywhere in the province due to the migratory nature of birds and their potential role as vehicles for infected ticks. The interesting perspective of this clinician was noted, particularly when

they indicated that they order Lyme disease tests "very cautiously, because I don't want to create and fuel Lyme paranoia". Given the serious consequences of undiagnosed Lyme disease, the implications of this perspective are recognized as grave.

Amongst the disease-specific advocacy groups, the non-Lyme participants were generally aware that Lyme symptoms could be vague, that they are "very hard to figure out", and as a result cases might be misdiagnosed or "fall through the cracks". They indicated that lupus, fibromyalgia, arthritis, MS and Chronic Fatigue Syndrome were among the list of diseases they had heard of with similar symptoms to Lyme disease. The Lyme advocates had a lot to say about the difficulties associated with diagnosing Lyme disease. "Because of the fact that our doctors haven't been fully educated and have been told that the disease isn't here for so many years", one advocate suggested that fewer diagnoses occur. Throughout the interview process, this repetitive question surfaced in various forms – does a lack of education coupled with risk perception influence clinical diagnostic practices? Confidence in cases being missed as a result of our two-tiered testing system was also expressed by the Lyme advocates, along with the belief that clinicians had difficulties in making a clinical diagnosis. They expressed concerns like "doctors are so convinced that this is a rare disease that they are even missing the classic bull's eye rash and questioning it as significant". They believe that Lyme is downplayed in Canada, and "they put far too much emphasis on the requirements of an endemic area for a diagnosis, and they put far too much emphasis on tying it all together with serology that they know is nowhere near sensitive enough". This group commented on the narrow range of the first test used in diagnosis, the ELISA, indicating false negatives commonly result, and that there is a more expensive C6 ELISA that is a broader, better test available. The Lyme advocates are upset that people have to travel elsewhere for this test, and to find a knowledgeable doctor because "the infectious disease doctors are usually not willing to even look at Lyme because they feel that the Lyme isn't here". Further to this, they expressed "we want our physicians here to be educated and to know the disease". They concur that without adequate education, LD will continue to go unrecognized, and undiagnosed.

The different species and strains of *Borrelia* which cause Lyme disease generate symptoms that vary greatly, and play an important role in diagnostic difficulties. Strle, Ruzic-Sabljic, Cimperman, Lotric-Furlan and Maraspin (2006) indicated the most common cause of Lyme neuroborreliosis in Europe is *Borrelia garinii*, followed by *Borrelia afzelii*, and *B. garinii* causes a "typical early Lyme neuroborreliosis" compared to *B. afzelii* infections, where clinical features are much less specific and more difficult to diagnose. O'Connell discussed difficulties in clinical and serological diagnosis given various genospecies cause different disease manifestations as well as differing immunological responses (1998). The variety of the *Borrelia* species causing the infection may alter test results, or allow for a wide-ranging clinical picture to the diagnosing physician. The literature suggests research in Canada has been confined to *Borrelia burgdorferi,* and doesn't include other *Borrelia* species, which is an obvious concern. The "question as to whether we would properly diagnose patients who have been infected with European *Borrelias* such *as B. garinii and B. afzelii* is very relevant. Not all North American tests would necessarily pick up antibodies, and our North American western blot would likely fail" (Artsob, 2008). This would clearly exacerbate a patient's chance of misdiagnosis.

When a question regarding the potential of misdiagnosis was posed, and key informants were asked of their knowledge and understanding of potential diseases that could mimic Lyme, we learned that there was fairly minimal knowledge amongst the participants. The scientists interviewed had nothing to offer on the topic, while the policy makers brought up Chronic Fatigue and Bell's palsy as having similar symptoms or being associated with Lyme, and raised the "highly controversial" issue according to one policy maker, of chronic Lyme infections and long-term antibiotic treatment. The clinician group indicated that arthritis along with some neurological findings appeared with Lyme, but had no knowledge of variants of *Borrelia* such as cysts, which were thought to be associated with complicated cases of Lyme that had gone misdiagnosed as MS and other diseases. One I.D. clinician did comment however, when asked about the presence of *B. garinii* infections matching the global distribution of MS and being isolated in ticks on birds in Newfoundland, with "Well I think it raised the possibility of a different pathogen

being involved in MS. [...] I am not surprised that there is a potential epidemiological link with something like that so it is worthwhile looking at". It was apparent listening to all groups, that the power to make the right decisions, and to diagnose accurately, comes from in-depth research and knowledge about the disease. The advocacy groups expressed this more frequently than any other group, along with the desire to be informed through appropriate channels, particularly when it related to their disease interest. Advocates for MS were not aware that there were issues with testing in early Lyme infections, or of the controversies with the testing, or that the MRI of a Lyme patient can parallel those found in MS for example. Recognizing the need for the effective communication of current science is critical so that all people have access to pertinent information.

**Research Question Eight: Are you aware of any difficulties associated with treating Lyme disease?**

The discussions with the participants regarding the treatment of Lyme disease were quite brief. Despite being outside the realm of many scientists' expertise, they were generally aware that if diagnosed early, Lyme disease responded well to treatment, and that later stages are more difficult to treat. One of the expert scientists indicated that he was aware there were controversies associated with the long-term antibiotic treatment of LD, and over whether or not chronic Lyme disease was a reality. This scientist mentioned that he followed the literature from New England in the United States, and was of the opinion that long-term antibiotic treatment for Lyme disease was unnecessary. The policy makers shared a similar low-level knowledge base, as did the clinicians, with some participants in both groups aware of certain controversies associated with long-term antibiotic treatment for later stages of LD. The implications of the brief responses to this question on public health might be significant. Without a well-versed medical system in the treatment of Lyme, if and when more cases are diagnosed, treatment issues never seen previously in Manitoba might surface. Difficulties in treatment might be seen in time.

Amongst the disease advocacy groups, the non-Lyme groups didn't think there were any concerns at all with treatment. This group expressed full trust in the medical community to appropriately treat anybody diagnosed with an infection, Lyme included. The Lyme advocates on the other hand were well aware of treatment controversies in the United States, and certain difficulties locally. One expressed the general nature of local problems to be centered around "our doctors are given a very limited antibiotic that they can give to the patients even if they feel this patient needs a longer treatment, they are told they cannot give a longer treatment [...] even with tuberculosis you would never take a patient off until their symptoms say they are clear of this disease". This response was derived from personal experience, and given the very few diagnoses of LD in the province, mirrors the controversy of long-term antibiotic treatment occurring in the United States.

Despite the nature of this question as a somewhat peripheral topic to this study, the value here is found in the lack of information – without the diagnosis of Lyme, there is no experience with treatment. Future issues in treatment will likely emerge as more LD cases surface locally.

**Research Question Nine: Do you believe the risk of Lyme disease is appropriately portrayed in the province?**

Participants were deliberately asked this question later in the interview, so as to separate it from the earlier question regarding their personal perception of risk. This question was intended to draw the participants' perceptions on communication to the public, and to minimize confusion, was positioned two questions later.

The scientists all agreed that the information from their group was portrayed appropriately, and delivered through the necessary channels. They collectively expressed the view that the necessary science associated with LD risk was making its way to the policy makers, but concurred that they carefully consider how LD risk should be presented. One expert Lyme scientist indicated "It's a balance; the risk is portrayed with

enough wiggle room." The notion of wiggle room seems to indicate that the scientists present a relatively broad or general risk statement to be used toward policy development. The single element presented most frequently by the scientists regarding LD risk is the identification of established *Ixodes scapularis* or *Ixodes pacificus* endemic sites. In Manitoba, this means that the message delivered is primarily that risk of LD exists in the southeastern portion of the province, and elsewhere in Canada where endemic sites have been identified. Although the Lyme expert scientists also indicated that they have presented information that exposure can exist outside of endemic areas as a result of a variety of factors. What seems to be heard down the line though, is that risk only exists in endemic areas. One scientist indicated this by "The front line is the doctors, and I hear "you can't have Lyme disease because these ticks aren't found here", so at some level we're not getting through to everyone…Manitoba Health. The I.D. people are excellent with communicating, and the media for the most part is balanced." Clearly there is some recognition by the scientists that their message regarding risk is being overlooked in some way.

The policy makers expressed a firm trust in the information received from the scientific community. They rely on the scientists to accurately present a picture of risk, which can then be used to create public health policies for the disease. Some concerns were expressed however that the message extending to physicians and the public might not be getting through. One policy maker indicated "I don't think necessarily the health departments are releasing the information readily to the public", while another responded "As far as appropriately, do enough people know about it or is the communication good enough, I don't know […] does the public know enough, do doctors know enough, probably not for various reasons, mainly people are too busy and unless you have commercials on TV people will not […] they probably don't know very much about Lyme disease." Some confusion expressed by the policy makers in how well the messages are getting through is recognized.

The clinician responses varied – some thought that risk is appropriately portrayed, but one clinician indicated "No, it is greatly overplayed". If the general message to public

Manitoba that LD risk is very low is being overplayed, then this clinician's perception that risk is lower than very low, likely has some influence over their practice. Overall, the clinicians expressed trust in receiving accurate information for use in their practices, and were generally under the impression that they didn't have to be too concerned since they understood the risk of LD to be very low. Furthermore, they generally believe that endemic sites present an isolated risk of exposure for their patients, and that most people will not be exposed to this risk. This research suggests that this, and other perceptions of risk held by practicing clinicians might directly influence the number of LD cases diagnosed, and reported.

Members of the Lyme advocate group strongly believe that Lyme disease risk in Manitoba and in Canada is downplayed. They understand risk to be highest within endemic areas, yet that it exists across the nation to a lower degree outside of these sites. They suggest that the scientists present this message, but are concerned that decision making groups, and clinicians do not recognize it, and the public is facing difficulties as a result. The non-Lyme advocacy members believed that LD risk was being appropriately portrayed, suggesting they had no reason to question why it wouldn't. They generally thought there is very little risk of acquiring Lyme disease while living in Manitoba, a view likely shared by the rest of the public.

**Research Question Ten: Are you confident that information regarding Lyme disease risk in Manitoba as delivered through the public health care system reflects current knowledge?**

This question posed to participants received a wide range of responses. The scientists, who are on the front line of delivering the message, seem to collectively echo the words of one scientist, who replied "Yes and no, there could always be more". The interviews with the scientists led to information that there is a single Lyme expert scientist currently working in Quebec on a variety of issues related to the emergence of LD in Canada, one federal entomologist working directly with the *Ixodes* ticks known to cause Lyme and the mammal hosts associated with those ticks, and one special pathogens expert who has

been immersed in Lyme projects and the identification of endemic LD sites in Canada. Very little research is being conducted locally in Canada, but certain global cutting-edge research has been over-viewed by our national scientists, and has been generally incorporated into the message they want to deliver to Canadians. Considering the resources in Canada directed toward LD research, including research scientists and funding are slim, it seems that our scientists are fluent with much of the global science, and with utilizing and applying this science to our local risk management protocols. The scientists were not confident however, that this current science is appropriately channeled through the public health line after it leaves their hands. The policy makers were all confident that they are receiving current scientific knowledge from the scientists they rely on, and strongly indicated that our national scientists set a firm foundation at the top of the hierarchy for risk information. They had nothing else to offer, aside from indicating they couldn't be entirely sure how individual clinicians would continue to deliver, and work with the most recently acquired information on LD.

The clinicians were generally unsure if the LD information delivered to them was current, and expressed some concerns over the necessity of time for information to fan out successfully. One clinician replied "No", that they didn't believe the message of LD risk reflected current knowledge, while another indicated "it's like anything that is published is always going to be a bit behind the times". Another replied from a different perspective, indicating "Yes, otherwise it would sort of undermine the basic principles of public health". Certain comments like this one seemed to portray the notion entertained by some clinicians that we have excellent public health practices, and that they should not be critiqued. Despite the fact that group should be receiving the same information through the same channels, their significantly varied responses in terms of perceptions of risk suggests that perhaps that the same message is not filtering through. The implications of this difference might be expressed through different clinical practice regimes regarding Lyme disease.

The non-Lyme advocates expressed complete confidence that the information they receive on LD risk reflects current knowledge, while the Lyme advocates explained their

belief that the knowledge being passed down is far from current, and contains important omissions. The view of the latter group focused strongly on the need for much further research, and enhanced connections to bring the current science into our local doctors' offices.

**Research Question Eleven: Do you suspect the risk of Lyme disease to increase or decrease in Manitoba during the next decade?**

Following the recognition of the participants' perception of risk in the province, this study attempted to determine their further perception as to how this might change with time. To set the stage for this question, a quick perusal of selected literature at this time will help in our analysis of the participants' responses, in particular with reference to the involvement of changing climate.

*B. burgdorferi* is documented as an accomplished traveler, and the new geography of infectious disease is being driven by shifting weather patterns that herald warmer temperatures, by environmental changes induced by humans, and by escalating international travel (Regush, 2000). Acknowledging climatic influences is important to studying any emerging infectious disease, and climatic change issues are evident locally. Bacterial zoonoses evolve along with these changes in climate, and with society and lifestyles (Phillipon, 2006). Forest biomes and tick-borne disease systems are highly susceptible to climatic influences, however climatic change has not been correlated to the increased incidence of tick-borne diseases in many parts of Europe over the past two decades (Randolph and Gern, 2004). Casimiro, Calheiros, Santos and Kovats (2006) estimated the future risk of zoonoses using ecologic scenarios to describe future changes in vectors and parasites, and concluded that higher temperatures may increase the transmission risk of zoonoses that are currently endemic to Portugal, such as Lyme disease. Year-to-year variability in Lyme disease cases in the U.S. has prompted improved understanding of the causes of such variability, and precipitation and mild winters seem to emerge as plausible causes for such variation (Subak, 2003). A Canadian biologist, Snetsinger reported "the number of ticks and associated risk depends primarily

on environmental factors such as temperature, moisture and population of deer" (Flegg, 2007). However, a recently reported human exposure risk study on the abundance and infection prevalence of vectors indicated "that precipitation in the current year and temperature in the prior year had only weak effects on entomological risk (Ostfeld et al., 2006). An increased incidence of various tick borne diseases in many parts of Europe due to documented climatic changes, as well as anthropogenic influence on habitat structure have been documented (Zajkowska, Malzhan, Kondrusik, Grygorczuk, Pancewicz, Kusmierczyk, Czupryna and Hermanowska-Szpakowicz, 2006). This increasing health burden of zoonotic diseases in Europe has been specifically linked to vector biology and disease transmission changes as a result of climate change (Gray et al., 2009). In Canada, Ogden et al. (2006) indicated climate change projections suggest that the range of *I. scapularis* will expand northward from its current range in southeastern Canada. A study was undertaken to determine whether or not this tick species could however establish itself in more northerly woodland types. The researchers concluded that *I. scapularis* could indeed successfully expand into more northerly woodlands in Canada (Ogden et al., 2006), and recent studies indicate the expanding range of *I. scapularis* is predicted to accelerate with climate change (Ogden et al., 2009). Resulting risk in Canada will clearly increase over time.

The scientists, with a firm understanding of these principles involved in risk, all expect that risk will only increase in Manitoba. The ranges of the vectors are expanding, increased numbers of endemic areas are expected, and increased exposure is expected. Climate change will influence this further. There is no question that they are communicating this information, and one of the national level Lyme experts is currently using modeling to identify patterns and enhance prediction. The policy makers clearly have received this message, all suspecting that risk will increase over time for the same reasons. Amongst these two groups, there was a solid and quick response to this question, leaving the firm impression that we can expect to see LD risk increase over time.

It was indeed surprising that the clinicians replied "Don't know", "it would depend", or that "it is impossible for me to predict what is going to happen". It was questioned whether or not they had truly been privy to 'enough' of the current scientific information, when the previous two groups seemed quite convinced that risk will definitely increase over time. The concern for public health lies in the perception held by the clinicians that the LD risk they currently believe is very low, is not going to change, and that they don't have a true sense of this documented emerging infectious disease in Canada.

The non-Lyme advocate group had a similar response with being generally unsure, while the Lyme advocates followed the scientific information, firmly suspecting risk would increase in time. An intriguing response following a different line of thought was heard from this group. "We are going to have an increase in the number of diagnoses just because of awareness", suggesting that as people are made more aware of Lyme, the number of reported cases will climb, and the perception of risk will follow suit. As previously presented, the perception of LD risk that is held by certain individuals is based on their knowledge of the number of cases documented. Considering the likelihood of improved awareness of Lyme in Canada over time, we might therefore expect an increased sense of risk if more LD cases are actually captured by clinicians operating under heightened awareness.

**Research Question Twelve: What preventive methods are currently employed to minimize Lyme disease risk to Manitobans, and Canadians?**

Preventing infectious disease by minimizing risk requires a focus on reducing exposure to the pathogen. The research has indicated the primary way to reduce exposure to Lyme disease is to reduce human contact with the vectors. Aultman, Walker, Gifford, Beard, Scott and Severson (2000) pointed out that attention has been drawn to reducing vector populations through community-based environmental modifications that reduce vector development or survival, through genetic manipulations of vectors to render the arthropods incompetent to transmit pathogens or alter their breeding systems, or through the use of natural enemies and microbial pathogens for biological control. Voigt (2006,

p.1) however reported "it is not possible to control ticks in nature... the first step should be an effective prevention". A variety of large-scale prevention methods are cited in the literature, some viewed more practical than others. Piesman (2006) reported testing of forest products as barriers to nymphal *Ixodes scapularis* to see if they could potentially be used to define high and low density tick zone borders on residential properties in Lyme disease endemic regions of North America. Three wood products were identified, which effectively act as barriers to nymphal *I. scapularis*: Alaska Yellow Cedar sawdust, Alaska Yellow Cedar woodchips, and cellulose, with the first most effective. The authors suggested creating barriers may someday play a role in integrated campaigns to reduce Lyme disease risk. Given exposure to the nymphal stage of the *Ixodes* life cycle is the most dangerous LD risk factor, prevention methods like these might become useful in Canada with further emergence of the disease. To date however, it seems that our assessment and perception of very low risk have limited any need for prevention strategies beyond the individual, as our interview responses display.

The scientists all indicated that general messaging to educate the public on tick avoidance was the key to prevention. The personal protective suggestions by this group included the avoidance of tall-grass habitats or wooded areas, wearing long pants and a long-sleeved shirt, tucking in clothing, using an appropriate mosquito repellent with DEET, doing tick inspections and removing ticks as soon as possible. Only one of the scientists pointed out the importance of "being aware that the tick is very small, freckle size, and to be vigilant for it", yet this is likely one of the most important pieces to communicate to the public since most people are usually concerned with large, adult ticks they can easily spot.

The policy makers shared the view that the use of personal protective measures when in a certain area was important, stressing endemic areas in particular. They generally indicated the same kind of prevention methods mentioned by the scientists. They also pointed out that physicians need to ask appropriate questions when they think they have a patient with Lyme disease, and make a diagnose quickly to facilitate effective treatment. This was not seen as a direct method of disease prevention, but somewhat of a preventive

method for reducing the chances of misdiagnosed LD cases that might surface as more serious diseases down the road.

The clinicians all concurred that education of health care providers and the public through public health messages is crucial to minimizing risk, and preventing disease. Education must incorporate the most current and accurate scientific knowledge into an effective form of communication for both clinicians, and the public. The clinicians are responsible for identifying this difficult disease when it presents itself in a variety of forms, while having little or no experience with it. This extremely difficult role should be supported with the ongoing dissemination of critical information to assist clinicians who are on the front lines. Media-based education to date has dominated, and has included letters to clinicians, posters, and public newspaper advertisements. One clinician indicated that perhaps television would be a useful tool for delivering messages on LD risk and prevention methods. Overall, this group seemed generally dissatisfied that Canadians and Manitobans had been delivered all of the necessary information and known strategies to protect themselves.

The non-Lyme advocate group identified the media as their source of information on general tick awareness, while the Lyme advocates felt that the media misses a critical role in delivering up-to-date and important information on LD transmission to the public, so that they can take personal precautions. They feel the messages are too general and simplistic, and neglect to relay a true picture of risk as a result. For example, one Lyme advocate felt that the media's focus on tick prevention while out in tick habitat was misleading given it is possible to acquire Lyme disease in our backyards. Furthermore, this advocate suggested "we have got to start debriefing the public from everything they know about ticks", because people have been mainly exposed to dog ticks or wood ticks "and they'll say oh that's a big tick, I would know if that was on me", and are not looking for the tiny little speck of a nymphal tick. It seems that the scientists stress they are delivering this message, so the information is being lost somewhere in communication to other groups. The data in this study seem to suggest barriers are at play somewhere

between those delivering policy, and the receipt of information by the clinicians and the public.

**Research Question Thirteen: Do you have a role in assessing the risks associated with this disease in Manitoba, and in Canada?**

Following a thorough evaluation of key informant risk perceptions, this study identified those individuals whose LD knowledge base and perceptions of risk were gathered formally for provincial and national risk determination. Two groups of individuals were identified to have involvement with risk assessment of LD in Manitoba and Canada: the Lyme expert and academic scientists, and policy makers. The latter group includes Medical Officers of Health (MOHs) and members of the Public Health Issues Committee and Zoonosis Disease Steering Committee. The level of LD knowledge and understanding was most evident in the former group, who as indicated earlier, presented a perspective of LD risk in the province to be slightly higher, and more variable than those with less LD knowledge. Given their professional opinions are highly regarded amongst all of the informant groups, it might follow that parallel perceptions of risk should exist in all groups. A contrary position was expressed during the interview process however. Those groups with less LD knowledge generally expressed a perception of risk that had been minimized following communication from those involved with assessing LD risk.

**Research Question Fourteen: Do you feel at risk for Lyme disease living in Manitoba?**

**Research Question Fifteen: Do you worry about you or your family contracting Lyme disease, and have you taken any personal precautions in the past year to prevent yourself from exposure to Lyme disease?**

Due to the congruent nature of question 14 and 15, analysis of the responses was completed concurrently. Starting with the non-Lyme advocacy groups, we heard little concern, and minimally adopted precautions were mainly centered on removing ticks

from their dogs. It is expected that this would be the normal practice followed by the public. The Lyme advocates on the other hand are absolutely concerned, primarily due to personal experience with the disease. They and their families exercise the utmost of precautionary measures, which extended beyond tick removal to avoiding tick habitats, tucking pants into socks, using insect repellants regularly, and educating their families and friends to follow suit. Individuals in each of these groups take precautionary measures according to their perception of LD risk.

The clinicians as a group did not feel at risk for LD personally, particularly if not visiting an endemic area. Their responses, including "I don't feel at all at risk in Manitoba" and "I guess I have never really thought about it" showed this group overall to be the least concerned about personal risk. The scientists' message that LD risk can exist outside of an endemic region has either not been directed to, or not been taken seriously by the clinicians. If the clinicians themselves do not feel that there is much or any risk to them and their patients, they may never consider LD with their patients. It is apparent in the data that personal perspectives might provide an operating foundation for professionals, and if clinicians are not considering LD as a serious possibility in the province, then it makes sense that there are few reported cases. The clinicians' perceptions in part, are a product of their knowledge base, coupled with the public health risk information channeled to them. Clearly this group has a minimized perception of risk when compared to the expert scientists assessing LD risk, and the implications for public health include fewer LD diagnoses, and patients facing misdiagnoses.

There was consensus amongst the policy makers, those who ultimately create the communication pathway to the clinicians, that they are not particularly concerned about Lyme disease. One indicated since they had never personally seen a deer tick, "I think that influences my attitude which could probably be characterized as complacent". Another demonstrated their lack of awareness that nymphal ticks were largely responsible for vectoring Lyme disease. Knowledge of nymphal tick size was not factored into any of the policy maker's perceptions, yet this is a crucial message that should be getting out to clinicians, and to the public.

The scientists at the top of this information hierarchy, felt they were at an increased risk for LD given their occupational exposure, and generally that personal precautions were important when doing field work. The personal precautions this group and their families exercised during leisure activities were however expressed as minimal, and this group indicated they don't feel the same level of risk at all in an urban setting. One scientist indicated "In general I feel the Lyme disease risk is low, so I am not personally concerned. [...] If the risk was increased, I would be more diligent but right now..." Even the scientists who are aware of all exposure pathways, and understand LD risk, adopt an "it will never happen to me" relaxed attitude to this serious disease. It was remarkable that this group did not comprehensively consider their working knowledge of LD, and merge information on nymphal tick size or urban exposures due to birds, with their personal strategies for protection. This seems convincingly indicative of the broadly adopted perception that Lyme disease risk is so low, that nobody gets Lyme here.

A substantial epidemiological knowledge base within each group seems to be key to the effective control of the disease, and was seen to influence participants' risk perceptions. Throughout the concurrent interviewing and grounded analysis process, coding successfully allowed for repetitive and frequent ideas to surface, as well as identified certain omissions, which linked the groups together. The development of the core category in this research emerged during data collection, through the use of the 34 identified codes, rising above all other categories in importance: this was that knowledge about Lyme disease epidemiology is central to determining the perception of LD risk held by each group, and key to bringing all groups together for effective control of the disease. The category map presented in Chapter Two visually introduced the framework and hierarchy of subcategories unfolding from the data.

Five subcategories flowed from the core category identified in this analysis. These were: *a lack of knowledge and understanding* of Lyme disease; *LD transmission*; *application of LD knowledge;, individual roles*; and *personal perspectives*. Further subcategories unfolding from these five, were created given ample data to support them.

A *lack of knowledge and understanding* of LD was identified as the first important subcategory in this study due its significant role in potentially creating barriers to the effective control of LD. Without knowledge, the power to generate policy efficiently, and the ability of clinicians to successfully treat patients would be compromised. As a whole, the data suggested fairly early in the interview process that a lack of understanding was hindering these two particular groups, the policy/decision makers and the clinicians, from providing superior public health. The lack of understanding in the data was further divided into the subcategories of research, and research funding. Multiple references to the need for further research to understand LD were expressed during the interview process, with all groups agreeing on the need for increased funding, but at the same time recognizing that priorities in health care must be established, and that there may not be sufficient resources to support all interests.

The second subcategory identified was *LD transmission.* Given the complexity of this disease, in-depth knowledge and understanding of all aspects of the epidemiology and ecology of Lyme disease coupled with likelihood of human exposure is required amongst the groups to effectively determine risk. When coded data were sorted, another hierarchical subset appeared. The six further subcategories derived from LD transmission included: the pathogen; the hosts; endemicity; known risk factors; disease mimicry; and LD vectors, the latter being further subdivided into three other categories, which included vector stage, other potential vectors and mechanical transmission. Each of these subcategories play a significant role in some facet of Lyme disease transmission, and exploring the participants' knowledge base of these categorical topics proved critical to identifying gaps in understanding between the informant groups.

The third subcategory, *application of LD knowledge,* became apparent following the identification of the variety of LD transmission factors, and the need for the complete information to be delivered to appropriate public health groups, and applied. Further subdivision unfolded by referring to the various ways to apply LD knowledge: diagnosing LD; testing for LD; treating LD; communication/messaging; education; prevention; and the larger umbrella of risk management. Each of these subcategories was

created to serve the data analysis in determining if the informant groups as a whole were effectively carrying out their specific public health role. Specifically, they helped determine if the scientists' expertise at every level was appropriately delivered to the policy makers, if policy was being effectively created and communicated from this, and if the clinicians were receiving important and comprehensive direction on Lyme disease to best serve the public.

As an analytic aid for working with a large volume of data, *individual roles* branching from the core category were described as the fourth subcategory. These differ slightly from the informant groups in that certain cases, informants identified in one group could serve two roles. For example, certain scientists are involved in various levels of decision/policy making, and certain policy makers also act as clinicians. In both cases they provided data that were analyzed within the context of the particular role served by the question. Five roles from this subcategory were identified: the scientists, policy/decision makers, the medical community, disease-specific advocates, and a fifth group stretching beyond the key informants, the public. It was considered important to draw out this final group, as the data indicated that the public plays a key role in prevention, as part of LD risk management.

A fifth and final subcategory, *personal perspectives*, was derived to sketch out the variety of feelings and emotions from the groups, which established the personal base for the application of LD knowledge within their given role. During the first few interviews, some initial coding and observer's comments were created, since it became quickly obvious that the wide-ranging backgrounds of the individuals interviewed, and their wide-ranging beliefs warranted organizational labels. The informants' responses to the questions posed were analyzed not only for content, but also for their emotions and underlying beliefs, and for the implications of these beliefs on their role in LD epidemiology. After careful consideration of the codes originally designed to fit the data, a reworking of these codes during final analysis to best serve the variety of perspectives led to nine subcategories: Perception of risk, trust of information, uncertainty of information, concern or worry, lack of concern or worry, frustration,

upset/anger, controversy and helpful. It was important to identify a lack of concern or worry, as ample data fitting this category become apparent, not simply meaning concern was not expressed, but rather a definite lack of concern was expressed. Each of these subcategories allowed for the informants' responses to be appropriately coded, and ultimately analyzed for their frequency or omission, which helped to provide a clearer picture of the operating perspectives of the informant group as a whole. These operating perspectives play a role in guiding the groups as they facilitate LD public health.

Problems with information dissemination were evident as data were collected and analyzed. Varied responses to the fifteen questions were heard within and between the participant groups, and significant informational gaps were identified between the groups. These gaps seem to affect the ability of each particular group to efficiently carry through with their specific public health role.

# Chapter VI: Conclusions

This thesis set out to examine the current understanding of the epidemiology of Lyme disease, the nature of the disease in Manitoba, and the perception of its risk to the population of the province. The understanding of the disease, and the impacts of perceived LD risk in Manitoba are important for the development of appropriate provincial public health policies, for guiding clinicians in their practices, and for general public awareness to minimize risk to the population. This study is one of few created to determine risk perceptions associated with a disease, and is an important contribution to future LD epidemiology in the province. The three research objectives that were central to this study, and the main findings are described.

This study first reviewed the current scientific understanding of the ecological and epidemiological issues influencing the risk of Lyme disease, including: The pathogen *Borrelia burgdorferi*; Lyme disease vectors; life cycles and competency; and the hosts and reservoirs of the disease. The science behind LD has progressed tremendously in recent years, and appears much more complex than generally understood. The global research on LD during the last decade has been extensive, and critical aspects of this science are new to public health in Manitoba. Key informant scientists are familiar with much of the current research, are leading some of the research in Canada, and recognize that crucial details must be communicated for effective LD control.

In 1987 we read in *Scientific American* that much remained to be learned about Lyme disease (Habicht, Beck and Benach, 1987). In November 2003 we read from the California Communicable Disease Control that despite 25 years' experience with Lyme borreliosis, much still remained to be learned about this complex zoonosis (Fritz and Kjemtrup, 2003), and much learning is still required. Understanding the risks, and how to prevent or control Lyme disease can only be effective if all vectors, hosts and reservoirs of the bacterium are identified and controlled. Current research in West Nile virus for example has taken heed of this, as Lawrie, Uzcategui, Gould and Nuttall (2004) reported that although mosquitoes are the primary vectors, West Nile virus (WNV) has

been repeatedly isolated from ticks. They indicated that although ticks are unlikely to play a major role in WNV transmission, some species have the potential to act as reservoirs for the virus, and until all vectors and reservoirs are identified, control of WNV is not possible (Lawrie et al., 2004). In general, considerable scientific research is necessary to effectively reduce the incidence of all "vector-borne" disease (Suss and Schrader, 2004), including Lyme disease. The ecological dynamics require much further attention according to Booth (2005), and the challenge according to Ostfeld (2005) is to figure out how much of this enormous complexity we need to understand, in order to manage the pathogenesis and risk of Lyme disease. In Manitoba, the critical research surrounding the *Borrelia* pathogen, recognized LD vectors, and suspect LD vectors are examples of new science important to understanding risk in the province. Very little current science has been incorporated into risk assessment within the province, with much evidence from the interviews conducted in this study.

Little understanding of the complexity of the pathogen or the variety of genospecies is evident from discussions with the policy makers and the clinicians within the province, and the related complications to diagnosis and treatment. Important details of Lyme disease vectors, their life cycle and life stages, and suspected possible vectors are lacking within groups who should have considerable awareness and understanding of the current research. Important epidemiological information that is critical for policy makers and clinicians is not, to a certain degree, being relayed effectively or in timely fashion, from the scientific community.

Secondly, this study provided a descriptive spatial analysis of the reported incidence of Lyme disease from a global, regional and local perspective, with reference to LD symptoms and other regional diseases that might invite confusion. Questions about disease distribution surfaced when investigating the official statistics of LD in the province. Global and European patterns of disease were compared with patterns in North America, and Manitoba. The incidence of disease appears to be higher elsewhere, including certain regions adjacent to Manitoba. The question of why Lyme disease is minimally reported in Manitoba prompted an exploration of other diseases for which LD

has been misidentified. Multiple sclerosis, lupus, arthritis, fibromyalgia, Chronic Fatigue Syndrome and others have created difficulty for health care providers, since many of the symptoms overlap with those found in LD, leading to misidentification. Questions of potential cases of misidentification in the province unfold when the incidence of these diseases are compared against the low incidence of Lyme disease in Manitoba.

Thirdly, this thesis investigated risk perception for this disease in the province from the perspective of regulatory and/or academic scientists, decision/policy makers, medical clinicians, and disease-specific advocacy groups, through twenty-three key informant interviews. A question of the critical science reaching the front-line health workers who are responsible for initiating the process of disease identification emerged. Moreover, concerns of the degree to which the practices of front-line health workers may have been influenced by their knowledge base, and their risk perceptions emerged.

Difficulty remaining abreast of the extensive research supporting the complex ecology of the Lyme disease pathogen, the vectors, the hosts and the reservoirs was evident throughout the interviews, and participants expressed their lack of knowledge might be the result of limited resources. Certain frustrations were expressed by a number of participants that further research was necessary, and that the communication of this research to all interested parties was key to effective LD control in the province.

Minimal understanding of the variety of *Borrelia* genospecies was clear in most of the interviews, and the policy makers and clinicians might be better able to interpret local LD test results if aware of the potential that other genospecies might be present, but are not incorporated into local testing protocols. Different, wider-ranging tests are used here if patients have traveled to Europe where the varieties of genospecies such as *Borrelia garinii* or *Borrelia afzelii* are recognized, yet clinicians are not directed to ask if their patients have been to Europe. With the identification of *Borrelia garinii* in birds in Newfoundland, it might be practical to consider the potential for this genospecies to cause disease in Canada, and to test for it.

Evidence of minimal understanding of the potential for vectors other than the solely recognized *Ixodes scapularis* within Manitoba was seen during the interviews, and the potential for other vectors possibly contributing to disease transmission was entirely dismissed. A number of participants, including some involved in policy making or in clinical practice also indicated some confusion with vector life stages, and their contributions to disease exposure and risk. The understanding of risk factors associated with nymphal *Ixodes* ticks, as known to the scientists, was not part of the policy makers or clinicians LD knowledge base. Seasonal exposures resulting from different life stages are crucial to determining patient potential for Lyme disease. Most of the policy makers, clinicians, and disease advocates were unaware that the nymphal ticks present the highest risk for people, and are as small as the typed period at the end of this sentence. Effective disease control might be hampered when key facts like this are not part of the working knowledge base of all public health groups.

Interest in the higher incidence of Lyme disease in adjacent regions both nationally and provincially was not expressed during the interviews, nor has it been incorporated into our understanding of local risk in Canada or Manitoba. The documented low incidence of LD within the province seems to be accepted, based on the general understanding or perception that Lyme disease might be emerging locally with the recent identification of endemic Buffalo Point. The potential that other diseases may have been misidentified, since Lyme disease is a great imitator, has not been considered to play a role in the low incidence of LD within the province or nation.

Throughout data collection and analysis, the 34 codes utilized allowed one major category: knowledge and understanding of LD to emerge from the data. Five further sub-categories also emerged: lack of knowledge and understanding of LD, application of LD knowledge, LD transmission, individual roles, and personal perspectives. Each category reached saturation as data became repetitive throughout the collection and analysis process. In keeping with standard application of grounded theory, three themes were established along with a summative theory that was ultimately derived from this study.

The first theme was that a barrier exists, preventing the complete dissemination of information from the scientific community through to the public. This study suggests that the scientists' messages, and their delivery to the policy/decision makers, most importantly those working within MB Health, are reportedly complete and frequent. As such, the disruption in communication would seem to occur somewhere along the information pathway from the decision makers to the medical community and the public. Where this disruption occurs however, seems in part to depend upon the perception of the individuals ultimately receiving the information, and how they use it. If a medical clinician has little awareness of LD, or an established perception of extremely low LD risk in the province for example, it seems that the flow of information may stop with them, never enter into their practice, and ultimately never make it to the patient. The data provided in the interviews suggest that barriers at this level of communication were not uncommon. A second disruption in communication seems to occur between the policy makers and what is delivered directly to the public via bulletins and related media paraphernalia. The scientific message is lost to some degree in translation through the bulletins created for the lay public. Furthermore, the message of risk tends to be minimized, which might in itself perpetuate a public perception of low LD risk in the province, and ultimately influence precautionary practices.

The implications of the existence of these barriers and the resulting suboptimal flow of information on LD may have important consequences. Control of the disease, regardless of its current level within the province, will require the combined efforts of the scientists, public officials, the frontline medical doctors and the public. The foundation of any control program would be current, correct and appropriate scientific information, which enable physicians to properly judge the risk of LD in their patients and permits the public to prevent exposure and to seek appropriate help when necessary. Problems with the effective dissemination of information between the groups evident in the data, thus may hamper LD control in the province.

The second theme identified from this research is that operating knowledge levels, along with personal perceptions of risk seem to directly affect or influence professional

practice. For example, it is evident from the data that individuals with a larger scientific knowledge base and understanding of this disease, identify the risk of disease in the province to be more significant than those who know less. The scientists who are knowledgeable of all aspects of LD transmission conclude that the risk in Manitoba is generally low, and recognize a number of potential influences that might elevate this risk in certain areas, or as a result of certain habits such as camping. On the other hand, individuals who lack knowledge and understanding of this disease, for example in areas of vector competency, LD testing or otherwise, more frequently perceive the risk to be extremely low, rare, or exceptionally rare.

Throughout analysis of the data, it became apparent that one's perception of LD risk in Manitoba might have significant impact on their practices, professional or otherwise. This was most evident in the clinician group interviewed. For example, clinicians operating with the personal perspective that some risk of LD is present within the province, expressed their desire to thoroughly investigate when their patients presented with LD symptoms. On the other hand, if a clinician believes that the risk of LD in urban or rural Manitoba is zero or minimal, the evidence in the data suggests the impact on their professional practices is contrary. For example, if certain obstacles are encountered in diagnosis, such as testing issues including false negatives, a physician not believing their patient could have LD might not pursue further investigation into Lyme disease, and might not treat this patient for LD. Or, an MD might not even consider LD as a possibility because their patient hadn't been in a reported endemic area, didn't have an EM rash, or didn't have a history of a tick bite. Clearly, this is problematic to effective LD control in the province, and all individuals need to be equipped with the best information to determine their risk.

A third theme apparent in the data is that the risk of Lyme disease might be greater overall than is generally understood, and perceived. For example, other possible vectors not considered to pose risk, might actually allow for a certain amount of disease transmission. As presented in Chapter Three, other tick species such as *Dermacentor variabilis,* and other insects such as *Aedes vexans* questioned to transmit Lyme might

play some role in disease transmission in Manitoba. Furthermore, knowledge and acceptance of certain low-risk transmission pathways, such as blood transfusion, congenital contraction, or mechanical transmission from vectors have not been factored into risk assessments or messages to date. As identified by one clinician, it is also important to recognize that with little or no experience with diagnosing or treating LD patients, MDs are not acquiring the experience they might need to make this difficult diagnosis. This, coupled with the clinical perceptions that might lead to disease misidentification, we might expect lower numbers of LD cases being reported in Manitoba. If the numbers of LD cases in the province are actually higher than what is reported, this in itself might impact the perceptions of risk held by people in the province, and further enhance the perception of low risk. And if this is the case, an imprecise perception of low risk will continue to impact practices, both professionally and otherwise. This might create difficulties for effective LD control in Manitoba.

These three themes, as they emerged from the data, directed this research toward a theory, which is well grounded in the data. This research revealed that more scientific research and an increased knowledge of LD, along with improved communication amongst all interested parties in the province might lead to the best perception of risk in Manitoba, and overall improved public health. And as a result, an increase in the number of Lyme disease diagnoses within the province might be expected to follow.

Further Research and Policy Implications

This research exposed specific gaps to effective Lyme disease control that might provide opportunity for further research. It was evident in the data that there are three potential directions new research might take.

First, surveillance research in the province is required to establish sufficient baseline data that would be helpful to future LD management. Specific areas requiring attention include the pathogen and the variety of emerging genospecies, the vector(s) and their competency, and the variety of hosts and reservoirs within the province.

Given the global variety of *Borrelia* species, and the limitations of testing within the province to the specific genospecies *Borrelia burgdorferi* s.s., scientists should expand their research to include other species, and their potential in both recognized endemic sites within the province, and other potential sites in Manitoba. Without research, the understanding of *Borrelia* pathogens that might be present in the province is largely speculative. Serosurveys of residents in regions lacking a known Lyme vector might further help to establish a better understanding of exposure, to which vector research could follow.

Vector research in Manitoba should consider other potential vectors present in the province that have been named elsewhere for possible disease transmission. It might be possible that certain vectors have been overlooked due to imperfections in knowledge about the disease, or the thought that ecological conditions might be lacking in Manitoba, and these might be important for understanding disease transmission and risk within the province. Surveillance studies to date in the province have only included *Ixodes scapularis*, and have been limited to isolated areas of endemic concern. The competency of vectors, and potential for mechanical introduction of disease, particularly given the ability of both *Dermacentor variabilis* and *Aedes vexans* to carry LD, should also be studied further within the local context. If these two species present even a minor risk for LD transmission, their enormous populations in Manitoba might suggest an elevated risk

for the people in the province. Vector sampling might be done throughout the entire province to collect baseline data useful for ongoing research. Particular areas of interest or concern could include provincial ecosystems that support the pathogen, vector and hosts, and are located where people might be exposed. Cottage communities, rural towns, and urban environments are all important areas to at the very least, conduct some preliminary research. Long-term sampling at sites identified to have significant vector populations of concern could allow Lyme research to extend to other areas outside of endemic Buffalo Point on a continual basis. Given annual fluctuations in supporting ecology, regular surveillance is important for identifying localized pockets of potential disease exposure.

Hosts and reservoirs have not been studied in the province, aside from localized research at Buffalo Point recently. Deer, mice and other large and small mammals might be playing an important role in maintaining the pathogen, or the vector species involved in LD transmission, but without research directed toward this, we are left assuming that white-tailed deer, along with deer mice are the primary hosts and reservoirs simply because they are elsewhere. Research to explore all potential hosts and reservoirs in Manitoba is needed, so human exposures and risk information data is complete.

The possibility exists that a general lack of knowledge and understanding of Lyme disease in the province might contribute to an inaccurate perception of risk, and significantly impact professional practice. Education and updating of the medical community and of local physicians who might not recognize the potential for the transmission of Lyme disease in the province, based on their limited understanding of LD and perception of risk, is important.

A second direction new research could take is to focus on the relationship, or possible misidentification between Lyme disease and MS, and other symptomatically overlapping diseases. If revised risk analysis suggests that it is warranted, medical testing might be expanded to incorporate testing for LD whenever any potential for misidentification exists. Given the potential to contract LD within the province, newly diagnosed MS or

other patients might be closely examined for Lyme disease. Criteria to trigger a more in-depth patient examination or LD testing might include a variety of multi-system symptoms not common to MS, arthritis or lupus alone. More specifically, overlapping LD and MS symptoms are neurological, but if a patient experienced other symptoms, such as joint involvement, further investigation might be warranted. Since there are a number of documented concerns over LD testing reliability in the province, perhaps greater emphasis should be directed toward MB Health's directive that Lyme disease is a clinical diagnosis. Following this, if questionable cases of MS or other commonly misidentified diseases are identified, they might form an important research group for a clinically diagnosed antibiotic trial for Lyme disease. Manitoba Health or some other group might conduct this type of research, to explore the possible connections between these diseases, or misidentification that might be taking place within the province.

Finally, this research suggests the need for better communication of current LD science from the scientists to the policy/decision makers, the clinicians, and the public. Barriers to effective information dissemination were identified between the groups, and it was recognized throughout the interviews that key informants were unsure how to improve the flow of information. Could the scientists, or the policy makers create a more effective means of communicating information, or more powerful tool to ensure their messages are heard? Research is needed to determine how to deliver important health messages effectively, so that improved communication is realized to clinicians and the public, and ultimately better disease control is achieved. It was suggested by one policy maker/clinician that perhaps television advertisements are the best way to go, since they might target the largest audience, given that MB Health bulletins were obviously not getting the message out as intended.

The implications of the important science not reaching those who can apply it are enormous. In order to address this, government, research scientists, health care workers and other appropriate parties should develop a common system that enables leading-edge scientific findings to be disseminated in appropriate form in order to facilitate further research, public health, and public education in the province.

Communication delivered directly to the public should be created to effectively portray a scientific and thorough understanding of risk in Manitoba. Effective prevention and control strategies among the public will only be implemented in response to the perception that within Manitoba, all persons are at risk for this disease.

Should policy changes be implemented, in order to judge the effectiveness of interventions, this study might be revisited in three to five years time to see how the situation, and flow of information might have improved.

Glossary of Terms (in alphabetical order)

*Arthropod* – The largest phylum of animals which includes the insects, crustaceans, and arachnids, the latter of which the ixodid (hard) ticks belong to.

*Borrelia burgdorferi* sensu lato complex – The group of spirochetal bacteria that collectively act as Lyme disease pathogens.

*Borrelia burgdorferi* sensu stricto, *Borrelia afzelii, Borrelia garinii* – Specific strains of the *Borrelia* spirochete that most commonly cause Lyme disease.

*Bridging Vector* - Transmits the infection to humans without necessarily maintaining the agent of disease in nature.

*Competent/Important Vector* – This describes ticks which acquire the bacterium while feeding on infected reservoir hosts, maintain the spirochetes in their midgut while they moult, and successfully pass the infection on to the next host.

*Experimental Vector* - Shown to be capable in the laboratory of acquiring a pathogen while feeding, maintaining it until the following blood meal, and transmitting the infection to a host.

*Haematophagous* – Requiring a blood meal from a host.

*Incompetent Vector* – Vectors observed to be unable to maintain spirochetes through a moult, and unsuccessful in transmitting infection to a host.

*Intermediate host* – Sustains the pathogen in some vector-borne diseases, Lyme disease included, and can serve as a blood meal for at least one of the vector's life stages.

*Ixodes ricinus complex* - Includes four hard tick species which serve as the primary bridging vectors of Lyme disease spirochetes to humans: the castor bean tick, *Ixodes ricinus* and the taiga tick, *Ixodes persulcatus* in Eurasia, and the blacklegged tick, *Ixodes scapularis* along with the western blacklegged tick, *Ixodes pacificus* in North America.

*Maintenance host* – Animal serving as part of the enzootic maintenance of the disease cycle. For example, deer are maintenance hosts for tick populations, keeping the tick population healthy.

*Monophyletic* - Refers to a taxon or clade with any group of organisms that includes the most recent ancestor of all those organisms and all the descendants of that common ancestor (i.e. insects, mammalia).

*Non-reservoir host* – These hosts may have contact with infected ticks, but are unable to transmit the infection to ticks.

*Primary Vector* – Refers to the most successful vector for transmitting a particular pathogen (i.e. mosquito for West Nile, tick for Lyme disease).

*Reservoir host* – Animal serving as part of a reservoir population, to exist and maintain the pathogen population in nature. These hosts are a proven natural source of infection for ticks or insects.

*Secondary Vector* - Gray et al. (2002) suggest this term be applied to vectors that are not necessarily considered the most important or competent vectors in transmitting disease, but do play a secondary role in transmission, and have potential to be a part of the enzootic cycle of disease.

*Scutum* – Shield-like exoskeleton hard covering on the back of ticks.

*Spirochete* – A corkscrew-shaped prokaryotic (lacking a nucleus and various organelles) bacterium. The Lyme disease pathogen is a spirochetal bacterium.

*Transstadial transmission* – Transmission of the bacterial pathogen through one or more of the tick's developmental stages (egg → larva → nymph → adult).

*Transovarial transmission* – Transmission of the pathogen from the adult female tick to the eggs.

*Vector* – A living organism, usually an arthropod, responsible for transmission of the pathogen (usually a microorganism) and resulting disease to humans.

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