Studies on the Vector Ecology of the American Dog Tick, *Dermacentor variabilis,* (Say) (Acari: Ixodidae) in Manitoba, Canada

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Abstract

The American dog tick, *Dermacentor variabilis* (Acari: Ixodidae), is a blood-feeding arthropod known to vector pathogens of medical and economic importance. This tick is widely distributed throughout eastern North America. In Manitoba, this tick is near its northern distributional limit, complicating its biology and capacity to act as a reservoir and vector for pathogenic agents. After the detection of an outbreak of bovine anaplasmosis in southeastern Manitoba in 2008-2009, I conducted a study to determine what, if any, role arthropod vectors had in the maintenance of the infection in the outbreak region. *Dermacentor variabilis* is the only biological vector of *Anaplasma marginale*, the causative agent of bovine anaplasmosis, in the region. During the season of adult tick activity, in 2011 and 2012, a total of 2056 ticks were collected and screened using real time PCR for the presence of *A. marginale*. Additionally, the mouthparts of 560 horse flies (Diptera: Tabanidae) collected in 2011 were also screened, as these flies are considered to be possible mechanical vectors. None of the ticks or fly mouthparts tested positive. I also screened 1044 adult *D. variabilis* from 10 localities across the province (averaging 95 ticks per locality) for Spotted Fever Group Rickettsia, using real time PCR, to establish a baseline prevalence of infection and distribution throughout the province. The ticks were infected with only one rickettsia species, *Rickettsia montanensis,* at a prevalence of infection of 9.8% (range, 0.00-21.74% among the localities) based on sequence analysis of the gene encoding for OmpA. Male and female ticks were equally infected; however, prevalence of infection was lower in ticks collected from the north compared to ones from the south. Finally, I assessed the ability of field-collected, unfed, questing adult ticks to survive an additional winter in Manitoba. Adult American dog

ticks in more temperate climates can survive through more than one vector season. I found that 39.4% (SE ± 2.50) and 19.9% (SE± 1.14) of these ticks collected in May in Manitoba, and placed in outdoor enclosures, survived until January and April, respectively. There was no difference in survival between males and females. Therefore American dog ticks may act as pathogen reservoirs in a greater capacity than previously expected.

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I wish to dedicate this thesis to my father, Wilfred Yunik. He has been supportive of all my endeavors throughout my life and taught me the importance of higher education.

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Formatting Note

This thesis is written in a manuscript format, with the three scientific chapters written as if they were stand-alone scientific journal articles. Species that are introduced for the first time in each chapter have their complete scientific name followed by the taxonomic authority. A complete list of literature cited for all chapters is provided at the end of the thesis.

Chapter 1: General Introduction

Ticks are obligatory, blood-feeding, ectoparasitic mites that require blood meals for sustenance, development and in most cases, gamete production. They are a diverse group of arthropods, with over 900 recognized species, that infest every lineage of terrestrial vertebrate (Balashov 1972, Barker and Murrel 2008). Ticks are also arguably the most successful of arthropod vectors, transmitting the greatest variety of pathogens, including viruses, protists, nematodes, and bacteria. In terms of impact from a public health and veterinary standpoint, ticks are considered to be the penultimate disease vector, second only to the mosquitoes and pose serious risks to humans, livestock and wildlife.

The American dog tick, *Dermacentor variabilis* (Say), is likely the most abhorred tick found in Manitoba. In the adult stage, these large, conspicuous ectoparasites readily feed on humans, livestock, pets and numerous other medium to large-sized mammals. They are distributed, often in dense populations, throughout the southern portion of the province and can be encountered in rural and urban settings. Once attached to their host, females can feed for several days, engorging with blood and swelling multiple times in size. The feeding pool that is generated under the host's skin often forms large, unsightly and intensely itchy welts that are prone to secondary infection. These inflamed feeding sites offer ideal conditions for the transmission of numerous blood-borne pathogens that can result in debilitating disease.

Members of the bacterial order Rickettsiales are obligate intracellular parasites. Numerous genera within this order (*e.g.*, *Rickettsia* and *Anaplasma*) include tick-borne pathogens, some of which that are spread by the bite of *D. variabilis*. Some of these

pathogens are considered to be the causative agents of newly emerging and re-emerging diseases on the global level, including the tick-borne spotted fevers (Raoult and Roux 1997, Walker and Ismail 2008, Wood and Artsob 2012). Some of these emerging and re-emerging spotted fevers have been detected in North America (McQuiston *et al.* 2012, Wood and Artsob 2012). Understanding more about the ecology of the American dog tick in Manitoba will provide insight that can be used to evaluate policy and manage these diseases if and when they arise in the province.

The causative agent of bovine anaplasmosis, *Anaplasma marginale* (Theiler), is an example of a pathogen that has caused epizootic outbreaks in Canada. Globally, it is biologically vectored by a wide range of hard ticks, including *D. variabilis*, in tropical and subtropical regions around the world. The bacterium infects and reproduces within the erythrocytes of many species of ungulates, including but not limited to white-tailed deer, *Odocoileus virginianus* Zimmerman, elk, *Cervus canadensis* (Erxleben) and New and Old World bison and buffalo. Greatest pathology and economic loss occur when cattle herds become infected. The disease can present an acute phase, resulting in up to 90% of the red blood cells being infected and removed by the host's endoreticular system. Symptoms can include fever, loss of weight, icterus, respiratory distress, abortion and death (Jones and Brock 1966). Animals that survive after being infected become chronic carriers of the bacterium and pose the risk of infecting ticks, their calves and they may serve as a reservoir allowing for iatrogenic transmission (Kieser *et al.* 1990). Ticks can only become infected by feeding on an infected host, as transovarial transmission is not known to occur (Kocan *et al.* 2010). Due to the significant economic importance of *A. marginale*, many review papers covering the biology, as well as clinical information,

have been published (Jones and Brock 1966, Kocan *et al.* 1989, 2010, Davidson and Goff 2001, Aubry and Geale 2011). At this point in time, the Canadian Food Inspection Agency has classified *A. marginale* as a reportable pathogen. As a result, infected animals are euthanized and measures are taken to eradicate the bacterium from outbreak regions. There have been only a few outbreaks of this disease in Canada; however, these outbreaks have greatly affected the producers involved and require considerable resources to control (Howden *et al.* 2010, Aubry and Geale 2011). The outbreak that occurred in 2009 in a small community in southeast Manitoba is central to this thesis and resulted in the culling of over 700 head of cattle. There has been a limited amount of research conducted in the field on the ecology of this organism.

Some members of the genus *Rickettsia*, including the spotted fever group rickettsia (SFGR), are considered to be the causative agents of medically important zoonotic diseases and are associated with *D. variabilis*. Members of the SFGR rely on tick vectors and infected rodents to maintain their existence in a transmission cycle (Azad and Beard 1998). Human infections occur through the transmission of the bacteria via the bite of an infected tick. There is a broad range in pathogenicity, host specificity, and variations of the biology observed among the SFGR. This has resulted in fascinating and complicated ecology that we have only recently been able to study intensely through the development of molecular techniques.

Establishing a baseline of prevalence and distribution of zoonotic disease-causing agents in a given region, whether endemic or emerging, is essential for understanding the ecology of the diseases. This can now be more easily accomplished for vector-borne diseases with the use molecular techniques, rapidly screening known vectors for the

presence of the pathogens. Since the biology of a vector species can differ from region to region, an in-depth understanding of the vectors is also critical. In this thesis, I describe the prevalence and distribution of *A. marginale* and SFGR in American dog ticks in Manitoba. Additionally, I assess winter survival and the potential role this tick may play as a reservoir of disease agents near its northern distributional limits.

Chapter 2: Literature Review

Parasitic blood-feeding arthropods occupy a complicated and unique niche. Often they are highly reliant on their vertebrate host from which they imbibe blood for sustenance or for gamete development. They have evolved numerous traits that allow them to seek a host, break through the barrier of skin, counter immune responses and avoid detection and removal by the host. The success of the evolution of blood-feeding arthropods has also allowed for the co-evolution of a wide array of blood-borne endoparasites that utilize the blood-feeding events as a method of sustaining their population, transferring back and forth between the invertebrate and vertebrate hosts. These endoparasites include viruses, bacteria, protists, and helminths that replicate inside their hosts, often with little or no consequence. However, the indiscriminate nature behind blood-feeding, along with many other factors, can result in a wide degree of pathology presented by the vertebrate host. Pathogens that spread and maintain themselves in this manner may cause vector-borne diseases. Because of the intricate nature of the vector, pathogen and host relationships, the ecology of vector-borne diseases can be very complicated. Biotic and abiotic factors that affect any member of this triumvirate, whether at the individual or population level, will directly affect the other members. There are countless studies to look at stressors such as the effects of climate, population structure, weather, various behaviours and their effects on the ecology of vectored pathogens (Randolph 2004, 2008, 2009, 2010, Sumilo *et al.* 2008, Tabachnick 2010).

Arguably the most successful group of arthropod vectors is the ticks (Order: Ixodida). Many factors contribute to this success and the broad variety of pathogens they transmit. Firstly, members of this group are obligate blood-feeders during all active stages

of their life. They require at least one complete blood meal before advancing through each stage of their lifecycle, a lifecycle that can often last more than two years. As a result, ticks feed repeatedly and often on numerous hosts. Also, ticks can be specialists or generalists and infest a large range of hosts. Every lineage of terrestrial vertebrate has at least one species of tick that infests it (Balashov 1972). Additionally, many species of ticks can be found infesting numerous lineages of hosts throughout their life and geographic range. For instance, juvenile blacklegged ticks, *Ixodes scapularis* Say, are often found infesting small mammals and birds in the northern portion of its range (James and Oliver 1990). However, in southern areas, they may also infest reptiles (Whalley 1999, Durden *et al.* 2002). As adults, they can be found on numerous species of hosts, including, but not limited to white-tailed deer, *Odocoileus virginianus* Zimmermann, larger rodents such as porcupine, *Erethizon dorsatum* (L.), and carnivores such as the racoon, *Procyon lotor* (L.), and American black bear, *Ursus americanus* (Pallas) (Watson and Anderson 1976, Richardson *et al.* 1994, Yabsley *et al.* 2009). This wide array of hosts has in part allowed *I. scapularis* to extend its range. The method in which ticks feed contributes to their vector potential. Unlike most blood-feeding arthropods, ticks can feed on their host for days before becoming sufficiently engorged, allowing a greater time for pathogen exposure to the vertebrate host (Atwood and Sonenshine 1967, Balashov 1972). They achieve this by utilizing chelicerae to penetrate the host's skin while a barbed hypostome provides anchorage. They then secrete a large volume of saliva comprised of various elements including antigens which may be toxic, anticoagulants, immunosuppressants, vasodilators and if certain conditions are met, pathogens (Gregson 1959, Kemp *et al.* 1982, Ribeiro *et al.* 1985). Ticks also lack a peritrophic membrane, a

physical barrier that lines the midgut epithelium in some insects, allowing for easier dissemination of pathogens throughout the tick's body (Balashov 1972).

There are three families of ticks. Soft ticks (Family: Argasidae) lack a hard exterior dorsal component to their exoskeleton, referred to as the scutum, and have ventral mouthparts. Hard ticks (Family: Ixodidae) have a scutum and rostral mouthparts, and the family Nuttalliellidae, the most basal group of ticks; there is only one extant species in this family, *Nutalliella namaqua* Bedford, found in Africa. In North America, ixodid ticks are responsible for the vast majority of tick-vectored pathogens of medical and veterinary importance (Sonenshine 1993).

Of the Ixodidae of North America, one genus stands out for the most significant hindrance to man and livestock. The genus *Dermacentor* has a large geographic distribution throughout North America (Sonenshine 1979). In Canada, the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles, ranges from British Columbia to south-central Saskatchewan, where it is sympatric with the American dog tick, *Dermacentor variabilis* (Say). The range of *D. variabilis* extends from south-central Saskatchewan east to Nova Scotia (Wilkinson 1967, Sonenshine 1979, Dergousoff *et al.* 2013), with disjunct but stable populations in parts of southern Ontario. The winter tick, or moose tick, *Dermacentor albipictus* (Packard), is distributed coast to coast (Wilkinson 1967) and is tightly correlated to the distribution of its main hosts the moose, *Alces alces* (L.), the white-tailed deer, and the caribou, *Rangifer tarandus* (L.). The winter tick is unique among the North American ixodids as it can complete its lifecycle on one host, making them poor vectors of pathogens (Howell 1940). In contrast, *D. variabilis* and *D. andersoni* are three-host ticks. Juvenile ticks are often found on rodents, while adult ticks

feed on a wide array of hosts, primarily larger mammals including larger rodents, carnivores, but also on ungulates and humans (Smith *et al.* 1946, Burachynsky and Galloway 1985). Each blood meal of this tick typically comes from a different host. This gives the tick a chance to acquire a pathogen during the larval or nymphal instars and infect a new host upon subsequent feeding. Adult male ticks are known to take multiple blood meals and even feed on more than one host (Zaugg *et al.* 1986, Kocan *et al.* 1992b, Lysyk 2013). This obligation to feed on multiple hosts and their capability to feed on humans and livestock makes them a vector of significant importance. *Dermacentor variabilis* is the tick humans encounter most often in Manitoba, on themselves and their companion animals (Gkoroba 1980).

The lifecycle of *D. variabilis* varies slightly from region to region (Garvie *et al.* 1978, Sonenshine 1979, McEnroe 1986). In Manitoba, eggs hatch approximately two weeks after being laid during the active season of the adult tick in late spring until fall (approximately May through to mid-August) (Sonenshine 1993). Six-legged larvae emerge and enter a state of dormancy in order to overwinter. Once the snow has melted in approximately mid-May, the larvae quest in the leaf litter before attaching to a rodent host (Burachynsky and Galloway 1985). The larvae then feed to repletion, drop back into the leaf litter and moult into a nymph. The process of questing, feeding and moulting, this time to the adult stage, is repeated. The majority of these adults enter dormancy and overwinter until the next spring. From the thawing of the snow in the spring until approximately the end of August adult ticks can be found questing in low lying vegetation (Gkoroba 1980). Adult ticks that successfully find a host will feed and mate on the host before the female falls off the host to lay her approximately 4000-6500 eggs on the soil

(Smith *et al.* 1946). Egg clutch size can vary greatly depending on the quality and size of the final blood meal along with other biotic and abiotic factors (Balashov 1972). Adult ticks that are near their northern distribution limit and are unsuccessful in host acquisition were assumed to die before winter (Wilkinson 1967, McEnroe 1975, Sonenshine 1979, McEnroe and Specht 1987). In climates more temperate than Manitoba, less than 10% of unfed adults can survive to quest in the next vector season (Sonenshine 1973, 1979, McEnroe 1984, 1986). Surviving multiple winters in Manitoba would allow ticks to act as a vector and as a reservoir for some pathogens.

Two closely related genera of bacteria, *Rickettsia* and *Anaplasma*, are obligate intracellular parasites that require arthropod vectors for natural transmission. The rickettsiae are the more diverse group, consisting of at least 28 species, almost all exclusively transmitted by ticks (Azad and Beard 1998). There are four groups of rickettsiae, three of which contain known human pathogens. These groups are the spotted fever group rickettsiae (SFGR), consisting of about 20 species, including *R. rickettsii* (Wolbach), the causative agent of Rocky Mountain spotted fever, the typhus group consisting of *R. prowazekii* da Rocha-Lima and *R. typhi* (Wolbach and Todd), the ancestral group consisting of *R. bellii* Philip *et al.* and *R. canadensis* McKiel *et al.*, and the transitional group composed of *R. akari* Huebner *et al.*, vectored by the house mouse mite, *Liponyssoides sanguineus* (Hirst), and *R. felis* Bouyer *et al.*, transmitted by the cat flea, *Ctenocephalides felis* (Bouché), and the hedgehog flea, *Archaeopsylla erinacei* (Bouché) (Gillespie *et al.* 2008, Wood and Artsob 2012). Historically, the transitional group was considered to be part of the SFGR; however, there is enough divergence to justify classifying these rickettsiae into a separate group (Gillespie *et al.* 2008, Wood and

Artsob 2012). These groupings were at one time based primarily on the diseases caused and serological tests, but have been confirmed by gene sequence analysis of highly conserved proteins including, OmpA, OmpB, gltA and 16sRNA (Anderson and Tzianabos 1989, Roux and Raoult 1995, 2000, Roux *et al.* 1997, Fournier *et al.* 1998, Gillespie *et al.* 2008). It should be noted that the rickettsiae are seen as emerging human pathogens, with more than nine tick-vectored SFGR pathogens being discovered since 1991 (Wood and Artsob 2012). Many species have yet to be cultured and have not been formally described, while others, such as *R. montanensis* (Lackman *et al.*), have only just recently been recognized as human pathogens (McQuiston *et al.* 2012). More extensive field studies are required to understand precisely what species of SFGR are present in North America, their distribution, and their main host associations.

Each of the various SFGR can have different transmission pathways. Ticks can be infected once they have fed on an infected amplifying host, such as a vole, *Microtus* spp. (Azad and Beard 1998). Once the ingested bacteria have penetrated the tick's tissues, the tick becomes chronically infected. Transstadial transmission occurs when the tick moults to its next instar. The tick can then transmit the bacteria to the next host (Azad and Beard 1998). Some SFGR, such as *R. peacockii* Niebylski *et al.*, also have the ability to be transovarially transmitted, that is, from an infected female tick to its offspring (Wood and Artsob 2012). Inside the mammalian hosts, the rickettsiae reproduce primarily in endothelial cells and readily move through the blood stream (Walker and Ismail 2008). Infections can elicit a powerful immune response, resulting in vascular permeability of infected tissues with the vast majority of morbidity arising from cerebral and non-cardiogenic pulmonary oedema (Walker and Ismail 2008).

There are two main factors affecting the distribution of SFGR in North America, one being the distribution of the species of tick vector. Some SFGR exhibit a high degree of vector specificity. For instance, *R. montanensis* is usually only associated with *D. variabilis*, while *R. peacockii* is usually only associated with *D. andersoni*, even in localities where the tick species are sympatric (Niebylski *et al.* 1997, Ammerman *et al.* 2003, Dergousoff *et al.* 2009, Wood and Artsob 2012). The other main factor is the presence of other SFGR in the same region. Seldom are ticks superinfected with more than one species of *Rickettsia* (Burgdorfer 1975, Burgdorfer and Anacker 1981, Niebylski *et al.* 1997, 1999, Ammerman *et al.* 2003, Dergousoff *et al.* 2009, Wood and Artsob 2012). This is likely because of competitive exclusion within tick tissues. It is thought that once a tick's tissues are infected, alteration of the expressed surface proteins may prevent adhesion and subsequent absorption of a second species of *Rickettsia* (Macaluso *et al.* 2002). As a result, prevalence is usually higher for SFGR that are without or with low pathogenicity to their host and vector and are more widely distributed, while more pathogenic species remain at a lower prevalence with a more confined distribution (Walker and Ismail 2008). This was classically documented in the studies conducted in the Bitterroot Valley, Montana. There was a noticeably higher prevalence of Rocky Mountain spotted fever on the west side of the valley than on the east. However, a large proportion of the ticks on the east side were infected with what was then called the East Side Agent, now *R. peacockii* (Burgdorfer and Anacker 1981, Niebylski *et al*. 1997). This led to the development of the interference hypothesis, where it is thought *R. peacockii* was preventing the spread of *R. rickettsii* to the east. *Rickettsia peacockii* is a non-pathogenic SFGR that is transovarially transmitted, and does not significantly affect its tick host. This results in a larger proportion of ticks being infected and potential

amplifying hosts surviving with anti-SFGR antibodies (Niebylski *et al.* 1997, Kurtti *et al.* 2005, Walker and Ismail 2008, Wood and Artsob 2012). In contrast, *R. rickettsii* is highly pathogenic, causes morbidity and mortality to its tick and rodent hosts, is not very successfully transovarially transmitted, and results in reduced fecundity of infected female ticks (Niebylski *et al.* 1999, Walker and Ismail 2008, Freitas *et al.* 2009, Wood and Artsob 2012).

A limited amount of research has been done on the distribution of SFGR in tick populations in Canada (Dergousoff *et al.* 2009, Wood and Artsob 2012). In Canada, there are four main species of hard ticks that regularly infest humans (Gregson 1956), of which only two, *D. andersoni* and *D. variabilis* are known vectors of SFGR. The SFGR associated with these ticks are *R. rickettsii* and *R. montanensis*, known human pathogens, and *R. peacockii* and *R. rhipicephali* (Burgdorfer), with no known or unknown pathogenicity (McQuiston *et al.* 2012, Wood and Artsob 2012). Dergousoff *et al*. (2009) studied the distribution of SFGR across Alberta to Ontario, with emphasis on tick populations in Saskatchewan. A total of 508 *D. andersoni* and 818 *D. variabilis* were screened for SFGR from 15 localities. No *R. rickettsii* or *R. rhipicephali* were found; however, 76% (range 61-96%) of *D. andersoni* tested positive for *R. peacockii* while 8% (range 0-33%) of *D. variabilis* tested positive for *R. montanensis*. Only one of the seven localities where the ticks were sympatric had both SFGR, detected at prevalences of 86% and 2% for *R. peacockii* and *R. montanensis*, respectively (Dergousoff *et al.* 2009). In this study, *R. peacockii* was only found in *D. andersoni*, while *R. montanensis* was only found in *D. variabilis*.

The sister family to the Rickettsiaceae, the Anaplasmataceae, is also composed entirely of obligate intracellular parasites and are vectored by ticks (Davidson and Goff 2001). The type species of this family, *Anaplasma marginale* Theiler, is the causative agent of bovine anaplasmosis (Theiler 1910). This is an infectious, but not contagious, blood-borne pathogen that has a tropical and subtropical distribution and affects many ruminants, most importantly domesticated cattle. Bovine anaplasmosis is seen as one of the most significant tick-vectored pathogens of cattle in the world and is responsible for constraints on trade and significant economic loss to the American herd (McCallon 1973).

The distribution of the disease is largely correlated with the distribution of the species of ticks that are biological vectors for the pathogen. In Canada, the primary tick vectors are *D. variabilis* and *D. andersoni* (Lankester *et al*. 2007). Although the tick vectors are present and widely distributed in Canada, *A. marginale* remains, for the most part, absent from our national cattle herd (Howden *et al.* 2010). This is chiefly because bovine anaplasmosis is a reportable disease regulated by the Canadian Food Inspection Agency (CFIA). With this status, herds found to be infected, along with adjacent herds, are quarantined and a modified stamp-out program, consisting of multiple rounds of blood-testing and culling, is maintained until no infected animals remain. This disease has since been removed from the federally reportable disease list and placed onto the list of immediately notifiable diseases as of April 1 2014.

Transmission of *A. marginale* can be accomplished in a variety of ways, primarily through biological transmission by ticks. Once ticks ingest infected blood, the bacteria infect the cells lining the gut wall and reproduces. The organism eventually passes throughout the tick, infecting numerous tissues, including the salivary glands, taking a

reticulated form. In these tissues, the bacteria again reproduce forming large colonies and take the infectious dense form. This form allows the bacteria to persist for an extended period of time outside of a host cell (Kocan *et al.* 2009), allowing for transmission to a vertebrate host upon subsequent tick feedings (Kocan 1986, Kocan *et al.* 1989, 2004, 2008). Within the tick, transstadial transmission is typical; however, transovarial transmission is not. As a result, once a tick is infected, it will remain infected for the rest of its life (Kocan *et al.* 1989,1992a, 2010). It is thought male ticks of the genus *Dermacentor* play the most important role in the biological transmission and maintenance of *A. marginale*. These ticks, once persistently infected, may feed multiple times and can infest new hosts, potentially exposing a larger proportion of the herd to the pathogen (Zaugg *et al.* 1986, Kocan *et al.* 1992a,1992b, Lysyk 2013).

Vertebrate hosts may also be infected with *A. marginale* via mechanical transmission. This is accomplished by direct inoculation of infected blood cells into a naïve animal. This can occur through multiple paths. Biting flies, including horse flies, deer flies and stable flies, *Stomoxys calcitrans* (L.), have been incriminated as potential mechanical vectors, carrying infected blood cells on their mouthparts (Howell *et al.* 1941, Wilson and Meyer 1966, Ewing 1981, Hawkins *et al.* 1982, Teskey 1990, Scoles *et al.* 2005a). Although this mode of transmission may play a major role in areas where tick vectors are absent, fly transmission is significantly less successful when compared to tick transmission (Scoles *et al.* 2005a). Mechanical transmission may also occur through improper sterilization of tools such as needles, dehorning implements, and scalpels used in veterinary procedures (Reeves and Swift 1977). Calves may also become infected

through blood contact during the birthing process, or occasionally transplacentally (Grate *et al.* 2013).

In the vertebrate host, *A. marginale* infects red blood cells, often mature ones (Jones and Brock 1966). Although *A. marginale* is known to infect a wide array of ruminants, including but not limited to elk, *Cervus canadensis* (Erxleben), white-tailed deer, *O. virginianus*, mule deer, *O. hemionus* (Rafinqesque), and giraffe, *Giraffa camelopardalis* (L.), the exact role these wild hosts play in disease maintenance remains understudied (Howe *et al.* 1965, Kuttler *et al.* 1967, 1968, Augustyn and Bigalke 1974, Zaugg 1986, Chomel *et al.* 1994, Zaugg *et al.* 1996, Davidson and Goff 2001). Wild hosts often exhibit low parasitaemia and as a result, low morbidity. This also makes some wild hosts poor reservoir hosts maintaining the infection in a sylvatic cycle (Keel *et al.* 1995). In contrast, the effects of bovine anaplasmosis infection in cattle can range from subclinical, to highly pathogenic and can result in death. The age of the animal when it is first infected appears to play a key role in the course of the disease, with young calves being least susceptible. Calves under the age of one year often experience a subclinical or mild disease, while animals one to two years old may experience an acute but often non-fatal form of the disease (Aubry and Geale 2011). Cattle over the age of two may experience clinical disease with mortality that ranges from 30-50% (Richey 1991).

After exposure, *A. marginale* will undergo an initial incubation period of 7-60 days before infecting red blood cells. The bacteria then undergo reproduction, forming colonies of four to eight bacteria per blood cell. The ungulate's reticuloendothelial system attacks infected blood cells, resulting in release of some of the bacteria along with the cellular components of the red blood cells (Kocan *et al.* 2010). During the initial phase of

the infection, the number of infected red blood cells doubles every 24 hours and can reach a concentration of 10^9 infected red blood cells per mL of whole blood (Richey and Palmer 1990). Infection presents clinically when 15% of an animal's erythrocytes are infected (Aubry and Geale 2011). Symptoms can include icterus, fever, respiratory distress, weight loss, abortion and death. Animals that survive the acute infection, even if subclinical, become persistently infected carriers of the bacteria and are chronically infected for the remainder of their lives, acting as reservoirs for the pathogen (Kieser *et al.* 1990). The chronic carrier state is maintained by antigenic modulation, the shuffling and expression of hypervariable regions of the MSP2 and MSP3 genes that alter the exterior protein profile of *A. marginale*, and requires the host to mount a new immune response (French *et al.* 1998, 1999, Brayton *et al.* 2003). This evasion of the immune system leads to a rise in parasitaemia that is then suppressed in a cyclical fashion that occurs approximately every two weeks (Kieser *et al.*1990). There are no antibiotics or vaccines registered against *A. marginale* for use in cattle in Canada (Aubry and Geale 2011).

The first outbreak of bovine anaplasmosis in Canada occurred in Manitoba in 1968. A year later, the disease was made federally reportable. Since then, there have been five additional outbreaks: 1979 in Quebec, 1983 in Saskatchewan, 1996 in Ontario, 2000 in Saskatchewan and in 2009 in Manitoba. In the thesis, I shall examine the 2009 outbreak in southern Manitoba. In all cases, the pathogen was eradicated from the country (Howden *et al.* 2010). From October 2009 to April 2010, 590 animals in Manitoba were diagnosed with bovine anaplasmosis but only one animal presented with clinical

symptoms. Mean prevalence of infected animals in infected herds ranged from 0.04% to 66.00% (Howden *et al.* 2010).

With the recent advent of a wide array of highly efficient molecular tools, large scale studies of the prevalence and distribution of SFGR and *A. marginale* in their tick host populations can be conducted. Understanding how these bacteria and their tick hosts are geographically distributed throughout endemic and outbreak regions can allow researchers to assess risk and suggest measures to control enzootic and epidemic outbreaks.

Chapter 3: Active surveillance of *Anaplasma marginale* in populations of arthropod vectors (Acari: Ixodidae; Diptera: Tabanidae) during and after an outbreak in livestock herds in southern Manitoba

Abstract

The infectious but not contagious disease of ruminants, bovine anaplasmosis, is classified as a reportable disease in Canada. The Canadian government mandates that a modified stamping-out regimen be instituted upon identification of an infected herd. This can result in significant reduction of herd size, economic loss for producers and requires veterinary time and resources. The disease is caused by the blood-borne pathogen, *Anaplasma marginale*. This proteobacterium is biologically vectored by over 20 species of hard ticks (Acari: Ixodidae), but can potentially be spread through mechanical transmission by biting flies or fomites involved in veterinary procedures. After the identification of an infected animal in 2008, an outbreak was detected in southeastern Manitoba. The modified stamping-out program was initiated in 2009 and concluded in the fall of 2011. Fourteen herds were identified as having reactor animals. Active surveillance of potential arthropod vectors was conducted in 2011 and 2012 using molecular techniques to detect if the bacterium was present. Screening for *A. marginale* was conducted on 2056 American dog ticks, *Dermacentor variabilis*, collected from pastures over the two years. In 2011, 520 horse flies of at least eight species (Diptera: Tabanidae) were also screened. *Anaplasma marginale* was not detected by real time PCR from DNA extracted from any of the arthropods.

Introduction

The gram-negative bacterium, *Anaplasma marginale* (Theiler), is the causative agent of bovine anaplasmosis, a disease that infects ruminants around the globe (Theiler 1910, Kocan *et al.* 2010). This proteobacterium belongs to the order Rickettsiales and is an obligate, intracellular parasite. *Anaplasma marginale* is an obligate intra-erythrocytic bacterium that chronically infects wildlife, including bison, *Bison bison* (L.) (Zaugg 1986), some cervid species including elk, *Cervus canadensis* (Erxleben) (Howe *et al.* 1965, Zaugg *et al.* 1996), white-tailed deer, *Odocoileus virginianus* Zimmermann (Kuttler *et al.* 1967, 1968), and mule deer *Odocoileus hemionus* Rafinesque (Howe *et al.* 1965, Chomel *et al.* 1994). Infections in wildlife may not cause any measurable level of morbidity or reach parasitaemia levels that allow them to serve as wild reservoirs for the pathogen (Davidson and Goff 2001). Clinical signs can develop in domesticated cattle including anaemia, splenomegaly, enlargement of the gallbladder, icterus, reduced weight gain, abortion and in severe cases, death (Jones and Brock 1966, Potgieter and Stoltsz 1994). Animals that survive acute infections of *A. marginale* become chronically infected, resulting in periodic elevations of parasitaemia caused by antigenic modulation requiring a new immune response to be mounted before the animal can suppress the infection (Kieser *et al.* 1990). This pathogen is of economic importance in Canada, and has resulted in some trade restrictions being imposed on Canadian cattle for export (Kocan *et al.* 2010, Aubry and Geale 2011). In Canada, bovine anaplasmosis is a reportable disease and, under the *Health of Animals Act*, requires producers with infected herds to undertake a modified stamping-out program until there are no longer infected animals in the region (Howden *et al.* 2010, Aubry and Geale 2011).

All species of the genus *Anaplasma* have strains that utilize a tick as a biological vector, undergoing a migration and maturation inside the tick before becoming infectious to the definitive vertebrate host (Kocan 1986, Kocan *et al.* 1992b). Ticks of the genus *Dermacentor* are the main biological vectors of *A. marginale* in North America (Kocan *et al.* 1981, 1985, Kocan 1986, Stiller *et al.* 1989). Ticks become infected only after feeding on an infected host. Cells lining the gut and Malpighian tubules are infected first. Here the bacteria reproduce forming large colonies before dispersing to other tick tissues including fat bodies, muscles, and salivary glands. Once infected, the tick will be infected for the remainder of its life. Transstadial transmission, but not transovarial transmission, of *A. marginale* occurs within *Dermacentor* ticks (Kocan *et al.* 1980, Stich *et al.* 1989, Stiller *et al.* 1989). This genus of tick is widely distributed throughout North America (Sonenshine 1979). In Canada, the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles, ranges from British Columbia to south-central Saskatchewan, where it is sympatric with the American dog tick, *Dermacentor variabilis* (Say) (Dergousoff *et al.* 2013). The range of *D. variabilis* extends from Saskatchewan east to Nova Scotia (Wilkinson 1967, Sonenshine 1979, Dergousoff *et al.* 2013). The winter tick, or moose tick, *Dermacentor albipictus* (Packard), is distributed coast to coast (Wilkinson 1967) but is believed to play only a minor role in the epidemiology of anaplasmosis as it typically completes its lifecycle on one host (Howell 1940). In contrast, *D. variabilis* and *D. andersoni* are three-host ticks. This means that each active life stage requires a blood meal that will likely be acquired from three different hosts. This gives the tick a chance to acquire a pathogen during a blood meal in a larva or nymph and infect a new host on subsequent feedings. Adult male ticks of this genus are also known to take multiple blood meals and even feed on multiple hosts (Zaugg *et al.* 1986, Kocan *et al.* 1992b, Lysyk 2013). This obligation of

blood-feeding numerous times, along with the long duration of the blood meals, increases the vector potential of these ticks, making them suitable vectors for a wide array of microbes, including *A. marginale*.

Anaplasma marginale may be transmitted by other means. Vertical transmission can occur between an infected cow and naïve calf through trans-placental infection or through blood contact during the birthing process (Zaugg 1985, Grate *et al.* 2013). Additionally, mechanical transmission can also occur. This occurs through inoculation of uninfected animals with infected erythrocytes. Poor herd management techniques, such as improper sterilization of veterinary tools (*e.g.*, needles, ear tag applicators) between animals is a major route of *A. marginale* transmission in some regions (Reeves and Swift 1977). Under some circumstances, biting flies, including deer flies and horse flies (Diptera: Tabanidae) and stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), may serve as mechanical vectors, transferring infected blood cells on their mouthparts between animals with varying degrees of success (Howell *et al.* 1941, Wilson and Meyer 1966, Ewing 1981, Foil 1989, Scoles *et al.* 2005a).

The CFIA conducts a Bovine Serological Survey (BSS) every three to five years to affirm that the national herd remains free of bovine anaplasmosis, brucellosis and blue tongue virus. Blood samples are collected from federally inspected abattoirs, stratified by province, and are subjected to a variety of serological assays looking for antibodies to these pathogens. The results of the 2007-2008 BSS indicated that the Canadian herd had a prevalence of infection higher than the acceptable 0.02% for bovine anaplasmosis (Pare *et al.* 2012). A portion of the positive samples were traced back to Manitoba and resulted in increased awareness and surveillance amongst the veterinary community. This resulted in

the detection of an outbreak in southeastern Manitoba and involved animals belonging to 13 beef and one bison producer. The mandated modified stamp-out program was initiated in 2009 and concluded in the summer of 2011 (Howden *et al.* 2010). This outbreak was unique among Canadian outbreaks in that detection of infected animals occurred through multiple summers, requiring multiple rounds of culling, indicating that maintenance of the infection could have been occurring. At the initiation of the modified stamp-out program, the cumulative beef cattle herd size of the producers who participated in this study was approximately 1278 (range of head per farm was 40 - 373), of which 15% were culled in the first screening. Prevalence of *Anaplasma marginale* infection ranged from 2.7 - 32.0% per herd. In the spring of 2011, seven additional animals were culled from a herd of 115, while one additional animal was culled in the summer from a herd of approximately 30. These data are based on results from a questionnaire filled out by participating producers in 2011 that was distributed by the CFIA district officer in Steinbach (Appendix A).

The exact role that arthropods, especially ticks, play in the epidemiology of bovine anaplasmosis is understudied (Kocan *et al.* 2010). Certain strains of *Anaplasma marginale* react differently in the vertebrate and arthropod hosts (Wickwire *et al.* 1987, de la Fuente *et al.* 2003a, 2007, Kocan *et al.* 2004, 2008, Palmer and Brayton 2013). Additionally, the behaviour, physiology, and life history of the tick vectors can vary throughout their geographic range (Sonenshine 1993, Scoles *et al.* 2005b). Only recently have we had the molecular tools to conduct large-scale, active surveillance of arthropod vectors in a pastoral setting, which has been suggested as a key element to understanding the ecology of bovine anaplasmosis (Sonenshine 1993). The objective of this study was to

assess what if any role arthropod vectors of *A. marginale* had in the potential maintenance of the infection in the outbreak region in Manitoba.

Materials and Methods

In April 2011, personnel at the Steinbach district office of the Canadian Food Inspection Agency (CFIA) were contacted, as they were the primary responding veterinarians responsible for the control of the anaplasmosis outbreak the southeastern Manitoba. The district office provided essential historical background on the presence of *A. marginale* in the region over previous years and initiated contact with producers who owned the pastures that contained the infected animals. Of those contacted, 10 producers initially expressed interest in this arthropod vector surveillance study; however, only nine producers participated in the study that year. During the summer of 2012, only seven of the original producers were included in the study. Two producers did not participate in 2012 as their farms suffered extensive damage from prairie fires.

Ticks were collected by the dragging technique (Kohls 1937), using a piece of white flannel (1.3 X 0.70 metres), spread by a plastic spar and dragged through the pastures by researchers. The flannel, along with clothes of the researchers, were examined for ticks approximately every 10 metres. Ticks were removed and placed in a 15 dram plastic vial with a perforated snap-top lid. When leaving the pasture, date and location of collection were recorded and the vials were placed in a plastic bag with a paper towel moistened with water, and then placed in an insulated container for transport.

Tick dragging was conducted on non-rainy days when the air temperature was above 10°C, after snow had melted in April, until August when questing ticks were no

longer present. These practices increased the efficacy of the flannel, as ticks do not grip wet flannel well, and ensured that environmental conditions would favour tick questing behaviour (Harlan and Foster 1990, Schulze *et al.* 2001). The time allotted for drag sampling the over 18 km^2 of pasture was based on suitability of tick habitat, recent herd history, including number of animals culled per herd and pasture utilization, and producer interest. In total, 1013 (487 males; 526 females) and 1043 (493 males; 550 females**)** ticks were collected in 2011 and 2012, respectively. At least 100 ticks were collected from land owned by each producer involved in the study. Biosafety precautions were observed when moving between pastures including, but not limited to, disinfecting boots when entering and leaving pastures and using separate drags for separate pastures. Typically, ticks were collected from only one producer's pastures per day.

Once returned to the lab, the exterior of the ticks was sterilized through a series of washes to increase accuracy of later reactions and prevent contamination from microbes that the ticks may have picked up in the environment. Ticks were washed in four different solutions and kept separated based on the location from which they were collected. The first solution consisted of one drop of Tween® 80 per 10 mL of 0.5% bleach. The second solution was 0.5% benzalkonium chloride. The third solution was 70% ethanol. The final solution was purified water. All ticks collected from the same pasture on the same day were placed in 50 mL centrifuge tubes and approximately 45 mL of the first solution was added. The tubes were then sealed and slowly and repeatedly inverted for three minutes. The solution was decanted and the next solution added. This process was repeated for all solutions. After decanting the purified water, the ticks and centrifuge tube were dried with

a paper towel; the ticks were replaced into the tube and frozen at -80°C for long term storage.

Horse flies were collected using a Manitoba Horse Fly Trap (Thorsteinson *et al.* 1964). In 2011, a trap was operated for four days when fly populations appeared to be at their highest in the first week of June. The trap was placed for the first two days on a pasture where fly intensity appeared to be the highest and where previously infected animals had been maintained. On subsequent days, the trap was placed in close proximity to the herd that most recently contained a cow that had an active *A. marginale* infection. Trapping was performed from 0900-2000h. Once removed from the field, flies were killed by being placed in a freezer at -5°C for approximately 20 minutes before being transferred to a sterile plastic bag and placed in a -80°C freezer for long term storage. Because of the success of CFIA's quarantine, test and culling program, the probability of collecting infectious horse flies in the summer of 2012 was seen as negligible. Therefore, no flies were collected. The potential for *A. marginale* to be harboured in overwintering juvenile or adult ticks (Chapter 6) or in potential wild reservoir hosts justified a second year of tick collecting in 2012.

All diagnostic work done on ticks and flies was conducted in a biosecurity level two laboratory in the National Microbiology Laboratory, 1015 Arlington Street, Winnipeg, Manitoba, Canada. Ticks were removed from -80°C storage and sorted by date, location and by sex into pools of no more than five. A total of 217 (104 of males; 113 of females) pools were screened in 2011 and 224 pools (106 of males; 118 of females) in 2012. These pools were given unique identifications to connect them with a database containing information regarding each herd's history and pasture information.

Each tick was then cut in half sagittally using a scalpel on a sterile Petri dish in a biosafety cabinet. The scalpel was disinfected between ticks by washing in 10% chlorine bleach solution followed by a rinse in 90% ethanol. Half of each tick was frozen individually in a SARSTEDT 2.0mL micro tube, while the other half remained in a designated pool (with the other four halves of ticks from that pool). This ensured that individual positive ticks found in pools that tested positive for the presence of *A. marginale* could be identified. The ticks in each pool were then cut further into smaller pieces using a sterile scalpel to enhance DNA extraction.

Extraction of DNA from the pools was conducted using the DNAeasy 96 extraction kit from Qiagen with no modification of the blood and tissue protocol. All extracts were then stored in a -80°C freezer. Once all extractions were complete, they were subjected to screening for *A. marginale* using real time PCR with primers and a probe that have been successfully used in previous studies targeting the 16S rRNA gene (Reinbold *et al.* 2010). The PCR was conducted using 96 well fast plates on an Applied Biosystems VIIA™ 7 Real Time PCR system. The thermo-cycling regime consisted of an activation stage lasting for two minutes at 50°C, an initial denaturation lasting 10 minutes at 95°C and 40 cycles of 95°C and 60°C lasting for 15 seconds and one minute, respectively. The total reaction volume was 30µL per well. Forward and reverse primers had a final reaction concentration of 0.667 µM, while the probe had a reaction concentration of 0.125µM. Each reaction contained 12.5 µL of TaqMan® Universal Master Mix (2X) and 5 µL DNA extract. The remaining volume consisted of nuclease-free water. An isolate of *A. marginale* from an infected cow from the Manitoba outbreak in 2008 was used as a positive control. During the extraction process, aliquots of the

buffers provided in the Qiagen kit remained open in micro-centrifuge tubes in the biosafety cabinet. These buffers served as negative controls to ensure that contamination was did not occur.

In total, 520 horse flies were caught. Because horse flies are potential mechanical vectors, only mouthparts were tested for *A. marginale* as bacteria in the remaining part of the fly body should not be transmittable. Initial tests were conducted to determine if whole fly heads could be used, but extracts from these tests contained a substance, presumably eye pigments from the compound eyes, which affected PCR efficiency. Flies were sorted by the date they were collected and to the lowest taxonomic level possible in an expedient manner (Teskey 1990, Thomas 2011). Mouthparts were removed from the flies and pooled in groups of no more than five keeping each trap date and taxonomic group separate. The pools of mouthparts were subjected to the same DNA extraction and PCR protocols that were used to screen the ticks for *A. marginale*.

Voucher specimens of both flies and ticks were deposited in the J. B. Wallis / R. E. Roughley Museum of Entomology in the Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada.

Results

In total, 520 tabanids were collected over the four trapping days in 2011. The total collection consisted of two genera: *Tabanus* (n=116) and *Hybomitra* (n=404) (Table 1). *Tabanus similis* Macquart was the most abundant species of the genus *Tabanus*; however, three *T. vivax* Osten Sacken were also collected. Six species of *Hybomitra* were

identified, *H. illota* (Osten Sacken) (n=61) and *H. pechumani* Teskey and Thomas (n=10). In order to minimize the amount of time between collection and testing for *A. marginale*, the remaining four *Hybomitra* species (n=333) were not sorted to species level. However, *H. nuda* (McDunnough), *H. lasiophthalma* (Macquart), *H. frontalis* (Walker) and *H. epistates* (Osten Sacken) were all present in the samples. These are all widely distributed and abundant species in Manitoba (Teskey 1990).

In 2011, 217 pools of *D. variabilis* and 110 pools of flies were tested, while in 2012, 224 pools of ticks were screened for the presence of *A. marginale*. All ticks and flies tested were negative for *A. marginale*; positive controls always tested positive. The producers involved were informed by phone or in person of the results as they became available and were pleased to participate in the study.

Discussion

There are numerous factors that may have been responsible for our failure to detect *A. marginale* in horse flies and ticks. The greatest contributing factor may have been the success of the modified stamp-out program that CFIA enacted in 2009 and concluded in 2011, when all herds that had been infected and herds in proximity of infected herds were deemed free of anaplasmosis. This success by CFIA dramatically reduced the probability that *A. marginale* would be detected in the arthropod populations, particularly the biting flies.

If during the trapping period there were in fact infected cattle in close proximity to the fly trap, there are other reasons why *A. marginale* was not detected on the mouthparts of the flies. The probability of detection drops with a decrease in the amount of bacteria

circulating in the herd. If flies are feeding randomly, and only a small portion of the herd is infected, then the likelihood of catching a partly fed contaminated fly is low. Additionally, because parasitaemias fluctuate greatly in chronically infected animals, a fly feeding on an infected animal may not have been contaminated by a detectable amount of bacteria if parasitaemia is low. The size of the fly population also may have had an effect in this study. Overall fly pressure on the animals was quite low. This is reflected by the relatively small daily trap catches (Table 3.1) and by observations made on the cattle's lack of response to fly attack (Ralley *et al.* 1993). Increasing the number of flies caught could have increased the probability of detecting *A. marginale*. Finally, the method used to trap the flies may not have facilitated catching infectious flies. The Manitoba Horse Fly Trap uses a large glossy black sphere as a visual target (Thorsteinson *et al.* 1964). As a result, primarily host-seeking flies are caught. This means the only infectious flies caught would have to be flies that had partially fed on a chronically infected animal, which had high enough circulating bacteria, and then were interrupted during the feeding process before flying into the trap. Although this seems unlikely, this method of trapping more accurately represents how flies would transmit this pathogen as compared to other methods, such as catching feeding flies on the cattle with a sweep net or by using flight intercept traps as host only seeking flies are caught.

In addition to the removal of the reactor animals, there are also other contributing factors that may have limited our ability to detect *A. marginale* in the ticks. When this study was initiated in 2011, there were only a few reactor cows present (Howden *et al.* 2010), there was still the possibility that the bacteria may have spilled over into the tick population and the ticks may have acted as a reservoir (Kocan *et al.* 1981, 1992a, 1992b).

Exposure of the ticks to infected animals is the only way for the ticks to acquire the infection; therefore any factor that decreases exposure also reduces the probability of detection of *A. marginale* in the ticks. For instance, if there was a low density ticks during the height of *A. marginale* activity, there would be a smaller probability of collecting these ticks.

Exposure could also be reduced by the absence of infected wild reservoirs. Although the exact role wild reservoirs play in the maintenance of bovine anaplasmosis is understudied, populations of both *O. virginianus* and *C. canadensis* are present in southeastern Manitoba and are known to come in close proximity to livestock and pose a disease risk (Vander Wal *et al.* 2012, Brook *et al.* 2013). These populations may also frequently cross the international border into Minnesota where anaplasmosis is classified as an endemic, non-regulated pathogen (Howden *et al.* 2010). However, the herd of *C. canadensis* that routinely migrates between Minnesota and the outbreak region in Manitoba has been monitored for *A. marginale* in the past. On the basis of serological tests, the herd has been considered free of anaplasmosis since at least 2004 (Hildebrand *et al.* 2010).

A lack of competency of the tick vector for the strain of *A. marginale* that was detected in Manitoba may also have played a role in the lack of detection. Several geographic isolates of *A. marginale* have been identified in North America, of which at least four appear not to be transmitted by ticks (Smith *et al.* 1986, Wickwire *et al.* 1987, de la Fuente *et al.* 2003a, 2003b). This is caused by variation in the adhesive properties of MSP1a, the protein thought to be used to invade erythrocytes in cattle and various tissues in the tick (de la Fuente *et al.* 2001, 2003a, 2003b, 2007). If ticks were exposed to a strain

that was not transmissible by ticks, the bacteria would not have been able penetrate the gut wall to invade the tick's tissue. This could cause death of the bacteria and may reduce the likelihood of detection when compared to a truly infected tick that would contain numerous colonies of the bacteria (Kocan 1986, Kocan *et al.* 1989, 1992b).

This study is the first large scale surveillance study of arthropod vectors of *A. marginale* in an outbreak region in Canada. The lack of detection of the bacteria affirms the effectiveness of the current CFIA policy to control anaplasmosis in areas with abundant biological vectors.

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Additional and special thanks go to the producers who allowed access to their pastures and offered firsthand experience of the *Anaplasma* problem in southeastern Manitoba.

Tables and Figures

Table 3.1. List and number of horse flies caught in the endemic area in southeastern Manitoba in 2011 and that were tested for the presence of *Anaplasma marginale* separated by trapping day. The numbers of pools (five flies per pool) of flies tested are indicated in parenthesis beside each count.

Fly species	Day 1	Day 2	Day 3	Day 4	Total flies caught per species
Tabanus similis	60 (12)	21 (5)	19 (4)	13 (3)	113 (24)
Tabanus vivax	2 (1)	1 (1)	0 (0)	0 (0)	3 (2)
*Hybomitra spp.**	83 (17)	95 (19)	84 (17)	71 (15)	333 (68)
Hybomitra pechumani	7 (2)	3 (1)	0 (0)	0 (0)	10 (3)
Hybomitra illota	35 (7)	4 (17)	5 (1)	4 (1)	61 (13)
Daily total catch	187 (39)	137 (30)	108 (22)	88 (19)	520 (110)

*=Pools of *Hybomitra* that were not sorted to species level but consisted of at least *H. nuda, H. lasiophthalma, H. frontalis* and *H. epistates.*

Chapter 4: Assessment of Prevalence and Distribution of Spotted Fever Group Rickettsiae in Manitoba, Canada, in the American Dog Tick, *Dermacentor variabilis* (Acari: Ixodidae)

Abstract

Little is known about the distribution and prevalence of the spotted fever group rickettsiae in Canada. I conducted active surveillance for tick-associated rickettsiae in 10 localities in Manitoba. A total of 1044 adult American dog ticks, *Dermacentor variabilis* (Acari: Ixodidae), were collected and screened for spotted fever group rickettsiae. *Rickettsia montanensis* was the only species of rickettsia detected. The mean prevalence of infection was 9.8% (range, 0.00 - 21.74% among localities). The proportion of infected male and female ticks was not significantly different; however, tick populations near the northern limit of *D. variabilis* distribution in Manitoba had lower prevalence of infection compared to tick populations from more southern localities in the province.

Introduction

Rickettsia is a genus of small, gram negative alphaproteobacteria that are obligatory intracellular parasites. Rickettsiae, including the spotted fever group rickettsiae (SFGR), are vector-borne pathogens of significant human and animal importance (Burgdorfer and Anacker 1981, Azad and Beard 1998, Raoult and Parola 2007). Rickettsioses have recently risen to the attention of physicians as important emerging diseases in North America and around the world (Wood and Artsob 2012). Numerous factors contribute to this. Humans are encroaching into areas where SFGR are enzootic and are being exposed (Wood and Artsob 2012). Humans are also altering the landscape

for agricultural development, causing movement and population fluctuations of hosts, altering the potential range of the tick and pathogens (Tabachnick 2010, Dergousoff *et al.* 2013). Range expansion of the SFGR is also attributed to the increase in the range of the tick vectors (Wilkinson 1967, Dergousoff *et al.* 2013). Long distance movement of infested domestic and large wild animals, including white-tailed deer, *Odocoileus virginianus* Zimmermann, are known to spread various diseases, parasites and vectors in Manitoba (Lankester 1974, Shury and Bergeson 2011, Vander Wal *et al.* 2012). Additionally, abiotic factors caused by changing climate may facilitate a change of prevalence and geographic distribution of SFGR by altering survivorship and population density of vectors and hosts involved in the disease cycle (Tabachnick 2010). Establishing a baseline for any disease, whether endemic or emerging, serves an important role in public health, conservation and for future epidemiological studies.

There are four different groups of rickettsiae separated by disease presentation and genetically determined virulence factors. The SFGR consist of approximately 20 species and many are confirmed human pathogens (Wood and Artsob 2012). Other members of the SFGR are implicated in emerging disease, or have unknown pathogenicity. These rickettsiae still play an important role in transmission ecology interfering with transmission of other more pathogenic SFGR (Azad and Beard 1998, Randolph 2004). This group of rickettsiae is transmitted by a variety of species of hard ticks (Acari: Ixodidae). In North America, this includes ticks of many genera including *Haemaphysalis, Rhipicephalus*, *Amblyomma,* and *Dermacentor.* The most infamous of the SFGR is *Rickettsia rickettsii* (Wolbach), the causative agent of Rocky Mountain spotted fever.

Additional complications must be considered when examining the ecology of SFGR in comparison to other tick-borne agents. Presence or absence of one species of SFGR in a geographic area may greatly impact the ecology of another. For example, *R. rickettsii* is considered to be a more pathogenic organism because it causes higher levels of morbidity to the infected vertebrate and tick host (Burgdorfer and Anacker 1981, Azad and Beard 1998, Niebylski *et al.* 1999, Raoult and Parola 2007, Wood and Artsob 2012). This species also appears to have higher infection prevalence in areas where it is the only endemic *Rickettsia* (Wood and Artsob 2012). These facts alone could limit the population of hosts and vectors, reducing the possibility of transmission of other less prevalent species (Niebylski *et al.* 1999). However, in locations in North America where less pathogenic or non-pathogenic SFGR are present, such as *Rickettsia peacockii* Niebylski *et al*., there appears to be an absence or very low prevalence of *R. rickettsii* (Burgdorfer *et al.* 1966, Azad and Beard 1998, Niebylski *et al.* 1999, Wood and Artsob 2012). This phenomenon can be explained by at least two possibilities. Naïve hosts may be exposed to the less pathogenic SFGR and develop a sufficient immune response to prevent future transmission of *R. rickettsii*. Additionally, competitive exclusion has been observed among the SFGR. This means that ticks that are previously infected with some species of SFGR rarely are superinfected with *R. rickettsii* (Niebylski *et al.* 1999, Macaluso *et al.* 2002).

Lower prevalence of human cases of rickettsioses has been reported in Canada compared to the United States (Dergousoff *et al.* 2009, Wood and Artsob 2012). Additionally, there are few published data on the presence of SFGR and cases of rickettsioses in Canada, even though the primary vector species are present and abundant

(Gregson 1956, Wilkinson 1967, Wood and Artsob 2012). Recently, the number of human and domestic animal cases of diseases caused by a wide array of tick-borne pathogens has increased (Wood and Artsob 2012). This is attributed to range expansion of tick populations, changes in landscape and climate and more accurate diagnostic testing (Randolph 2004, Ogden *et al.* 2007, 2008a, 2008b, Tabachnick 2010, Wood and Artsob 2012). Manitoba offers ideal habitat for a variety of rodent hosts that can harbour rickettsiae, especially the suspected main amplifying host, the meadow vole, *Microtus pennsylvanicus* (Ord) (Reich 1981, Wood and Artsob 2012). Southern Manitoba is also within the northern limit of the range of the American dog tick, *Dermacentor variabilis* Say, an important vector of SFGR in North America, including *R. rickettsii* (Wood and Artsob 2012). Little work has been conducted exploring the distribution and prevalence of SFGR in Canada, especially in Manitoba (Dergousoff *et al.* 2009). All ecological components appear to be in place to allow for the transmission and maintenance of these organisms. The range of *D. variabilis* in Manitoba has, in recent history, expanded northward encroaching into the boreal region of the province (Dergousoff *et al.* 2013). These newly established populations may have different profiles of infection by various *Rickettsia* spp. when compared to southern tick populations that have been endemic and potentially infected for a much longer period of time. The objective of this study was to establish what rickettsial agents are present in American dog ticks in Manitoba and if prevalence of infection varies among tick populations from different geographic regions.

Materials and Methods

Host-seeking ticks were collected from crown land, provincial parks, and private property by drag sampling, as described in Chapter Three. A total of 1044 adult American dog ticks were collected from 10 different localities from across the province (Figure 4.1; Table 4.1). Drag sampling was performed from April to August at all localities in 2012 while preliminary sampling was also conducted from one locality (Arbakka) in 2011. Ticks were disinfected, sorted and stored as described in Chapter Three. The same DNA extraction technique, as described in Chapter Three, was used; however, for this work, DNA was extracted from individual ticks rather than in pools. All molecular work was conducted at the National Microbiology Laboratory in Winnipeg, Manitoba, Canada. Voucher specimens of ticks were deposited the J. B. Wallis / R. E. Roughley Museum of Entomology in the Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada.

Ticks were screened for SFGR using a ViaXII™ (Applied Biosystems) Real Time polymerase chain reaction (PCR) system. Two assays using different primers and probes were conducted, primer probe set CS and PanRick (Table 4.2), each targeting separate sections of the gene encoding for citrate synthase (*gltA*). Only DNA extracts that were reactive on both the CS and PanRick primer and probe sets were considered positive. Both reactions followed the same protocol and thermo-cycling conditions. Final reaction volume for the reactions was 25µL. Forward and reverse primers (Table 4.2) had a reaction volume of 0.75µL each with a reaction concentration of 0.3 µM. The probes (Table 4.2) had a reaction volume of 0.25µL at a reaction concentration of 0.1µM. Each reaction also contained 12.5 µL of TaqMan® Universal MasterMix (Applied Biosystems),

5 µL of DNA extract and 5.75µL of nuclease-free water. Thermo-cycling consisted of a 15-minute denaturation at 95°C and 40 cycles of 95°C, 55°C and 72°C for 15, 30 and 30 seconds, respectively. Samples that screened positive in both assays were subjected to a conventional PCR assay to produce amplicon that was submitted for sequencing.

The conventional PCR targeted a portion of the gene responsible for coding for an outer membrane protein, OmpA, a protein that is species-specific in the SFGR and is widely used for *Rickettsia* identification (Roux *et al.* 1996). Each reaction had a final volume of 50µL and consisted of 5µL of forward and reverse primers (Table 4.2), each with a reaction concentration of 1µM, 25µL of HotStarTaq Master Mix® (Qiagen) and 13 µL of nuclease-free water. The thermo-cycling conditions consisted of a 15-minute denaturation at 95°C, followed by 40 cycles of 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 1 minute. A final extension stage of 72°C for 10 minutes followed the cycling. This amplicon was sequenced at the National Microbiology Laboratory along with the primer set *Rr*190.70p - *Rr*190.602n (Table 4.2) used in the amplification.

Sequences were aligned and edited using Geneious software. The National Centre for Biotechnology Information Basic Local Alignment Search Tool was used to compare the aligned nucleotide sequences with sequences published in GenBank®. The accession number and identification of the search result with the highest degree of similarity to each sequence were recorded.

Statistical comparison of prevalence of infection among localities was done using Fisher's exact test computed through the statistical software Quantitative Parasitology 3.0 (Rózsa *et al.* 2000**)**.

Results

The only tick species collected was *D. variabilis.* In total, 1044 adult ticks were collected, 531 males and 513 females, from all localities dragged in Manitoba. Collections consisted of 88-182 ticks in each locality with a mean of 95 ticks per locality.

Of the ticks collected and screened, 9.87%, 41 males and 62 females, tested positive for the presence of SFGR DNA. Although more female ticks were infected, this result was not significant (p=0.1813) based on a log likelihood ratio test with Williams' correction (Sokal and Rohlf 1995). All infected ticks were infected with one *Rickettsia, Rickettsia montanensis* (Lackman *et al.*). This was confirmed by a 100% match of the amplicon sequences to a section of the complete genome of *R. montanensis* strain OSU 85-930 (accession number AY543682). The prevalence of tick infection ranged from 0.00 - 21.74% among the localities (Table 4.1). Spotted fever group rickettsiae were not detected in American dog ticks from only two localities, Porcupine Provincial Forest and Nopiming Provincial Park.

Discussion

The fact that *D. variabilis* was the only tick collected in this study known to be infected with SFGR is not surprising. The blacklegged tick, *Ixodes scapularis* Say, and other *Ixodes* spp. are present in the province at lower densities than American dog ticks, but are not associated with any known SFGR (Krakowetz *et al.* 2011). The winter tick, *Dermacentor albipictus* (Packard), and the rabbit tick, *Haemaphysalis leporispalustris* (Packard), are also present in the province (Gregson 1956) and are known vectors of

some *Rickettsia* spp.; however, due to their life history traits and behaviour, adult ticks are uncommonly collected by drag sampling (Gregson 1956). Also, they seldom feed on human hosts (Philip and Kohls 1951, Freitas *et al.* 2009). There are no published records for the collection of the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles, in Manitoba and none were collected. This tick's known range extends from central Saskatchewan west to British Columbia (Gregson 1956, Dergousoff *et al.* 2013). Absence of this tick may explain the absence of *R. peacockii*, as this bacterium appears to have a high degree of host specificity, even in areas where the two host populations overlap (Dergousoff *et al.* 2009). The pathogenic *R. rickettsii*, and non-pathogenic *Rickettsia bellii* Philip *et al.* are known to be transmitted by *D. variabilis* in the United States (Burgdorfer 1975, Philip *et al.* 1983) but *R. bellii* has not yet been detected in Canada (Dergousoff *et al.* 2009).

Dergousoff *et al.* (2009) reported on the prevalence and distribution of *Rickettsia* spp. in *Dermacentor* spp. in the prairies of Canada. In their study, American dog ticks were collected from 15 localities within Alberta, Saskatchewan, Manitoba and Ontario over a four year period. In areas where *D. andersoni* was present, the *Rickettsia* populations profiles looked different from what we observed due to the high prevalence of *R. peacockii* (range 36 - 96%). However, in 453 ticks screened from six localities across the three most eastern provinces sampled, where populations of *D. variabilis* are geographically separated from *D. andersoni*, there was an average infection prevalence of 9.17% (range 0.00 - 33.00%) with 63 of the 453 ticks testing positive for *R. montanensis*. The current study focused on 11 collections, ten localities, with the majority collected in 2012, conducted in one province where only *D. variabilis* was collected. The average

prevalence of infection per collection was 9.87% (range 0.00 - 21.7 %) with 103 of the 1044 ticks collected testing positive for *R. montanensis*. It is also notable that in both studies, the ticks from the localities closest to the northern distribution limit of *D. variabilis* were not infected with SFGR.

The distribution of *R. montanensis* appears to be patchy across the range of *D. variabilis* in the Canadian Prairie Provinces. It appears that populations of *D. variabilis* that are well below the historical distribution (Figure 4.1) and appear to be well established tend to have higher prevalence of *R. montanensis* when compared to those closer to the northern range limit of the American dog tick when not in sympatry with *D. andersoni*. Populations that are closer and even past the historical northern limit appear to have lower or undetected prevalence of infection with *R. montanensis*. It is possible that as *D. variabilis* expands its range in the province, and perhaps the country, the tick may not be dispersing with an immediately detectable prevalence of *R. montanensis*. This supports the evidence that *R. montanensis* is not transovarially transmitted at a sustainable rate and requires rodents as a reservoir (Niebylski *et al.* 1999). Greatest range expansion of *D. variabilis* is achieved by phoretic movement of adult ticks infesting large mammalian hosts. Even if these ticks were infected at a high prevalence, it is unlikely they would ever feed on a rodent amplifying host allowing for the maintenance of the infection. Only the movement of infected larvae or infected rodents into areas where *R. montanensis* is absent would allow establishment of the infection. This disjunction in movement of the ticks preceding movement of pathogens has been well documented in Canada with regards to *I. scapularis* and *Borrelia burgdorferi* Johnson, the causative agent of Lyme disease (Ogden *et al.* 2013a). *Borrelia burgdorferi* is not transovarially

transmitted and also requires rodent reservoirs. The requirement of a rodent reservoir may also explain the overall lower prevalence of infection by *R. montanensis* when compared to other rickettsiae, such as *R. peacockii*, that are transovarially transmitted (Kurtti *et al.* 2005). The exclusion of *R. rickettsii* by *R. montanensis* may hinder the emergence of Rocky Mountain spotted fever within Manitoba and has implications for the potential epidemiology of *R. rickettsii* if it ever emerges in Manitoba, as the province has abundant populations of vectors and hosts. For instance, emergence of *R. rickettsii* in the province may be more probable in areas where American dog tick populations have only recently expanded and where the transmission cycle of *R. montanensis* has yet to be established. However, exclusion of *R. rickettsii* would probably be more likely if a species of transovarially transmitted rickettsia was present in the province.

Although these data primarily represent the distribution and prevalence of SFGR in adult *D. variabilis* in Manitoba during 2012, we did have specimens to test from Arbakka, Manitoba collected in 2011. It is notable that the prevalence of infection for these two years did not differ significantly and occurred at a comparatively higher level than other localities. The sampling site in Arbakka was among the most southern sites in the study.

This study also has public health implications. *Rickettsia montanensis* is now known to be a human pathogen transmitted by the bite of infected *D. variabilis* (McQuiston *et al.* 2012). The disease causes less morbidity than other pathogenic SFGR. We provide critical, all be it preliminary, data that can be used to evaluate risk of this rickettsiosis in the province. Continued active surveillance conducted on an annual basis, at a wider array of localities would strengthen our understanding of the annual variation

in prevalence and distribution of *R. montanensis* in Manitoba. To gain a better understanding of the epidemiology of SFGR, additional research on the role of small mammal reservoirs from different geographic areas of the province would be informative. Although the burden of disease is unknown, physicians should also be aware that the bite from an American dog tick, the tick most commonly found infesting humans in Manitoba, may, in some instances, have health implications caused by under-recognized, tick-associated pathogens such as *R. montanensis*.

Acknowledgements

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Tables and Figures

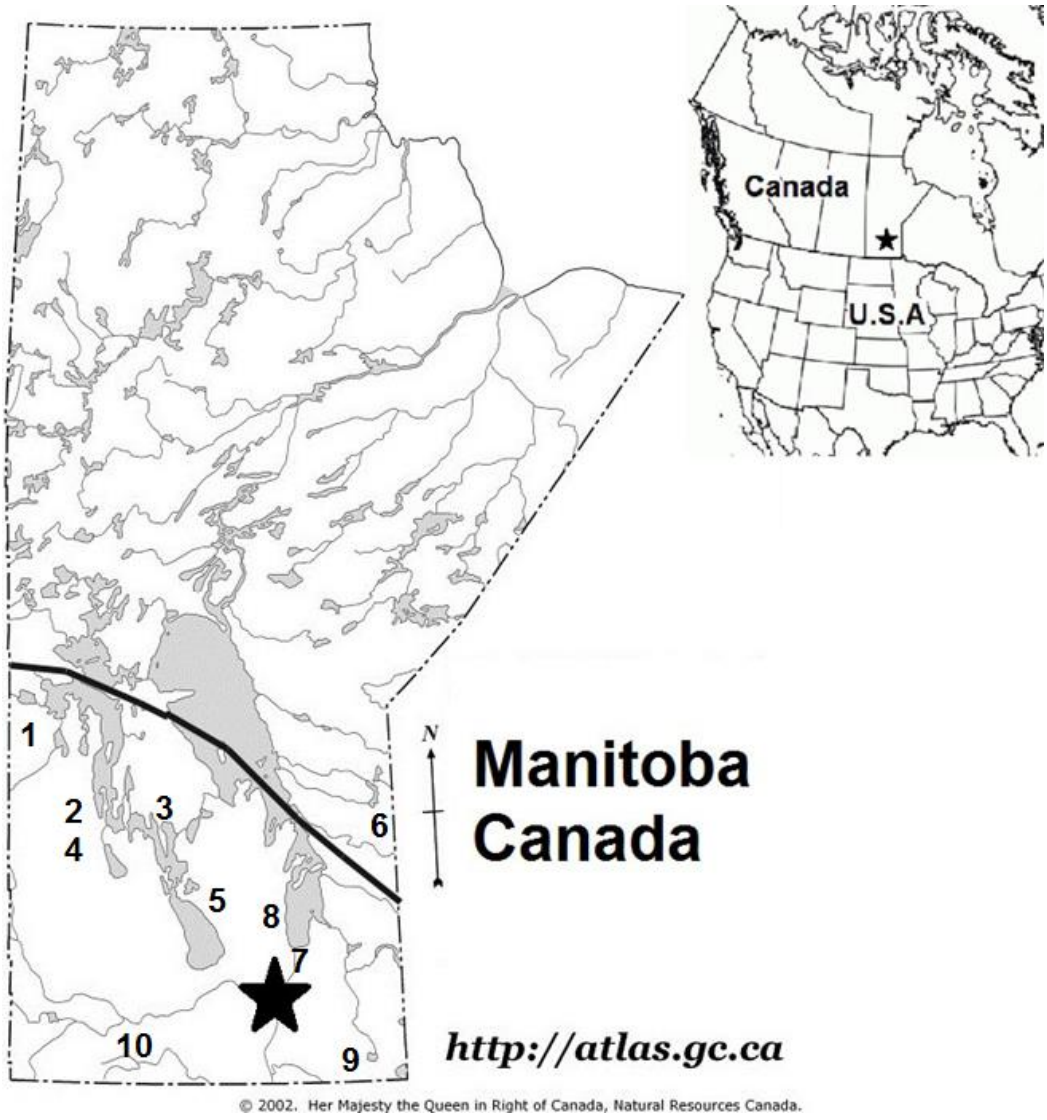

Figure 4.1. Map of Manitoba, Canada. The numbers signify approximate locations where American dog ticks, *Dermacentor variabilis*, were collected (Table 4.1); the star signifies the capital, Winnipeg. The dark line is the historical northern distribution limit of *D. variabilis* modified from Wilkinson 1967 and Dergousoff *et al.* 2013. Provincial borders are indicated with dashed lines, international borers have one small dash between two long dashes. Solid grey lines are maritime borders.

Table 4.1. List of localities with co-ordinates for collections of *Dermacentor variabilis* along with numbers of ticks collected at each site and the number infected with the Spotted Fever Group Rickettsia, *Rickettsia montanensis*. All sites were sampled in 2012, with the exception of Arbakka, where ticks were collected in 2011 and 2012. Values for the prevalence of infection with the same superscript are not significantly different ($P \leq 0.05$).

			Numbers of ticks tested for SFGR			Numbers of ticks infected with SFGR			
Localities	Site number	GPS co-ordinates	Males	Females	Total	Males	Female	Total	Prevalence %
Nopiming Provincial Park	6	50°46.103N 095°18.020W	92	90	182	0	0	0	0.0^{a}
Porcupine Provincial Forest	1	52° 27.172N 101°07.480W	23	19	42	0	0	0	0.0^{ab}
Ethelbert	2	51°33.463N 100°22.189W	47	45	92	0	3	3	3.3^{b}
Sandy Hook	8	50°33.178N 097°00.790W	47	45	92	0	3	3	3.3^{b}
Venlaw	4	51°18.067N 100°26.284W	47	45	92	2	4	6	6.5^{bc}
Homebrook	3	51°44.423N 098°46.261W	47	45	92	2	6	8	8.7^{bcd}
Dunrea	10	49°255.34N 099°4334.74W	40	44	84	10	3	13	15.5^{cde}
Libau	7	50°20.790N 096°42.189W	47	45	92	6	9	15	16.3^{cde}
Eriksdale	5	50°51.271N 097°57.849W	47	45	92	3	14	17	18.5^{de}
Arbakka 2011	9	49°03.237N 096°28.648W	47	45	92	10	8	18	19.6^{e}
Arbakka 2012	9	49°03.237N 096°28.648W	47	45	92	8	12	20	21.7^{e}
Total			531	513	1044	41	62	103	9.9

Table 4.2. List of sequence, 5' to 3', of primers and probes, with dye and quencher, used in the real time and conventional PCR assays for detection and sequencing of Spotted Fever Group Rickettsiae DNA collected from whole DNA exactions from tick tissue.

Primer set name with target gene	Source	Forward primer	Reverse primer	Probe
CS (g*ltA*)	(Stenos *et al.* 2005)	TCGCAAATGTTCACGGTACTTT	TCGTGCATTTCTTTCCATTGTG	FAM-TGCAATAGCAAGAACCGTAGGCTGGATG-Tamra
PanRick (g*ltA*)	(Wölfel *et al.* 2008)	ATAGGACAACCGTTTATTT	CAAACATCATATGCAGAAA	FAM-CCTGATAATTCGTTAGATTTTACCG-Tamra
*Rr*190.70p-Rr190.602n (*OmpA*)	(Regnery *et al.* 1991)	ATGGCGAATATTTCTCCAAAA	AGTGCAGCATTCGCTCCCCCT	NA

Chapter 5: Ability of unfed *Dermacentor variabilis* (Acari: Ixodidae) to survive a second winter as adults in Manitoba, Canada, near the northern limit of their range

Abstract

One thousand seven hundred unfed, field-collected adult *Dermacentor variabilis* (Say) were overwintered in 34 outdoor enclosures near the northern limit of their distribution in Manitoba, Canada. At the northern limits of the range of *D. variabilis*, it had always been assumed that unfed adult ticks questing in spring succumbed before the next winter and were not part of the population observed in the following year. Assessment of survival of the ticks was conducted on two occasions. In midwinter, an average of 39.4% (SE ±2.50) of the ticks were still alive while an average of 19.9% (SE ±1.14) survived to April. Male and female ticks had equal survivorship. The ability to survive an additional winter allows ticks to act in a greater capacity as reservoirs for tick-associated pathogens in this region.

Introduction

The American dog tick, *Dermacentor variabilis* (Say), is a three-host ixodid tick that spends the majority of its life off of its hosts. In Canada, eggs hatch giving rise to larvae throughout the late summer (Garvie *et al.* 1978). The vast majority of these larvae overwinter and are seen in April the following year, feeding predominantly on rodents (Burachynsky and Galloway 1985). After feeding, these larvae moult to nymphs in the leaf litter and the process of feeding, detachment and moulting, this time to the adult stage, is repeated, feeding typically on some small to moderate-sized mammalian host

(Garvie *et al.* 1978). These adult ticks will then overwinter and quest for a final host the next spring and summer. In more moderate climates, a small proportion of these ticks can endure a second winter as unfed adults if hosts are not found in the fall (Sonenshine 1979,1993, McEnroe 1984,1986). It was assumed that only one cohort of adult ticks is ever present at the northern limit of the distribution due to the complete die off of the previous year's adult cohort (McEnroe 1975, McEnroe and Specht 1984, Sonenshine 1993).

The tick's distribution is predominantly an eastern one, ranging from the Mississippi basin to the eastern seaboard of the United States (Sonenshine 1979). American dog ticks can be found from northern Mexico in the south, to the southern prairies of Canada in the north (Wilkinson 1967, Sonenshine 1979, Dergousoff *et al.* 2013). Populations are also known to exist in a small number of widely separated localities in Ontario, in western and central Nova Scotia, and disjunct populations have been established along the Pacific coast of North America, presumably caused by the movement of livestock (Wilkinson 1967, Dodds *et al.* 1969, Garvie *et al.* 1978, Sonenshine 1979, Furman and Loomis 1984). The tick populations in Manitoba were considered to be disjunct and in a location far north of the hypothetical breeding range in an area with harsher weather conditions including colder air temperatures and reduced humidity when compared to stable populations in the southeast (Wilkinson 1967, Sonenshine 1979). The severe cold of winter, resulting in dehydration, has been historically seen as the limiting factor for tick survival in the north (McEnroe 1978). Ticks were believed to be incapable of successful overwintering in locations where the mean winter temperature (December to February) remained above 0°C, or where there

were less than 2500 annual degree-days above 6.5°C (Wilkinson 1967, Sonenshine 1979). However, American dog tick populations are flourishing and appear to be expanding northward in Manitoba and Saskatchewan at a rapid rate (Dergousoff *et al.* 2013).

The northward range expansion of the tick may lead to the range expansion of the pathogens they transmit, as seen with other tick-borne infections in the province (Ogden *et al.* 2008c). The process of feeding on multiple hosts and surviving for more than one year makes *D. variabilis* a suitable vector and potential reservoir for a suite of microorganisms, some of which are pathogenic. In North America, *Dermacentor variabilis* is incriminated as the major biological vector of the medically important spotted fever group rickettsiae, including *Rickettsia rickettsii* (Wolbach), the causative agent of Rocky Mountain spotted fever, and *Rickettsia montanensis* (Lackman *et al*.), along with other *Rickettsia* species (Wood and Artsob 2012). Economically important ruminant pathogens, including strains of *Anaplasma marginale* Theiler and *Anaplasma ovis* Lestoquard, the causative agents of bovine and ovine anaplasmosis, respectively, are also transmitted by this tick (de la Fuente *et al.* 2008). Understanding the lifespan of tick vectors under various climatic conditions at the limits of their range can contribute valuable epizootiological information with regards to establishment and spread of pathogens and lead to more appropriate response measures.

The ability of adults of some species of *Dermacentor* to quest in two consecutive seasons has been demonstrated. These studies were conducted in environments that have more favourable winters in coastal areas such as British Columbia, Canada (Gregson 1951), and along the eastern seaboard of the USA (Sonenshine 1973, 1979,1993, McEnroe 1984), or well within the distribution limit of the tick (Eads and Smith 1983). It

was demonstrated in all these studies that approximately 10% of the adult tick cohort, either *Dermacentor andersoni* Stiles or *D. variabilis*, would survive to quest again the following spring. The majority of these were mark-recapture studies and as a result, survivorship may have been underestimated. One notable exception is a long term survivorship study of *Dermacentor reticulatus* (F.) kept in an enclosure at ground level in former Czechoslovakia. In this study, 54.6% female and 57.8% male *D. reticulatus* survived 399 days after being collected in the wild in April 1977 (Černy *et al.* 1982).

In 2012, we conducted a preliminary study to examine variations of questing behaviour of unfed adult *D. variabilis* throughout its vector season from various locations in Manitoba (Appendix B). In October, we noted that approximately 80% of the ticks in that study were alive, but in a dormant state and exhibiting negative geotropic and phototropic behaviour after being disturbed. This finding spurred us on to conduct the current field study, to examine the ability of unfed adult *D. variabilis* to survive through more than one winter in Manitoba.

Materials and Methods

Tickaria. Tickaria were constructed to house ticks for the duration of the experiment and were designed to simulate a natural outdoor enclosure. The main body of the tickaria was a four litre translucent white plastic bucket. For drainage, five circular holes, 2.5 cm in diameter, were cut in the bottoms of the buckets, one in the middle and the remainder equally distributed 5.5 cm from the centre. A circular hole with a diameter of 16 cm was cut into the centre of the lids of the tickaria to allow for air circulation and weather

exposure. Nitex® mesh screen with a mesh opening of 700 µm was cut and fastened using marine glue to cover the holes in the buckets and lids.

Field Plot. In early May, the tickaria were placed in a field plot adjacent to a wind break on the north side of a corn field at the Glenlea Research Station (49°39'1.40"N; 97° 9'15.29"W), Faculty of Agricultural and Food Sciences, University of Manitoba, located approximately 20 km south of the main University of Manitoba campus, Winnipeg, Manitoba. This habitat consisted of tall grasses guarded by rows of green ash, *Fraxinus pennsylvanica* Marshall, Siberian elm, *Ulmus pumila* L., American elm, *Ulmus americana* L. and a small number of white spruce, *Picea glauca* (Moench). Soil cores were dug in a square grid pattern approximately 30.5 cm apart. These cores were approximately as wide as the tickaria and approximately 9 cm deep. A core was placed in each tickarium and the tickaria were nested in the holes that this process generated. Vegetation in the tickaria continued to grow throughout the season. Adult *D. variabilis* were noted questing in the habitat surrounding the tickaria.

Tick Collection and Survival Assessment. Adult *D. variabilis* were collected from a field surrounding an abandoned homestead located in Sandy Hook, Manitoba (50°32'18.75"N; 96°59'46.46"W) in the first week of May, 2012. Ticks were collected using the dragging method with a rectangular piece of white flannel 1.33 m long by 0.673 m wide. Ticks were then sorted by sex and placed into groups of 25 inside clear plastic vials with a perforated lid. Each group of males was paired with a group of females (50 ticks in total) and randomly assigned to a tickarium and a treatment. To ensure that the ticks observed in the tickaria at the end of the study were truly the collected ticks, and not ticks that may have been present in the soil cores, half of the tickaria contained ticks that

were treated by marking them with a spot of paint on their scutum. Paint was applied using an extra fine-tipped DecoColor™ paint pen. Some ticks were retained in a refrigerated incubator at 10°C with 12 hours of light and darkness to assess acute toxicity of the paint. No mortality was observed over a six week period. Ticks were brought to the tickaria field plots and released into their randomly assigned tickaria on 9 May, 2012. Before being released, the vials of ticks were inspected for mortalities. No mortality was observed in either the painted or unpainted treatments. In total, 34 tickaria were placed each with 50 ticks added. A few vacant tickaria remained in the field plot controlling for the presence of ticks that may have been unintentionally added with the soil cores.

Subsets of tickaria were removed from the field on two occasions to assess tick survivorship. The tickaria were either collected in the winter (07-10 January, 2013), when they were buried beneath more than a metre of snow, or in the spring (29 March, 2013-01 April, 2013) when the snow had melted. Tickaria were allowed to sit at room temperature in a sink for 48 hours or until the soil cores were sufficiently well drained to process. Questing and ambulatory ticks were collected and counted as survivors from each tickarium. Ticks that appeared to be dead were also collected and held at room temperature for an additional two days. Total numbers of surviving ticks and recovered dead ticks were recorded for each tickarium. Vacant tickaria were inspected for ticks that may have been included during the placement of the soil core into the tickaria. A G-test was conducted to compare survivorship between the two sexes of tick after a value of 0.5 was added to each count to meet all criteria for the test (Sokal and Rohlf 1995).

Results

All tickaria inspected in winter, approximately 35 weeks after the ticks were collected, contained surviving ticks. The average percentage of live ticks at this point in time was 39.4% (Fig. 5.1) with 138 ticks surviving from the original 350. The percentages of live ticks in each tickarium ranged from 18-60% (16-52% males; 20-68% females) at this point in time. The number of surviving ticks declined in the spring, approximately 47 weeks after collection; all but one of the 27 inspected tickaria contained at least one live tick. The percentages of live ticks ranged from 0-48% (0-44% males; 0-68% females) with 269 surviving from the original 1350. The average number of surviving ticks found in the spring was 18.5% of the original total. Dead ticks were detected in all tickaria that had ticks deposited in them; however, due to the degrees of desiccation, fragmentation and decomposition, an accurate count could not be conducted. A G-test of independence with Williams' correction was performed to compare the male to female survivorship ratio and returned P-values of 0.9391 and 0.657 for the winter and spring counts, respectively. Thus there was no difference in the ability of males or females to overwinter. None of the vacant tickaria, or tickaria that contained painted ticks, contained ticks that were unaccounted for.

Discussion

At least 18.5% of adult ticks collected during the vector season of one year overwintered to the next vector season. This is significant since in much of the historical literature, American dog ticks were not expected to survive two vector seasons in Manitoba (Wilkinson 1967, McEnroe 1975, Sonenshine 1979,1993, Burachynsky and Galloway 1985). Although, in similar studies, unfed adult *Dermacentor* spp. were able to

overwinter elsewhere in North America, survivorship was estimated to be closer to 10% (Gregson 1951, Eads and Smith 1983, McEnroe 1984, Sonenshine 1993). However, these studies were conducted in localities with more temperate climates than the current study. The relatively high survivorship found in this study, along with the expansion of tick populations along its northern limits in Manitoba (Dergousoff *et al.* 2013) may be attributed to our early and extended snow cover (Dodds *et al.* 1969, McEnroe 1984), climate change (Ogden *et al.* 2008a, Tabachnick 2010), acclimation of the tick populations to more extreme environments, or simply because terraria were used as opposed to marking and recapturing ticks. Although winters in Manitoba are considerably colder and longer than the more temperate areas of the range of *D. variabilis*, the ticks themselves may not experience temperatures much below freezing and should be able to retain their moisture under the deep snow pack that accumulates early along forest and grassland ecotones in Manitoba where *D. variabilis* is most abundant (Sonenshine 1993).

There is a notable size dimorphism between male and female *D. variabilis*, with females typically being on average a few millimetres larger (Smith *et al.* 1946). Although there were more live females observed in both collection periods, this result was not significant. It appears that both sexes are equally able to survive through multiple winters.

The ability of this tick to overwinter a second year as adults, presumably in the pukak layer (Pruitt 1960), has many implications for its role as a disease vector. An increased lifespan can result in increased numbers of hosts to which the ticks, and pathogens they transmit, are exposed. This applies particularly to males of *D. andersoni* that are known to feed multiple times and switch hosts (Zaugg *et al.* 1986, Kocan *et al.* 1992a, 1992b, 2004, Lysyk 2013), or ticks that are dislodged during feeding. We presume

male *D. variabilis* behave in a similar way, but research is needed to confirm this. Increased winter survival could result in an increase in risk of pathogen transmission in years where ticks have greater overwintering success. Additionally, by being exposed to multiple large hosts at the adult stage, there is an increased chance of range expansion of the tick as infested hosts disperse over wide areas, a key method for tick dispersal (Madhav *et al.* 2004).

The results of this research also may impact some Disease Prevention and Control Guidelines, particularly in this region of Canada. In 2009, an outbreak of Bovine Anaplasmosis occurred in southern Manitoba (Howden *et al.* 2010) in pastures that contained large populations of *D. variabilis* (M.E.M.Y., unpublished). This is a reportable disease in Canada. The Canadian Food Inspection Agency mandates that a quarantine, test and cull program be initiated once a herd tests positive for the presence of anaplasmosis under Section 5 of the *Health of Animals Regulations*. Outside of vector season, an infected herd is deemed to be free of anaplasmosis after all animals test polymerase chain reaction and competitive enzyme-linked immunosorbent assay negative 35 days after the first insect-killing frost. It may be possible for ticks that may have acquired the pathogen during the outbreak as either nymphs or adults to act as reservoirs for the pathogen until future years, potentially re-infecting a herd deemed healthy and allowing *A. marginale* to persist in the environment. To highlight this risk, future research should be conducted to examine the capability of partially engorged adult male ticks to survive additional winters.

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Tables and Figures

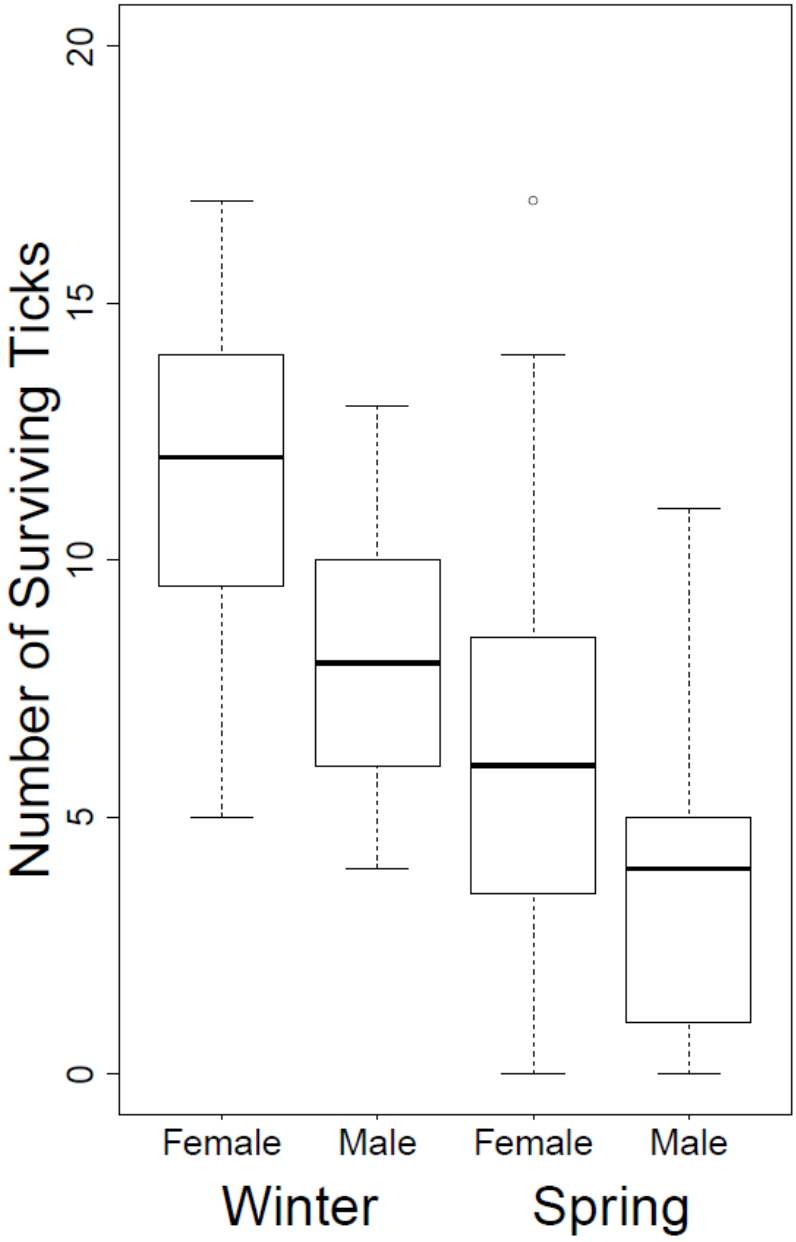

Figure 5.1 Box plots of counts of surviving ticks, separated by sex, from terraria retrieved in the winter and in the spring of 2012 from Glenlea Research Station in Manitoba. For the winter N=7 and for the spring N=27. N represents the number of terraria recovered that originally contained 50 live adult ticks

Chapter 6: General Discussion

Although there has been research in Manitoba studying population dynamics and infestation parameters of *Dermacentor variabilis* (Say) (Gkoroba 1980, Burachynsky and Galloway 1985), many questions surrounding the vector ecology of the American dog tick remain. Through the use of highly efficient molecular techniques, I was able to establish a baseline for the distribution and prevalence of vector-borne pathogens transmitted by *D. variabilis* in the province. Additionally, I was able to contribute to the understanding of the lifecycle of this tick near its northern distributional limit.

Absence of *Anaplasma marginale*

I was unable to detect the presence of *Anaplasma marginale* in field-collected ticks and horse flies. Infected animals were rapidly removed as mandated in the modified stamp-out program. Thus at the start of the field project, there were very few infected animals remaining in the initially infected herds. It is also possible that tests used to diagnose reactor animals had high enough sensitivity that the Canadian Food Inspection Agency (CFIA) veterinarians were able to detect DNA of *A. marginale* from blood samples, even though parasitaemia was too low to maintain the infection in the tick population or to have resulted in sufficient infected cells on the mouthparts of partially fed horse flies. Real time PCR detection limits for the assay used by the CFIA can detect as little as one bacterium per 250µL of plasma-free peripheral blood (Reinbold *et al.* 2010). Bear in mind only one of the 590 animals culled had clinical signs (Howden *et al.* 2010), more than 20 ticks removed from the reactor animal culled in the summer of 2011 that were tested by polymerase chain reaction (PCR) independently of this project and

were negative for the presence of *A. marginale* DNA, and no infected cells were detected in blood smears from the same animal, collected during the cull. It is important that we did not detect *A. marginale* in either the ticks or biting flies, indicating the true end of the outbreak with the culling of the last infected animals assuming arthropod vectors were involved in maintaining the pathogen in this region. Resolution of the outbreak would not be so clear cut if infected vectors had been found.

Detecting a positive pool of tabanid mouthparts would have been an extremely significant result. This would indicate there was likely at least one infected animal in the outbreak region during the trapping period. Because we used the Manitoba Horse Fly Trap, a trap that utilizes a target that catches primarily host-seeking flies (Thorsteinson *et al.* 1964), and because the trap could not be placed safely within the paddock, the probability of catching a fly that had partially fed on an animal that had a high enough parasitaemia before flying into the trap seems remarkably low. This probability would increase with the increase of the number of infected animals in the vicinity. It would have been difficult to determine what pasture contained the infected animals that had been the source of infected flies. The assumption that the pasture closest to the trapping location contained the infected animals is not a safe one, as tabanids are among the fastest flying insects and can disperse over kilometres in a short period of time (Hocking 1953, Foil *et al.* 1991). Additionally, the infectivity of the contaminated flies would also be unknown as we would only be able to determine if bacterial DNA was present.

Detecting positive ticks would also have been interesting from an epizootiological standpoint, but would not have provided as definitive a result as the positive fly mouthparts. A positive female tick would indicate there was at least one infected animal

on the pasture where the tick was collected the previous fall. This assumes that the tick was infected at the nymphal stage as female *D. variabilis* usually feed on one host until repletion unless they are groomed off (Smith *et al.* 1946). A positive male tick may indicate the same thing but because of the possible movement of male ticks from one animal to another, this result could also indicate that there were positive animals in that pasture at the time of tick collection (Smith *et al.* 1946, Zaugg 1986, Kocan *et al.* 1992b, Lysyk 2013). Although detecting *A. marginale* from the arthropod vectors would indicate the presence of the bacteria in the region, it would not indicate whether the bacteria came from an infected herd or wild reservoir hosts.

The lack of detection of *A. marginale* may also be caused by incompatibility between this strain of *A. marginale* and *D. variabilis* in southeastern Manitoba (Smith *et al*. 1986, Wickwire *et al*. 1987, de la Fuente *et al*. 2003a, 2003b). This could be a result of the bacterium being unable to penetrate the gut wall of the tick caused by changes in the tick's innate immune response and or the loss of genes required by the bacterium to infect the tick's gut epithelium. Comparing the migration of other tick-transmitted strains of *A. marginale* in Manitoba ticks may indicate whether or not these ticks were able to act as biological vectors.

Government Policy on Bovine Anaplasmosis

There is no safe vaccine available for prevention of bovine anaplasmosis registered in North America. Other countries, primarily in southern Africa, have allowed the use of a live vaccine. This vaccine is made by using whole blood from splenectomised cattle that have been inoculated with *A. centrale,* a less virulent *Anaplasma* that expresses

the same MSP5 antigen resulting in partial immunity. The risk of spreading other blood-borne pathogens and causing morbidity from *A. centrale* infection through the use of this vaccine are the major concerns limiting its use in North America. Although there is no cure for bovine anaplasmosis, treatment to keep parasitaemia low, limiting morbidity and chance of transmission can be accomplished through antibiotic treatment, typically with doxycycline or related pharmaceuticals. However, these antibiotics are not yet licensed for this use in Canada (Aubry and Geale 2011).

On 1 April, 2014, *A. marginale* will be removed from this list of federally reportable diseases and placed on the list of immediately notifiable diseases. This means that only laboratories will be required to report suspected or confirmed cases of anaplasmosis to the CFIA. Also, the CFIA will no longer respond to anaplasmosis cases or conduct surveillance to evaluate the status of the disease in the Canadian herd. This change in policy is being made with the intent of modernizing the CFIA's response to animal diseases and focus resources on more significant emerging and foreign zoonoses. It is believed that due to the endemic state of *A. marginale* in the USA, it is likely the bacteria will spill over the border and establish again in Canada and the cost of preventing this may not be reasonable.

I have found it rather cumbersome to navigate through the CFIA's policies with regards to bovine anaplasmosis, making it difficult for me to form a strong opinion on this change in policy. The chance for anaplasmosis to become established in Canada is present while the cost of combating it may indeed be unjustifiable. Because of the relatively low morbidity of the disease in cattle, and because the disease poses no risk to human health and low risk to Canada's wildlife, management of anaplasmosis as an immediately

notifiable disease may free some needed resources for the CFIA. However, a few points of policy remain unclear to me. According to the Organization of Economic Co-operation and Development's review, *Livestock Diseases Prevention Control and Compensation Schemes*[1], the CFIA outlined in the *Health of Animals Act* that immediately notifiable diseases will have no federal program for disease control or eradication, but there will be surveillance and monitoring programs within the country. However, on the CFIA website for the Anaplasmosis Program Adjustment, the agency states, "The CFIA will no longer conduct surveillance for anaplasmosis to verify Canada's status for the disease"[2]. Because of the low cost, high sensitivity and speed of cELISA or card agglutination testing, I feel *A. marginale* should remain on the list of screened pathogens during the upcoming BSS and at ports of entry.

Distribution and Prevalence of Spotted Fever Group Rickettsiae in Manitoba

The only species of spotted fever group rickettsiae (SFGR) detected in *D. variabilis* in Manitoba was *Rickettsia montanensis* (Lackman *et al*.). The prevalence of *R. montanensis* in Manitoba appears to be similar to what is observed in other *D. variabilis* populations allopatric with *D. andersoni* (Dergousoff *et al.* 2009). Male and female ticks were equally infected with a prevalence ranging from 0-22%. Of the ten localities sampled, no infected ticks were detected from two localities, Nopiming Provincial Park (n=200) and Porcupine Provincial Forest (n=42). These localities are north of the historic distribution limit of the American dog tick in Manitoba (Wilkinson 1967, Dergousoff *et al.* 2013). It was noted

[1] Agricultural policies and support. OECD.viewed on 12 March 2014. http://www.oecd.org/tad/agricultural-policies/livestockdiseasespreventioncontrolandcompensationschemes.htm#.UyCTgndRiP4
[2] Anaplasmosis Program Adjustments. Animals. Canadian Food Inspection Agency. viewed on 12 March 2014 http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/reportable/anaplasmosis/2013-02-24/eng/1361763159979/1361763263785

that more effort was required for collecting in these areas as questing ticks were not abundant and patches of ideal habitat were few. I have made the argument that the *D. variabilis* populations in these localities are newly established and were founded by individuals that had a low or no prevalence of infection of *R. montanensis*, a SFGR that probably requires lateral transmission through a vertebrate host for maintenance. As a result, amplifying hosts in the region may not yet have a high enough level of infection to maintain a detectable presence of *R. montanensis* in the tick population. A lag in time between establishment of a tick population and having levels of infectious agent reach a detectable level has been observed for other emerging tick-borne diseases including Lyme borreliosis (Ogden *et al.* 2013b).

Overwinter Survival of *D. variabilis* near its Northern Distribution Limits

That almost 20% of the adult ticks collected in 2012 overwintered to 2013 is remarkable and contributes significantly to the vector ecology of *D. variabilis* in Manitoba. If these ticks were transovarially, or as juveniles, infected with a pathogen, then there is the potential for them to act as a reservoir, maintaining the infection in a region longer than previously expected. However, this result does have its limitations. For instance, it is presumed that male *Dermacentor* spp. play the most significant role in biological transmission of *A. marginale* acquiring and transmitting the pathogen as adult ticks (Kocan *et al.* 2010). In this study, we presumed that the ticks collected were unfed and unmated. Having had a blood meal or having mated may significantly alter the physiology of the tick by depleting energy reserves, blocking cues that lead to dormancy or altering their osmolality resulting in higher mortality rates (Balashov 1972). Repeating

this study with fed ticks or even ticks we know are infected with SFGR or *A. marginale* would provide more information on potential risk of exposure.

Overwintering survival is often seen as a major limiting factor for the northern distribution of many species, including ticks (Wilkinson 1967, Dergousoff *et al.* 2013). Although this study was conducted near the northern distributional limits of *D. variabilis*, the survivorship may have been much lower in areas further north of this limit in Manitoba. Repeating this study in multiple areas in the boreal forest region of the province may demonstrate that the lifecycle of *D. variabilis* differs throughout the province. A drop in survivorship could be caused by numerous factors including varying climatic conditions or perhaps by lack of suitable overwintering microhabitat. Knowing more about the survivorship of the tick could be used to predict the potential for further movement of tick populations northward over the years.

Concluding Remarks

In this thesis, I was able to conduct a large-scale study to establish the baseline prevalence and distribution of two tick-borne pathogens in Manitoba, using molecular techniques. Although in this study *A. marginale* was not detected in the outbreak region, in the southeast of the province, occurrence of the bacteria may increase over time, as bovine anaplasmosis is slated to be removed from the federal reportable disease list in April, 2014. The population structure and lifecycle of *D. variabilis* and the SFGR in the province may also change with changes in climate and expanded distribution of the tick. I hope this thesis will contribute to charging the interest of future researchers to focus on the complicated ecology of tick-borne pathogens, as it has for me.

Literature Cited

Ammerman, N. C., K. Swanson, J. M. Anderson, T. R. Schwartz, G. E. Glass, and D. E. Norris. 2003. Prevalence of spotted-fever group *Rickettsia* in Maryland *Dermacentor variabilis*. Emerging Infectious Diesases 10:1478-1481.

Anderson, B. E., and T. Tzianabos. 1989. Comparative sequence analysis of genus-common rickettsial antigen gene. Journal of Bacteriology 171: 5199-5201.

Atwood, E. L., and D. E. Sonenshine. 1967. Dynamics of feeding of American dog tick, *Dermacentor variabilis*. Annals of the Entomological Society of America 60: 363-373.

Aubry, P., and D. W. Geale. 2011. A review of bovine anaplasmosis. Transboundary and Emerging Diseases 58: 1-30.

Augustyn, N. J., and R. D. Bigalke. 1974. *Anaplasma* infection in a giraffe. Journal of the South African Veterinarian Association 45: 229.

Azad, A. F., and C. B. Beard. 1998. Rickettsial pathogens and their arthropod vectors. Emerging Infectious Diseases 4: 179-186.

Balashov, Y. 1972. Bloodsucking ticks (Ixodoidea)-vectors of disease of man and animals (English Translation). Miscellaneous Publications of the Entomological Society of America 8: 161-376.

Barker, S. C., and A. Murrel. 2008. Systematics and evolution of ticks with a list of valid genus and species names. *In* A. S. Bowmen and P. Nuttall (eds.), Ticks, Biology Disease and Control. Cambridge University Press, New York.

Brayton, K. A., P. F. M. Meeus, A. F. Barbet, and G. H. Palmer. 2003. Simultaneous variation of the immunodominant outer membrane proteins, MSP2 and MSP3, during *Anaplasma marginale* persistence in vivo. Infection and Immunity 71: 6627-6632.

Brook, R. K., E. Vander Wal, F. M. van Beest, and S. M. McLachlan. 2013. Evaluating use of cattle winter feeding areas by elk and white-tailed deer: implications for managing bovine tuberculosis transmission risk from the ground up. Preventive Veterinary Medicine 108: 137-147.

Burachynsky, V. I., and T. D. Galloway. 1985. Seasonal dynamics and distribution of American dog tick, *Dermacentor variabilis* (Say), larvae and nymphs at Birds Hill Park, Manitoba. Canadian Journal of Zoology 63: 2748-2755.

Burgdorfer, W. 1975. Review of Rocky Mountian spotted fever (tick-borne typhus), its agent, and its tick vectors in the United States. Journal of Medical Entomology 12: 269-278.

Burgdorfer, W., and R. Anacker. 1981. Rickettsiae and Rickettsial Diseases. Academic Press, Inc., New York, New York.

Burgdorfer, W., K. Friedhof, and J. Lancaste. 1966. Natural history of tick-borne spotted fever in USA- susceptibility of small mammals to virulent *Rickettsia rickettsii*. Bulletin of the World Health Organization 35: 149-153.

Černy, V., S. Szymanski, F. Dusbabek, M. Daniel, and E. Honzakova. 1982. Survival of unfed *Dermacentor reticulatus* (Fabr.) adults under natural conditions. Wiadomosci Parazytologiczne 28: 27-31.

Chomel, B. B., M. L. Carniciu, R. W. Kasten, P. M. Castelli, T. M. Work, and D. A. Jessup. 1994. Antibody prevalence of eight ruminant infectious diseases in California mule and black-tailed deer (*Odocoileus hemionus*). Journal of Wildlife Diseases 30: 51-59.

Davidson, W. R., and W. L. Goff. 2001. Order Rickettsiales: anaplasmosis. Iowa State University Press, Ames, Iowa.

de la Fuente, J., E. F. Blouin, and K. M. Kocan. 2003a. Infection exclusion of the rickettsial pathogen *Anaplasma marginale* in the tick vector *Dermacentor variabilis*. Clinical and Diagnostic Laboratory Immunology 10: 182-184.

de la Fuente, J., A. Estrada-Peña, J. M. Venzal, K. M. Kocan, and D. E. Sonenshine. 2008. Overview: ticks as vectors of pathogens that cause disease in humans and animals. Frontiers in Bioscience 13: 6938-6946.

de la Fuente, J., J. C. Garcia-Garcia, E. F. Blouin, and K. M. Kocan. 2003b. Characterization of the functional domain of major surface protein 1a involved in adhesion of the rickettsia *Anaplasma marginale* to host cells. Veterinary Microbiology 91: 265-283.

de la Fuente, J., J. C. Garcia-Garcia, E. F. Blouin, S. D. Rodriguez, M. A. Garcia, and K. M. Kocan. 2001. Evolution and function of tandem repeats in the major surface protein 1a of the ehrlichial pathogen *Anaplasma marginale*. Animal Health Research Reviews 2: 163-173.

de la Fuente, J., P. Ruybal, M. S. Mtshali, V. Naranjo, L. Shuqing, A. J. Mangold, S. D. Rodriguez, R. Jimenez, J. Vicente, R. Moretta, A. Torina, C. Almazan, P. M. Mbati, S. T. de Echaide, M. Farber, R. Rosario-Cruz, C. Gortazar, and K.

M. Kocan. 2007. Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequences. Veterinary Microbiology 119: 382-390.

Dergousoff, S., A. Gajadhar, and N. Chilton. 2009. Prevalence of *Rickettsia* species in Canadian populations of *Dermacentor andersoni* and *D. variabilis*. Applied and Environmental Microbiology 75: 1786-1789.

Dergousoff, S. J., T. D. Galloway, L. R. Lindsay, P. S. Curry, and N. B. Chilton. 2013. Range expansion of *Dermacentor variabilis* and *Dermacentor andersoni* (Acari: Ixodidae) near their northern distributional limits. Journal of Medical Entomology 50: 510-520.

Dodds, D. G., A. M. Martell, and R. E. Yescott. 1969. Ecology of American dog tick, *Dermacentor variabilis* (Say), in Nova Scotia. Canadian Journal of Zoology 47: 171-181.

Durden, L. A., J. H. Oliver, Jr., C. W. Banks, and G. N. Vogel. 2002. Parasitism of lizards by immature stages of the blacklegged tick, *Ixodes scapularis* (Acari: Ixodidae). Experimental and Applied Acarology 26: 257-266.

Eads, R. B., and G. C. Smith. 1983. Seasonal activity and Colorado tick fever virus infection rates in Rocky Mountain wood ticks, *Dermacentor andersoni* (Acari: Ixodidae), north-central Colorado, USA. Journal of Medical Entomology 20: 49-55.

Ewing, S. A. 1981. Transmission of *Anaplasma marginale* by arthropods. Proceedings of the 7th National Anaplasmosis Conference. Mississippi State University, Starkville, Mississippi.

Foil, L. D. 1989. Tabanids as vectors of disease agents. Parasitology Today 5: 88-96.

Foil, L. D., D. J. Leprince, and R. L. Byford. 1991. Survival and dispersal of horse flies (Diptera: Tabanidae) feeding on cattle sprayed with a subleathal dose of fenvalerate. Journal of Medical Entomology 28: 663-667.

Fournier, P. E., V. Roux, and D. Raoult. 1998. Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA. International Journal of Systematic Bacteriology 48: 839-849.

Freitas, L. H. T., J. L. H. Faccini, and M. B. Labruna. 2009. Experimental infection of the rabbit tick, *Haemaphysalis leporispalustris*, with the bacterium *Rickettsia rickettsii*, and comparative biology of infected and uninfected tick lineages. Experimental and Applied Acarology 47: 321-345.

French, D. M., W. C. Brown, and G. H. Palmer. 1999. Emergence of *Anaplasma marginale* antigenic variants during persistent rickettsemia. Infection and Immunity 67: 5834-5840.

French, D. M., T. F. McElwain, T. C. McGuire, and G. H. Palmer. 1998. Expression of *Anaplasma marginale* major surface protein 2 variants during persistent cyclic rickettsemia. Infection and Immunity 66: 1200-1207.

Furman, D. P., and E. C. Loomis. 1984. The ticks of California (Acari: Ixodida). Bulletin of the California Insect Survey 25: 1-239.

Garvie, M. B., J. A. McKiel, D. E. Sonenshine, and A. Campbell. 1978. Seasonal dynamics of American dog tick, *Dermacentor variabilis* (Say), populations in southwestern Nova Scotia. Canadian Journal of Zoology 56: 28-39.

Gillespie, J. J., K. Williams, M. Shukla, E. E. Snyder, E. K. Nordberg, S. M. Ceraul, C. Dharmanolla, D. Rainey, J. Soneja, J. M. Shallom, N. D. Vishnubhat, R. Wattam, A. Purkayastha, M. Czar, O. Crasta, J. C. Setubal, A. F. Azad, and B. S. Sobral. 2008. *Rickettsia* phylogenomics: unwinding the intricacies of obligate intracellular life. Plos One 3(4), DOI: 10.1371/journal.pone.0002018

Gkoroba, K. G. 1980. A study of the seasonal population dynamics of the ticks *Dermacentor variabilis* (Say) and *Haemaphysalis leporispalustris* (Packard) in a marshland habitat. M.Sc. Thesis, University of Manitoba,Winnipeg.

Grate, H. E. G., N. A. da Cunha, F. G. Pappens, and N. A. D. Farias. 2013. Transplacental transmission of *Anaplasma marginale* in beef cattle chronically infected in southern Brazil. Revista Brasileira de Parasitologia Veterinaria 22: 189-193.

Gregson, J. D. 1951. Notes on the spring activity of the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles (Acarieni: Ixodidea). Proceedings of the Entomological Society of British Columbia 47: 4-7.

Gregson, J. D. 1956. The Ixodoidea of Canada. Canada Department of Agriculture, Ottawa, Ontario. Publication 930.

Gregson, J. D. 1959. Tick paralysis in groundhogs, guinea pigs and hamsters. Canadian Journal of Comparative Medical and Veterinary Science 23: 266-268.

Harlan, H. J., and W. A. Foster. 1990. Micrometeorological factors affecting field host seeking activity of adult *Dermacentor variabilis* (Acari: Ixodidae). Journal of Medical Entomology 27: 471-479.

Hawkins, J. A., J. N. Love, and R. J. Hidalgo. 1982. Mechanical transmission of anaplasmosis by tabanids (Diptera: Tabanidae). American Journal of Veterinary Research 43: 732-734.

Hildebrand, E., M. Carstensen, E. Butler, and L. Cornicelli. 2010. Preliminary results of herd health assessment for northwestern free-ranging elk from 2004-2009. Minnesota Department of Natural Resources Summaries of Wildlife Research Findings 2009: 135-149.

Hocking, B. 1953. The intrinsic range and speed of flight of insects. Transactions of the Royal Entomological Society of London 104: 223-345.

Howden, K. J., D. W. Geale, J. Pare, E. J. Golsteyn-Thomas, and A. A. Gajadhar. 2010. An update on bovine anaplasmosis (*Anaplasma marginale*) in Canada. Canadian Veterinary Journal 51: 837-840.

Howe, D. L., W. G. Hepworth, F. M. Blunt, and G. M. Thomas. 1965. Anaplasmosis in big game animals: experimental infection and evaluation of serologic tests. American Journal of Veterinary Research 25: 1271-1276.

Howell, D. E. 1940. The ecology of *Dermacentor albipictus* (Packard). Proceedings of the Sixth Pacific Science Congress 4: 439-458.

Howell, D. E., C. E. Sanborn, L. E. Roze-Boom, G. W. Stiles, and L. H. Moe. 1941. The transmission of anaplasmosis by horseflies (Tabanidae). Oklahoma Agriculture Experiment Station Technical Bulletin T-11: 1-23.

James, A. M., and J. H. Oliver, Jr. 1990. Feeding and host preference of immature *Ixodes dammini, I. scapularis* and *I. pacificus* (Acari: Ixodidae). Journal of Medical Entomology 27: 324-330.

Jones, E. W., and W. E. Brock. 1966. Bovine anaplasmosis - its diagnosis, treatment and control. Journal of the American Veterinary Medical Association 149: 1624-1633.

Keel, M. K., W. L. Goff, and W. R. Davidson. 1995. An assessment of the role of white-tailed deer in the epizootiology of anaplasmosis in the southeastern United States. Journal of Wildlife Diseases 31: 378-385.

Kemp, D. H., B. F. Stone, and K. C. Binnington. 1982. Tick attachment and feeding: role of the mouthparts, feeding apparatus, salivary gland secretions and the host response. Current Themes in Tropical Science 1: 119-168.

Kieser, S. T., I. S. Eriks, and G. H. Palmer. 1990. Cyclic rickettsemia during persistent *Anaplasma marginale* infection of cattle. Infection and Immunity 58: 1117-1119.

Kocan, K. M. 1986. Development of *Anaplasma marginale* Theiler in ixodid ticks: coordinated development of a rickettsial organism and its tick host. *In* J. R. Sauer and J. A. Hair (ed.), Morphology, physiology, and behavioral biology of ticks. John Wiley & Sons, New York, New York. pp. 472-505.

Kocan, K. M., S. J. Barron, S. A. Ewing, and J. A. Hair. 1985. Transmission of *Anaplasma marginale* by adult *Dermacentor andersoni* during feeding on calves. American Journal of Veterinary Research 46: 1565-1567.

Kocan, K. M., J. de la Fuente, and E. F. Blouin. 2008. Advances toward understanding the molecular biology of the anaplasma-tick interface. Frontiers in Bioscience 13: 7032-7045.

Kocan, K. M., J. de la Fuente, E. F. Blouin, J. F. Coetzee, and S. A. Ewing. 2010. The natural history of *Anaplasma marginale*. Veterinary Parasitology 167: 95-107.

Kocan, K. M., J. de La Fuente, E. F. Blouin, and J. C. Garcia-Garcia. 2004. *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology 129: S285-S300.

Kocan, K. M., W. L. Goff, D. Stiller, P. L. Claypool, W. Edwards, S. A. Ewing, J. A. Hair, and S. J. Barron. 1992a. Persistence of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) in male *Dermacentor andersoni* (Acari: Ixodidae) transferred successively from infected to susceptible calves. Journal of Medical Entomology 29: 657-668.

Kocan, K. M., J. A. Hair, S. A. Ewing, and L. G. Stratton. 1981. Transmission of *Anaplasma marginale* Theiler by *Dermacentor andersoni* Stiles and *Dermacentor variabilis* (Say). American Journal of Veterinary Research 42: 15-18.

Kocan, K. M., D. Stiller, W. L. Goff, P. L. Claypool, W. Edwards, S. A. Ewing, T. C. McGuire, J. A. Hair, and S. J. Barron. 1992b. Development of *Anaplasma marginale* in male *Dermacentor andersoni* transferred from parasitemic to susceptible cattle. American Journal of Veterinary Research 53: 499-507.

Kocan, K. M., D. Stiller, W. L. Goff, W. Edwards, K. B. Wickwire, W. Stich, T. N. Yellin, S. A. Ewing, G. H. Palmer.1989. A review of the developmental cycle of *Anaplasma marginale* in *Dermacentor* spp. ticks. Veterinary Hemoparasite Disease Research Workers, St. Louis, Missouri. pp.129-148.

Kocan, K. M., K. D. Teel, and J. A. Hair. 1980. Demonstration of *Anaplasma marginale* Theiler in ticks by tick transmission, animal inoculation, and fluorescent antibody studies. American Journal of Veterinary Research 41: 183-186.

Kocan, K. M., Z. Zivkovic, E.F. Blouin,V. Naranjo, A, Consuelo, R. Mitra and J. de la Fuente. 2009. Silencing of genes involved in *Anaplasma marginale*-tick interactions affects the pathogen developmental cycle in *Dermacentor variabilis.* BMC Developmental Biology 9: 42.

Kohls, G. M. 1937. Tick rearing methods with special reference to the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles. Dover Publications, Inc., Ithaca, New York.

Krakowetz, C. N., L. R. Lindsay, and N. B. Chilton. 2011. Genetic diversity in *Ixodes scapularis* (Acari: Ixodidae) from six established populations in Canada. Ticks and Tick-Borne Diseases 2: 143-150.

Kurtti, T. J., J. A. Simser, G. D. Baldridge, A. T. Palmer, and U. G. Munderloh. 2005. Factors influencing in vitro infectivity and growth of *Rickettsia peacockii* (Rickettsiales: Rickettsiaceae), an endosymbiont of the Rocky Mountain wood tick, *Dermacentor andersoni* (Acari, Ixodidae). Journal of Invertebrate Pathology 90: 177-186.

Kuttler, K. L., R. M. Robinson, and T. E. Franklin. 1968. Response to *Anaplasma marginale* infection in splenectomized deer. Journal of the American Veterinary Medical Association 20: 12-20.

Kuttler, K. L., R. M. Robinson, and W. P. Rogers. 1967. Exacerbation of latent erythrocytic infections in deer following splenectomy. Canadian Journal of Comparative Medicine 31: 317-319.

Lankester, M. 1974. *Parelaphostrongylus tenuis* (Nematoda) and *Fascioloides magna* (Trematoda) in moose of southeastern Manitoba. Canadian Journal of Zoology 52: 235-239.

Lankester, M. W., W. B. Scandrett, E. J. Golsteyn-Thomas, N. C. Chilton, and A. A. Gajadhar. 2007. Experimental transmission of bovine anaplasmosis (caused by *Anaplasma marginale*) by means of *Dermacentor variabilis* and *D. andersoni* (Ixodidae) collected in western Canada. Canadian Journal of Veterinary Research 71: 271-277.

Lysyk, T. J. 2013. Movement of male *Dermacentor andersoni* (Acari: Ixodidae) among cattle. Journal of Medical Entomology 50: 977-985.

Macaluso, K., R. Sonenshine, S. Ceraul, and A. Azad. 2002. Rickettsial infection in *Dermacentor variabilis* (Acari: Ixodidae) inhibits transovarial transmission of a second *Rickettsia*. Journal of Medical Entomology 39: 809-813.

Madhav, N. K., J. S. Brownstein, J. I. Tsao, and D. Fish. 2004. A dispersal model for the range expansion of blacklegged tick (Acari: Ixodidae). Journal of Medical Entomology 41: 842-852.

McCallon, B. R. 1973. Prevalence and economic aspects of anaplasmosis. *In* E. W. Jones (ed.), Proceedings of the Sixth National Anaplasmosis: Conference 1973, Las Vegas, Nevada. pp. 1-3.

McEnroe, W. D. 1975. The effect of mean winter temperature around 0°C on the population size of the American dog tick, *Dermacentor variabilis*, Say. (Acarina: Ixodidae). Acarologia 17: 208-219.

McEnroe, W. D. 1978. Winter survival microhabitat and constant density regulation of *Dermacentor variabilis*, Say (Acarina: Ixodidae). Acarologia 19: 406-413.

McEnroe, W. D. 1984. The effect of snowcover on an American dog tick, *Dermacentor variabilis* (Say.) (Acari, Ixodidae) population under a harsh winter environment. Zeitschrift für Angewandte Entomologie 97: 481-484.

McEnroe, W. D. 1986. Regulation of the American dog tick, *Dermacentor variabilis* (Say) (Acari, Ixodidae), two cohort population in the coastal area of Massachusetts. Journal of Applied Entomology 102: 370-379.

McEnroe, W. D., and H. B. Specht. 1984. The effect of winter stress on adult *Dermacentor variabilis* (Acari: Ixodidae). Folia Parasitologica 31: 375-378.

McEnroe, W. D., and H. B. Specht. 1987. Age effect analysis of *Dermacentor variabilis* (Say) adult populations during seasonal activity (Acari: Ixodidae). Canadian Journal of Zoology 65: 455-457.

McQuiston, J. H., G. Zemtsova, J. Perniciaro, M. Hutson, J. Singleton, W. L. Nicholson, and M. L. Levin. 2012. Afebrile spotted fever group rickettsia infection after a bite from a *Dermacentor variabilis* tick infected with *Rickettsia montanensis*. Vector-Borne and Zoonotic Diseases 12: 1059-1061.

Niebylski, M. L., M. G. Peacock, and T. G. Schwan. 1999. Lethal effect of *Rickettsia rickettsii* on its tick vector (*Dermacentor andersoni*). Applied and Environmental Microbiology 65: 773-778.

Niebylski, M. L., M. E. Schrumpf, W. Burgdorfer, E. R. Fischer, K. L. Gage, and T. G. Schwan. 1997. *Rickettsia peacockii* sp. nov., a new species infecting wood ticks, *Dermacentor andersoni*, in western Montana. International Journal of Systematic Bacteriology 47: 446-452.

Ogden, N., M. Bigras-Poulin, K. Hanincova, A. Maarouf, C. O'Callaghan, and K. Kurtenbach. 2008a. Projected effects of climate change on tick phenology and fitness of pathogens transmitted by the North American tick *Ixodes scapularis*. Journal of Theoretical Biology 254: 621-632.

Ogden, N., M. Bigras-Poulin, C. O'Callaghan, I. Barker, K. Kurtenbach, L. Lindsay, and D. Charron. 2007. Vector seasonality, host infection dynamics and fitness of pathogens transmitted by the tick *Ixodes scapularis*. Parasitology 134: 209-227.

Ogden, N. H., L. R. Lindsay, K. Hanincova, I. K. Barker, M. Bigras-Poulin, D. F. Charron, A. Heagy, C. M. Francis, C. J. O'Callaghan, I. Schwartz, and R. A. Thompson. 2008b. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. Applied and Environmental Microbiology 74: 1780-1790.

Ogden, N. H., L. R. Lindsay, and P. A. Leighton. 2013b. Predicting the rate of invasion of the agent of Lyme disease *Borrelia burgdorferi*. Journal of Applied Ecology 50: 510-518.

Ogden, N. H., S. Mechai, and G. Margos. 2013a. Changing geographic ranges of ticks and tick-borne pathogens: drivers, mechanisms and consequences for pathogen diversity. Frontiers in Cellular and Infection Microbiology DOI: 10.3389/fcimb.2013.00046

Ogden, N. H., L. St-Onge, I. K. Barker, S. Brazeau, M. Bigras-Poulin, D. F. Charron, C. M. Francis, A. Heagy, L. R. Lindsay, A. Maarouf, P. Michel, F. Milord, C. J. O'Callaghan, L. Trudel, and R. A. Thompson. 2008c. Risk maps for range expansion of the Lyme disease vector, *Ixodes scapularis*, in Canada now and with climate change. International Journal of Health Geographics 7: 24.

Palmer, G. H., and K. A. Brayton. 2013. Antigenic variation and transmission fitness as drivers of bacterial strain structure. Cellular Microbiology 15: 1969-1975.

Pare, J., D. W. Geale, M. Koller-Jones, K. Hooper-McGrevy, E. J. Golsteyn-Thomas, and C. A. Power. 2012. Serological status of Canadian cattle for brucellosis, anaplasmosis, and bluetongue in 2007-2008. Canadian Veterinary Journal 53: 949-956.

Philip, R. N., E. A. Casper, R. L. Anacker, J. Cory, S. F. Hayes, W. Burgdorfer, and C. E. Yunker. 1983. *Rickettsia bellii* sp. nov.: a tick-borne rickettsia, widely distributed in the United States, that is distinct from the spotted fever and typhus biogroups. International Journal of Systematic Bacteriology 33: 94-106.

Philip, C. B., and G. M. Kohls. 1951. Elk, winter ticks, and Rocky Mountian spotted fever: a query. Public Health Reports 66: 1672-1675.

Potgieter, F. T., and W. H. Stoltsz. 1994. Anaplasmosis. *In* J. A. W. Coetzer, G. R. Thompson and R. C. Tustin (eds.), Infectious Diseases of Livestock-With Special Reference to South Africa. Oxford University Press, Cape Town, South Africa. pp. 408-430.

Pruitt, W. O. 1960. Animals in the snow. Scientific American 202: 61-68.

Ralley, W. E., T. D. Galloway, and G. H. Crow. 1993. Individual and group-behavior of pastured cattle in response to attack by biting flies. Canadian Journal of Zoology 71: 725-734.

Randolph, S. E. 2004. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129: S37-S65.

Randolph, S. E. 2008. The impact of tick ecology on pathogen transmission dynamics. *In* A. S. Bowmen and P. Nuttall (eds.), Ticks Biology, Disease and Control. Cambridge University Press, United Kingdom. pp. 40-72.

Randolph, S. E. 2009. Tick-borne disease systems emerge from the shadows: the beauty lies in molecular detail, the message in epidemiology. Parasitology 136: 1403-1413.

Randolph, S. E. 2010. To what extent has climate change contributed to the recent epidemiology of tick-borne diseases? Veterinary Parasitology 167: 92-94.

Raoult, D., and P. Parola. 2007. Rickettsial Diseases. Informa Healthcare USA, Inc., New York, New York.

Raoult, D., and V. Roux. 1997. Rickettsioses as paradigms of new or emerging infectious diseases. Clinical Microbiology Reviews 10: 694-719.

Reeves, J. D., and B. L. Swift. 1977. Iatrogenic transmission of *Anaplasma marginale* in beef cattle. Veterinary Medicine & Small Animal Clinician 72: 911-914.

Regnery, R. L., C. L. Spruill, and B. D. Plikaytis. 1991. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. Journal of Bacteriology 173: 1576-1589.

Reich, L. 1981. *Microtus pennsylvanicus*. Mammalian Species 159: 1-8.

Reinbold, J. B., J. F. Coetzee, K. R. Sirigireddy, and R. R. Ganta. 2010. Detection of *Anaplasma marginale* and *A. phagocytophilum* in bovine peripheral blood samples

by duplex real-time reverse transcriptase PCR assay. Journal of Clinical Microbiology 48: 2424-2432.

Ribeiro, J. M. C., G. T. Makoul, J. Levine, D. R. Robinson, and A. Spielman. 1985. Antihemostatic, antiinflammatory, and immunosuppressive properties of the saliva of a tick, *Ixodes dammini*. Journal of Experimental Medicine 161: 332-344.

Richardson, D. J., L. A. Durden, and D. E. Snyder. 1994. Ectoparasites of the raccoon (*Procyon lotor*) from north-central Arkansas. Journal of the Kansas Entomological Society 67: 208-212.

Richey, E. J. 1991. Bovine anaplasmosis. Proceedings of the 24th Annual Conference of the American Association of Bovine Practitioners, Orlando, Florida. pp. 3-11

Richey, E. J., and G. H. Palmer. 1990. Bovine anaplasmosis. Compendium on Continuing Education for the Practicing Veterinarian 12: 1661-1668.

Roux, V., and D. Raoult. 1995. Phylogenetic analysis of the genus *Rickettsia* by 16S rDNA sequencing. Research in Microbiology 146: 385-396.

Roux, V., and D. Raoult. 2000. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (ompB). International Journal of Systematic and Evolutionary Microbiology 50: 1449-1455.

Roux, V., P. E. Fournier, and D. Raoult. 1996. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. Journal of Clinical Microbiology 34: 2058-2065.

Roux, V., E. Rydkina, M. Eremeeva, and D. Raoult. 1997. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the Rickettsiae. International Journal of Systematic Bacteriology 47: 252-261.

Rózsa L, J, Reiczigel, and G. Majoros. 2000. Quantifying parasites in samples of hosts. Journal of Parasitology 86: 228-232.

Schulze, T. L., R. A. Jordan, and R. W. Hung. 2001. Effects of selected meteorological factors on diurnal questing of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae). Journal of Medical Entomology 38: 318-324.

Scoles, G. A., A. B. Broce, T. J. Lysyk, and G. H. Palmer. 2005a. Relative efficiency of biological transmission of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) by *Dermacentor andersoni* (Acari: Ixodidae) compared with mechanical transmission by *Stomoxys calcitrans* (Diptera: Muscidae). Journal of Medical Entomology 42: 668-675.

Scoles, G. A., M. W. Ueti, and G. H. Palmer. 2005b. Variation among geographically separated populations of *Dermacentor andersoni* (Acari: Ixodidae) in midgut susceptibility to *Anaplasma marginale* (Rickettsiales: Anaplasmataceae). Journal of Medical Entomology 42: 153-162.

Shury, T. K., and D. Bergeson. 2011. Lesion distribution and epidemiology of *Mycobacterium bovis* in elk and white-tailed deer in south-western Manitoba, Canada. Veterinary Medicine International 2011: 1-11.

Smith, C. N., M. M. Cole, and H. K. Gouck. 1946. Biology and cotrol of the American dog tick. United States Department of Agriculture, Washington, D. C.

Smith, R. D., M. G. Levy, M. S. Kuhlenschmidt, J. H. Adams, D. L. Rzechula, T. A. Hardt, and K. M. Kocan. 1986. Isolate of *Anaplasma marginale* not transmitted by ticks. American Journal of Veterinary Research 47: 127-129.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. W. H. Freeman and Company, New York, New York.

Sonenshine, D. E. 1973. Ecology of American dog tick, *Dermacentor variabilis* in a study area in Virginia. 3. Studies on dispersal of immature ticks by small mammal-hosts. Annals of the Entomological Society of America 66: 44-46.

Sonenshine, D. E. 1979. Zoogeography of the American dog tick, *Dermacentor variabilis*. Recent Advances in Acarology 2: 123-134.

Sonenshine, D. E. 1993. Biology of ticks. vol. 2. Oxford University Press, Inc., New York, New York.

Stenos, J., S. R. Graves, and N. B. Unsworth. 2005. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group rickettsiae. American Journal of Tropical Medicine and Hygiene 73: 1083-1085.

Stich, R. W., K. M. Kocan, G. H. Palmer, S. A. Ewing, J. A. Hair, and S. J. Barron. 1989. Transstadial and attempted transovarial transmission of *Anaplasma marginale* by *Dermacentor variabilis*. American Journal of Veterinary Research 50: 1377-1380.

Stiller, D., K. M. Kocan, W. Edwards, S. A. Ewing, J. A. Hair, and S. J. Barron. 1989. Detection of colonies of *Anaplasma marginale* in salivary glands of three *Dermacentor* spp. infected as nymphs or adults. American Journal of Veterinary Research 50: 1381-1385.

Sumilo, D., L. Asokliene, T. Avsic-Zupanc, A. Bormane, V. Vasilenko, I. Lucenko, I. Golovljova, and S. E. Randolph. 2008. Behavioural responses to perceived risk of

tick-borne encephalitis: vaccination and avoidance in the Baltics and Slovenia. Vaccine 26: 2580-2588.

Tabachnick, W. J. 2010. Challenges in predicting climate and environmental effects on vector-borne disease episystems in a changing world. Journal of Experimental Biology 213: 946-954.

Teskey, H. J. 1990. The horse flies and deer flies of Canada and Alaska Diptera: Tabanidae. The Insects and Arachnids of Canada. Part 16. Publication 1838. Biosystematics Research Centre, Ottawa, Ontario.

Theiler, A. 1910. Gall-sickness of South Africa (anaplasmosis of cattle). Journal of Comparative Pathology and Therapeutics 23: 98-115.

Thomas, A. W. 2011. Tabanidae of Canada, east of the Rocky Mountains 2: A photographic key to the genera and species of Tabanidae (Diptera: Tabanidae). Canadian Journal of Arthropod Identification 13: 1-503.

Thorsteinson, A. J., W. Hanec, and G. K. Bracken. 1964. The Manitoba horse fly trap. The Canadian Entomologist 96: 166.

Vander Wal, E., P. C. Paquet, and J. A. Andres. 2012. Influence of landscape and social interactions on transmission of disease in a social cervid. Molecular Ecology 21: 1271-1282.

Walker, D. H., and N. Ismail. 2008. Emerging and re-emerging rickettsioses: endothelial cell infection and early disease events. Nature Reviews Microbiology 6: 375-386.

Watson, T. G., and R. C. Anderson. 1976. *Ixodes scapularis* Say on white-tailed deer (*Odocoileus virginianus*) from Long Point, Ontario. Journal of Wildlife Diseases 12: 66-71.

Whalley, H. D. 1999. *Ixodes scapularis* (Acari: Ixodidae) parasitizing coal skink (*Eumeces anthracinus*). Bulletin of the Maryland Herpetological Society 35: 76-78.

Wickwire, K. B., K. M. Kocan, S. J. Barron, S. A. Ewing, R. D. Smith, and J. A. Hair. 1987. Infectivity of three *Anaplasma marginale* isolates for *Dermacentor andersoni*. American Journal of Veterinary Research 48: 96-99.

Wilkinson, P. R. 1967. The distribution of *Dermacentor* ticks in Canada in relation to bioclimatic zones. Canadian Journal of Zoology 45: 517-537.

Wilson, B. H., and R. B. Meyer. 1966. Transmission studies of bovine anaplasmosis with horseflies *Tabanus fuscicostatus* and *Tabanus nigrovittatus*. American Journal of Veterinary Research 27: 367-369.

Wölfel, R., S. Essbauer, and G. Dobler. 2008. Diagnostics of tick-borne rickettsioses in Germany: a modern concept for a neglected disease. International Journal of Medical Microbiology 298: 368-374.

Wood, H., and H. Artsob. 2012. Spotted fever group rickettsiae: a brief review and a Canadian perspective. Zoonoses and Public Health 59: 65-79.

Yabsley, M. J., T. N. Nims, M. Y. Savage, and L. A. Durden. 2009. Ticks and tick-borne pathogens and putative symbionts of black bears (*Ursus americanus floridanus*) from Georgia and Florida. Journal of Parasitology 95: 1125-1128.

Zaugg, J. L. 1985. Bovine anaplasmosis: transplacental transmission as it relates to stage of gestation. American Journal of Veterinary Research 46: 570-572.

Zaugg, J. L. 1986. Experimental anaplasmosis in American bison: experimental infection and serologic study. Journal of Wildlife Diseases 22: 169-172.

Zaugg, J. L., W. L. Goff, W. Foreyt, and D. L. Hunter. 1996. Susceptibility of elk (*Cervus elaphus*) to experimental infection with *Anaplasma marginale* and *A. ovis*. Journal of Wildlife Diseases 32: 62-66.

Zaugg, J. L., D. Stiller, M. E. Coan, and S. D. Lincoln. 1986. Transmission of *Anaplasma marginale* (Theiler) by males of *Dermacentor andersoni* Stiles fed on an Idaho field infected, chronic carrier cow. American Journal of Veterinary Research 47: 2269-2271.

Appendix A: Questionnaire for Producers

A copy of the form circulated to the producers, by the CFIA district office, involved with the anaplasmosis outbreak in southeastern Manitoba.

Name:
Phone Number:
Address:
Land Location of homestead:

Participation: YES NO

Percentage of Positives:
Total Number of Cattle:

Number of Pastures:
Land Locations:

Directions: (if needed)

Pasture History:

Producer concerns/Requests:

Appendix B: Results from the 2011-2012 Overwintering Study

Fifty *D. variabilis* (25 males; 25 females) were collected from various localities in Manitoba and deposited into tickaria through the summer of 2011. Survivourship of the ticks was assessed in the second week of October (treatments 1 and 3) by arousing ticks that appeared to be in a dormant state. Surviving ticks were then replaced into new tickaria. Fall survivorship was not assessed for two tickaria (treatment 2). One tickarium was recovered from the field plot on 10 January, 2012 (treatment 3); the remaining tickaria were recovered 23 April, 2012. Survivorship of the ticks was assessed once the tickaria had thawed. The act of arousing the ticks in October may have resulted in removing the ticks from their selected microhabitat, reducing their chance of subsequent survival.

Date collected and deposited	Location	Treatment	Fall survivors (%)	Overwintered (%)
05-May-11	Beaudry*	1	31 (62)	0 (0)
05-May-11	Winnipeg	2	NA	10 (20)
03-Jun-11	Winnipeg	3	28 (56)	15 (30)
05-Jun-11	Beaudry*	1	22 (44)	0 (0)
05-Jun-11	Birds Hill*	1	4 (8)	0 (0)
05-Jun-11	Beaudry*	2	NA	4 (8)
14-Jun-11	Beaudry*	1	28 (56)	1 (2)
14-Jun-11	Birds Hill*	1	19 (38)	0 (0)
02-Jul-11	Beaudry*	1	24 (48)	1 (2)
02-Jul-11	Libau	1	27 (54)	0 (0)
07-Jul-11	Eriksdale	1	17 (34)	1 (2)
07-Jul-11	Beaudry*	1	32 (64)	0 (0)

*= Names of Provincial Parks

Appendix C: Detection of Unknown Sequence

What appeared to be false positives were detected when screening *D. variabilis* for SFGR using real time PCR for the gene encoding for gltA. When subjected to conventional PCR for the SFGR gene that encodes for OmpA, these false positives produced PCR product. This product's bands were approximately as bright and were the same size as the positive controls (approximately 400bp). Sequencing of this product produced the sequence given below. Performing a BLAST search on this sequence returned no significantly similar sequences. This sequence is not part of the genome of any organsim or virus that has had its genome sequenced, including *D. variabilis*, any of the SFGR, or any related organisms and viruses. Followup conventional PCR screening for the SFGR gene that encodes for the 17-kDa protean resulted in no amplification. This sequence was detected from DNA extracted from 41 ticks from nine localities. This sequence is included as an appendix note due to the frequent use of the *OmpA* gene in SFGR studies.

GTGGCTTGCAGTCTCACCCGCTACATTCGTATGTTGTAGCA
TGGGCCGTAGGGATATCAGTTAAAATCTAGTTCGTATATTT
TATCACGAGTCTGTTATCCCTACTGCAAAAACCGGCGTCTT
TCAGTGCCTTCCAGCGTGAAACCCGCTCGTTTTCTGACACT
GGATCGCACTGGGGACGCTGGCGCTGGCGCGGAGTCCCTA
GGACACCAGTGAGAACGCTGGATAAGAGCCGGGTCACAC
TGGACGAGAAGCCGGTTTCATTGCCATGACGCTGGGACAC
ACTGGTTTGTGCTGCATACACACAGGTTCTCGCTGCAGTTC
GCTGGCTCGGACATGAAACTGCCGCACTGGTTCCAGTAAG
CAAGGGCATAGATGCTGCCATTNAACGGAACA