University of Alberta

The species composition and distribution of Ixodidae from companion animals in Alberta, Canada.

by

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Dedication

For my wife Carrie, who found me in the middle and helped finish this. And for my daughter Kinley, who arrived and helped in her own way. And for my parents Pat and Mary Lou and my sister Joanna, who all helped from the start.

Abstract

This is the first major update since the 1950's to the composition and distribution of hard ticks in Alberta. Sixteen species were identified, the largest number of tick species in a single report from Alberta. The most common tick species identified from hosts which had not left Alberta were *Dermacentor albipictus*, *D. andersoni*, *D. variabilis*, *Ixodes kingi* and *I. scapularis*. A geographic distribution in Alberta for each of those five species was determined using Maxent. The distributions for *I. scapularis* and *D. variabilis* are the first ever determined for Alberta. It was found that one of three ticks on dogs went unnoticed by the owner and required a veterinary exam to detect. Twenty-two ticks tested positive for the presence of *Borrelia burgdorferi*, the causative agent of Lyme disease. Sixteen of these came from hosts that had not left Alberta. The implication of finding *I. scapularis* ticks and *B. burgdorferi*-positive ticks is discussed.

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I would like to thank my parents and sister for all their support throughout and for encouraging me to take this and then for encouraging me to finish it. Finally I would like to thank my wife and daughter for supporting me as I finished this.

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Chapter 1. General Introduction

1.1. Ticks: Their systematics and importance as disease vectors.

Ticks (Animalia, Arthropoda, Arachnida, Acari, Order Ixodida) are ancient pests that have only relatively recently (19th century) been identified as disease vectors (1-3). With an estimated origin in the Cretaceous period (65 to 146 million years ago) (2), ticks have been infesting hosts for a long time. These obligate haematophagous ectoparasites are now found in most parts of the terrestrial areas of the planet, feeding on hosts and potentially transmitting diseases. Ticks are closely related to mites and together the two orders comprise the Class Acari (1). The ticks have evolved into 899 described species (4) divided into three families: Argasidae, Ixodidae and Nuttalliellidae. There is only one member of Nuttalliellidae (*Nuttalliella namaqua* Bedford 1931) and I will not discuss it further. The Argasidae are known as the soft ticks (1) because of the relatively soft, leathery cuticle covering the exterior of their bodies. The Ixodidae are referred to as the hard ticks, a reference to the small sclerotized scutum (Latin for 'shield', a reference to the shape of the structure) found near the apical end of the dorsal surface of these ticks. My research is focused on the hard ticks (Ixodidae) so I will limit my discussion of the Argasidae. The Ixodidae consists of 12 genera (5), the largest of which is *Ixodes* (241 species). Other prominent genera include *Amblyomma*, *Dermacentor*, *Haemaphysalis* and *Rhipicephalus*.

Tick systematics is continually being updated and the nomenclature revised to reflect new research results. This can create confusion when older tick records are compared to newer records. For example, if historical records of the Lyme disease vector *Ixodes scapularis* Say 1821 are to be examined, then *I. dammini* should be included as in the past it was considered a distinct species even though it has since been synonymized under *I. scapularis* (6). A recent example is *Rhipicephalus (Boophilus) microplus* Canestrini 1888, a major vector of Anaplasmosis, which was previously named *Boophilus microplus* (5). The identification of a tick to the level of species is typically performed using dichotomous keys. The adult life cycle stage is normally used to identify the species. Identification of immature stages (larva, nymph) is less frequently performed as they are more difficult to identify to species and in some cases cannot be distinguished based on morphology. Many identification keys have been produced and for the identifications in Chapter Two I used mainly three high quality keys (7-9). For additional keys covering other parts of the world, see the excellent list compiled by Anderson (3). The genus and species names I will use comply with those set out in Horak et al. (5).

1.1.1. Tick anatomy and biology

A basic understanding of tick anatomy and biology is needed before further discussion of ticks can proceed. The seminal work on tick anatomy (and many other aspects of tick biology) is the two volume work by Sonenshine (1). I will start with a discussion of the physical characteristics of hard ticks and then discuss aspects of the life cycle. Keirans et al. (7,8) provide useful diagrams of basic structure. Sonenshine (1) provides a useful glossary of tick anatomy terms.

Like all arachnids, ticks have a segmented body. But the segments of a tick are highly fused. The anterior (prosoma) of the tick (commonly referred to, incorrectly, as the head) is the capitulum (or gnathosoma), which consists of the two palps (club like structures), the two chelicerae (knife like structures used to cut into the host), the hypostome (an anchoring structure with posteriorly directed spines reminiscent of a serrated knife) and the basis capitulum (the main structure connecting the others to the main tick body). The mouth is a canal formed between the chelicerae and hypostome. The posterior (fused opisthoma and podosoma) is the main body of a tick and is called the idiosoma. It contains all the internal organs and has different structures on the ventral and dorsal surfaces. The major feature on the dorsal surface is the scutum. The ventral surface contains the genital aperture and anus, and is where the legs attach. The legs attach to the body via segments referred to as coxae. The equivalent of a 'foot' at the distal end of the tick leg is a pair of segments referred to as the tarsus. The tarsus has a claw and a pad used for grasping and attachment. The main sensory organ used by a tick is called Haller's organ and it is located on the tarsus. The lateral surfaces of the tick contain the spiracles (within the spiracular plate) which are openings used in gas exchange that lead directly into the respiratory system.

The external morphology of the tick is used to distinguish species. The major features typically compared are the coxae, palps, hypostome, basis capitulum (various features of the ventral and dorsal aspects), scutum, anal groove (ridge in cuticle around anus), trochantor (the first leg segment,

attached to the coxa), genital groove (ridge in cuticle around genital aperture), spiracular plate and the festoons (posterior ridges in the cuticle). Colouration of a tick is not used in identification. Tick eggs cannot be distinguished morphologically. There are molecular methods to identify ticks (10), but distinguishing by morphology is much faster.

Adult ticks are distinguished from immature stage by having eight legs and a genital aperture. Only adult ticks are sexually dimorphic, the females being larger in overall size. The size of a female can change depending how engorged she is, with size potentially increasing tenfold (11) once fully engorged. The scutum does not expand and only covers a small area of the dorsal surface of an engorged female tick. The scutum on males covers the entire dorsal surface, preventing male ticks from noticeably increasing in size after feeding. Nymphs and larvae are distinct from adults. Neither of these immature life cycle stages have a genital pore and there is no sexual distinction at this point in the life cycle. Nymphs are the middle life cycle stage and are distinguished from larvae by having eight legs. Larval ticks have only six legs. Larval ticks are very small, approximately the size of a poppy seed. Nymphs are larger, typically being closer in size to adult ticks than larvae. After feeding nymphs are noticeably engorged. Soft ticks may have, depending on the species, multiple nymphal instars while hard ticks only have one nymphal stage.

1.1.2. Life cycles

Ticks progress over their lifetime from an egg to a larva to a nymph to an adult. In Figure 1.1 I present a simplified diagram of a typical three-host tick life cycle. Nearly all hard ticks have this three-host life cycle. One-host ticks,

such as *Dermacentor albipictus* Packard 1869, can molt from a larva to a nymph to an adult on a single host. Only females drop off the host and do so only to lay eggs. The hatched larvae then seek and attach to new hosts.

The life cycle of a tick can last up to three or even four years between hatching from an egg to the laying of eggs by a female (1,3). So surviving winter is necessary and ticks make use of behavioral diapause to achieve this. Usually ticks will over-winter on the ground under the leaf litter and in the soil (12). A typical three-host life cycle (Figure 1.1) in Alberta involves the larvae hatching from eggs and finding their first host sometime in the late spring/early summer. After feeding, a larva drops to the ground and may either molt (ecdyse) to a nymph or enter diapause and delay molting until spring. Newly molted nymphs in the fall will over-winter and then search for a host in the spring. Emergence of new adults in the late summer/early fall follows and the adults wait until the following spring to find a host, feed, mate, oviposit (lay eggs) and die.

Feeding occurs once every life cycle stage and typically involves a different host at each stage. Male ticks are an exception as they take multiple small meals and may even transfer to a different host, which has implications for disease transmission (13). In general ticks select a progressively larger size of host for each life cycle stage, with larger hosts being targeted as the tick molts to the later life cycle stages (3). For example a larva may feed on a mouse, a nymph on a rabbit and an adult on a deer. As one-host ticks in the larval stage must select a host suitable for the adult stage, they target large hosts (14).

1.1.3. Feeding

After finding a suitable site on the host, and using the claws and sticky pads on the tarsus to maintain a position, ticks use their chelicerae to cut through the outer layers of the host epidermis. The hypostome is inserted into this newly created wound which partially anchors the ticks. The attachment is secured by a secretion from the tick that hardens over the wound and parts of the capitulum, cementing the tick in place. The host fluids secreted into the site of the wound are ingested by the tick through the channel formed between the hypostome and chelicerae. During feeding the tick secretes saliva into the host through the wound (15,16) and this saliva has a number of compounds with a range of effects: anti-inflammation, immunomodullation, compounds which effect tissue repair, anti-coagulants, vasodilation and many other actions, all of which aid the tick in obtaining its meal. Feeding can last from two to 12 days depending on the species of tick and lifecycle stage (1), with immature ticks taking less time to feed. The ingested contents are deposited into the midgut but not digested immediately. Instead it is held in the midgut and digested, over time, intracellularly by the cells lining it. This ability to store a meal for future digestion is one feature of tick biology that allows ticks to persist for so long without feeding (such as the diapause phase during winter). Water balance is critical to a tick at all times but especially during feeding (1,3). The tick will extract water from the ingested contents and secrete it back into the host while feeding. The volume of the meal the tick retains in the midgut is much less than the total volume taken in from the host: a female tick can take in 10 times her final engorged weight over the course of a meal (1). Nitrogenous wastes are excreted as guanine which is a further way of

reducing water use. Once feeding is complete, the tick will extract its hypostome and chelicerae from the host and drop to the ground.

1.1.4. Reproduction

Ticks reproduce sexually and only adult ticks are capable of reproducing. The hard ticks mate on their hosts, with the exception of some members of *Ixodes* which sometimes mate off their hosts (1). A blood meal is required before the gonotrophic cycle begins (1) so only fed females will mate. The males take multiple meals, in between which they search for a female and mate. Males can occasionally transfer to a new host (13), but for the most part remain on their original host to mate and feed until death. Males locate females of the same species through pheromones and insert their hypostome and chelicerae into the female genital opening. Transfer of sperm to the female involves the male generating a sperm sac (spermatophore) from his genital aperture and contorting his body to place it on the genital opening of the female, moving his mouthparts from her genital opening only to finally position the spermatophore (1). The spermatophore then actively transfers itself into the female. The engorged, fertilized female then detaches from the host. She will lay (oviposit) up to 23,000 eggs on the ground. The number of eggs deposited varies by species. *D. variabilis*, for example, lays around 5,300 eggs (1) over the course of almost 24 days, with most deposited by day 10. The female tick dies shortly after depositing her eggs.

1.1.5. Tick hosts

The hosts that provide ticks with a meal can be terrestrial mammals, birds, reptiles or amphibians (2). The ticks I am focusing on for the most part make use of a range of hosts and do not limit themselves to a single host

species. This causes some confusion since many ticks have common names implying they have a single host type. Ticks such as *D. variabilis* (American dog tick), *Haemaphysalis leporispalustris* (the rabbit tick), *D. albipictus* (the moose tick), *I. scapularis* (the deer tick) and others can all be found on hosts other than what the common name implies. The type of host a tick is found on has a lot to do with how a tick finds a host.

The strategy a tick uses to find a host depends on the type, even species, of tick. I will discuss how these strategies are relevant to tick collection later on. Some ticks (soft ticks mostly) are nidiculous (nest dwelling) and find their hosts simply by carrying out their life cycle where the host sleeps (a nest, a den, etc.). Hard ticks must actively search for a host. Two strategies are used for this, referred to as questing (ambush) and hunting. Both strategies rely on the tick sensing a host via CO_2 concentrations in the ambient environment. A hunting style approach involves the tick actively moving toward the host, with the goal of tracking it down and climbing on. An ambush style is when a tick climbs vegetation (grass, shrubs, trees, etc.) and waits for a host to pass by. When a host is detected nearby, the tick will raise its anterior legs into the air and wave the tarsus. When a host brushes against the vegetation the tick immediately grabs hold and climbs onto the host. Ticks, such as *D. albipictus*, that use ambush strategies tend to form clumps of ticks on the vegetation. As mentioned previously, ticks tend towards smaller sized hosts during their immature life cycle stages. Ambush style ticks select different sized hosts by climbing to different heights on the vegetation (14).

1.1.6. Collection of ticks

Research on ticks begins with the recovery of ticks. The method used to recover ticks has an impact on which tick species are recovered. The most frequently used methods are flagging, trapping and collections from animals (such as captured wildlife, caged sentinel animals or domestic animals such as dogs). Flagging involves dragging a piece of cloth (flannel is typical) across a length of ground and then stopping and examining the fabric at predetermined points for any attached ticks. Trapping of ticks involves a stationary trap that is baited with dry ice (solid CO_2). Ticks are attracted to the CO_2, move into the traps and get stuck to the tape or glue surrounding the dry ice. A less common method of tick recovery involves examination and removal of ticks from the researchers themselves after having walked through tick habitat. Comparisons of different tick capture methods have been made (17-19) and flagging has been demonstrated as an effective method of tick recovery. Flagging is the method used when an estimation of tick density is desired. Traps baited with CO_2 were found to capture more mobile species of tick more frequently. The design of sampling strategies (20) can affect the degree of informative data collected on tick biology and distribution.

The use of animals to capture ticks can be an effective recovery method (21-25) and companion animals (such as dogs, cats, etc.) are often favoured (26-29). As Smith et al. (28) found, companion animals in particular are very useful if information on ticks relevant to humans is desired. Companion animals occupy the same geographic space as their owners and are more likely to come in contact with questing ticks in that space than their owners. Both Hamer et al. (27) and Smith et al. (28) found that testing of ticks recovered from dogs was a more efficient way of conducting surveillance for

a tick-borne pathogen compared to using a serosurvey of the dogs. Another major advantage of utilizing companion animals is that sampling for ticks is distributed across a larger area and a larger group of workers (veterinary clinics) than could be achieved by a lone group of researchers. Chapter Two will report on the use of a companion animal surveillance for ticks.

1.1.7. Major research on ticks

Research on ticks involves many fields of study but is focused mainly on questions arising from ticks as parasitic organisms capable of harbouring and transmitting numerous pathogens. More detail on tick-borne pathogens will be provided later in this chapter. There are some exceptions in tick research that focus on tick biology and morphology (1) but these tend to be less common. Tick saliva has been found to contain a large number and variety of compounds that serve several purposes (30). Sialomics, the study of the proteins and mRNA expressed by the salivary gland, has the potential to provide medicine with useful compounds. Research on ticks in Alberta has been underway for several decades (31,31-43). William Samuel has conducted much research in Alberta over the years on *D. albipictus* and the interaction of these ticks with moose. A large component of the research in Alberta concerning *D. andersoni* has dealt with the ability of this tick to transmit *Anaplasma marginale* Theiler 1910 to cattle. Much of the other research has dealt with the composition of species recovered in the province. My research will add to this knowledge and provide a province-wide perspective on ticks.

1.1.8. Tick distributions

Determination of the geographic distribution of tick species is a major area of tick research (32,34,36,37,44-53). As an active area of research stretching over many decades there have been many methods applied to determining tick distributions, from initially hand drawing maps to more current geographic information systems (GIS) approaches. The driving force behind determination of the distribution of tick species is to use that as a proxy for the distribution of tick-borne pathogens that may occur in an area or could potentially establish in an area. Some of the current research in Canada (48,49) has attempted to predict how the distribution of *I. scapularis* (the major Lyme disease vector) will change under different climate change scenarios. The overall prediction is that a warming climate will allow *I. scapularis* to establish in geographic areas where it currently does not persist (49). A gap in this research, which I address with my work, is that Alberta is not included in these predictions. This gap arises in part because the models are geared more towards Eastern Canada where there are established populations of *I. scapularis*. It also is due to modeling efforts being based, in part, on the environmental conditions at sites of tick recovery. Without field samples from Alberta, distribution models may not have the necessary information to assess the current situation in this province. Despite the limitations, Alberta is predicted to eventually be suitable for *I. scapularis* (49).

1.2. Ticks of Alberta

Future predictions of tick distributions are important, but one must also keep in mind which ticks are already known to occur in Alberta. Table 1.1 lists the hard ticks that have been reported from Alberta. There may be other species

that have been recovered in Alberta, but only those reported in the literature were included in the table. While some species have been recovered occasionally, such as *I. scapularis*, others were encountered more often. The most frequently encountered genera are *Dermacentor, Ixodes and Haemaphysalis*. In my reading of the literature, *D. andersoni* was the species reported most frequently. *Dermacentor albipictus*, *I. kingi*, *I. sculptus*, *I. angustus* and *I. spinipalpis* were commonly encountered. The earliest major work on ticks for Alberta (and the rest of Canada) is that by Gregson (36) in which point locations for several species within Alberta are identified. The work by Wilkinson (37) focused on the *Dermacentor* genus only but does try to associate point locations with environmental factors. Two of the three *Dermacentor* species mapped (*D. andersoni* and *D. albipictus*) are reported in Alberta. This association is an improvement on the earlier work of Bow and Brown (33,34) which was more arbitrary in delimiting the extent of distribution. The commonality amongst all the previous work in Alberta on the tick species present is that it either aggregates point locations of tick recovery from other sources or reports on unique recoveries from only a few sites. There is no single author that collected and identified ticks from across the province and no unique province-wide assessment of tick distributions. This is a gap in our knowledge to which I can contribute with my research. In Chapter Two I will report on the tick species recovered from hundreds of sites across Alberta. The distribution patterns in Alberta of the most common species will then be the focus of Chapter Three.

1.3. Tick-borne pathogens

There are many pathogens that can be transmitted by ticks. The variety of pathogens include bacteria such as *Rickettsia rickettsii* (etiological agent of Rocky Mountain Spotted Fever in humans), *Borrelia burgdorferi* (Lyme disease), *Francisella tularensis* (Tularaemia), *Ehrlichia chaffeensis* (Human Monocytotropic Ehrlichiosis), *A. marginale* (Anaplasmosis), *A. phagocytophilum* (Human Granulocytic Anaplasmosis), and many other bacterial species, sub-species and strains. There are also viruses vectored by ticks including the Colorado tick fever virus, the Powassan encephalitis virus, the Crimean-Congo hemorrhagic fever virus, tick-borne encephalitis virus, and others. Protozoa, most often a species of either *Babesia* or *Theileria*, can also be transmitted by ticks. Tick paralysis (54) is also a concern, but it is caused by a toxin in tick saliva and not a pathogenic organism. The pathogens ticks may harbor and transmit vary by geography, host range and tick species. A comprehensive discussion of all potential pathogens that ticks could vector is beyond the scope of my work and is better suited to textbooks. I found the work by Goodman et al. (55) to be particularly useful. I will limit my discussion here mainly to *B. burgdorferi* (and *A. marginale*, briefly) as these were part of the impetus for initiating my research. Also, with my focus on tick species composition and distribution, I will only briefly discuss disease pathology in the definitive host (which could be a human, dog, cow, horse, etc. depending on the pathogen).

1.3.1. Lyme disease

In the late 1970's there was an outbreak of what was thought to be a cluster of juvenile rheumatoid arthritis cases in Lyme, Connecticut (55,56). Further investigation of these human patients revealed an association with ticks and by 1982 Burgdorfer et al. had isolated a spirochete associated with Lyme

disease (57). The name *Borrelia burgdorferi* Johnson 1984 was assigned (58) to the spirochete bacterium Burgdorfer et al. (57) had isolated and *B. burgdorferi* was recognized as the etiological agent of Lyme disease. Also referred to as Lyme borreliosis (LB), it is currently the most common vector borne disease in the United States of America (59). Related to the spirochete that causes syphilis (*Treponema pallidum*), *Borrelia* are long, corkscrew-shaped, flagellated, gram negative bacteria. They exist solely as parasites within a host, where they can persist on extracellular surfaces, within vessels and move through tissues.

There are a several interesting physical characteristics of *Borrelia*. *Borrelia* are notable for having evolved a decreased dependency on iron (60), having eliminated genes for iron metalloproteins common to bacteria and replacing iron with manganese for those not eliminated. Its genome (61,62) is a linear chromosome, which is unique among bacteria, with covalently closed hairpin telomeres. There may be up to 21 "extrachromosomal DNA elements, the largest number known for any bacterium" (62), some of which are essential for the bacterium to survive. Highly evolved as a parasite, *B. burgdorferi* lacks several biosynthetic pathways and depends on the host for many compounds. Even essential compounds, such as nucleotides and fatty acids, must be obtained from the host as *Borrelia* cannot synthesize its own (63).

There have been different members of *Borrelia* that have been distinguished from *B. burgdorferi* over the years. There are at least 18 described species (64). Collectively this group is sometimes referred to as *Borrelia burgdorferi* sensu lato, with *B. burgdorferi* sensu stricto (s.s.) denoting the original *B. burgdorferi.* Most research on *B. burgdorferi* is carried out on laboratory

strain B31. Among the group of LB etiological agents, there are three species that are most often implicated in LB: *B. burgdorferi* (s.s.) (the North American agent of LB), *B. garinii* and *B. afzelii*. The former two are the main European LB pathogens (vectored by *I. dammini*). I will be focusing on North American LB and the associated vectors.

The vectors in North America of *B. burgdorferi* (s.s.) are the blacklegged ticks (*I. scapularis* in the east and *I. pacificus* along the west coast (65)). Between these two species, *I. scapularis* is of greater concern as it can be found in more places. Also, *I. pacificus* has a lower prevalence of *Borrelia*-infected adults than *I. scapularis* (66). Some established populations of *I. scapularis* have had *B. burgdorferi* reported at a prevalence of 58% (56) in adult ticks, while newly established populations have been reported at lower levels. There is a hypothesis that *I. scapularis* moves into a new geographic area first and that infection of the ticks by *B. burgdorferi* lags behind by a few years (67).

Within the tick, *B. burgdorferi* resides in the midgut. When a tick attaches to a host and begins to feed, *B. burgdorferi* begins to disseminate throughout the tick (55). While the estimated time varies from 24 to 72 hours, by about 48 hours after the tick begins to feed *B. burgdorferi* can be found in the salivary gland and is being passed to the host during the fluid exchange that occurs during feeding.

In the mammalian host, *B. burgdorferi* infection proceeds through three distinct phases (55). The pathogen initially begins replicating near the site of the tick bite, leading to the erythema migrans rash (bulls-eye rash) seen on the skin in 70 to 80% of human cases (68). From there *Borrelia* spreads

throughout the host during a dissemination stage, often characterized by malaise, fatigue, headache, fever. Untreated this can lead to neuroborreliosis, a condition where the spirochetes begin interfering with neurological function (55,69). At this point in the infection there is a risk of heart problems due to *Borrelia.* The final phase is a set of chronic conditions, such as arthritis-like symptoms and chronic neuroborreliosis, occurring much later (years later sometimes). In dogs, the infection by *Borrelia* is similar and can potentially involve arthritis, neurological symptoms and kidney and heart problems (70).

The pathology of Lyme disease can vary even among hosts of the same species and this makes diagnosis difficult (56,70). A diagnosis of Lyme disease is clinical, with diagnostic tests supporting that diagnosis. Often, clinical diagnoses require a determination of any geographic association with an area where *Borrelia* or its vectors (*I. scapularis* or *I. pacificus* in North America or *I. dammini* in Europe) are endemic (68,70). Response to antibiotics is often used as an indicator (70), although concurrent infection with a different tick-borne pathogen (such as *Babesia* or *Anaplasma*) can confuse results. Laboratory tests to detect *Borrelia* in a host include serological based methods, polymerase chain reaction (PCR) methods on a variety of matrices (serum, blood, CSF, urine) and culture methods that attempt to grow the *Borrelia*. Ticks can also be tested directly for the presence of *Borrelia* using PCR, culture or histology.

Lyme borreliosis in North America is considered endemic in areas of the eastern USA with several small foci in Canada (mainly in Ontario and Quebec) (46,47,59,70,71). In the USA there were over 20,000 human cases

of Lyme disease in 2010, the latest year data are available (59). Lyme disease became a nationally reportable disease in Canada in 2010 (72). There is less information on incidence rates in Canada, with only 69 confirmed cases reported between the late 1980's and 2003 (72). Some provinces have better data available than others. Estimates of yearly incidence among humans in British Columbia are low and considered comparable to the rate (<0.5/100,000) seen in several western states of the USA (59,73). For comparison, the 2010 incidence among humans in Delaware was 73.1/100,000 (59). Among dogs in Canada, the seroprevalence of Lyme disease is estimated to be low nationally at 0.72% (74) but is higher in regions with known endemic foci of *I. scapularis*. *Borrelia*-endemic regions arise in part because the spirochete is able to persist in reservoir hosts, such as white-footed mice (*Peromyscus leucopus* Thomas 1895) (75). Reservoir hosts are needed to maintain viable *Borrelia* that can infect new generations of vector ticks as *Borrelia* is not passed to the eggs from the ovipositing female tick. Finally, *B. burgdorferi* has been recovered in Alberta (76) but it is not considered endemic at present. For comparison, the 2010 incidence in Montana was estimated at 0.3/100,000 (59).

1.3.2. Anaplasmosis

While *B. burgdorferi* is the most prominent tick-borne pathogen and can infect a wide range of hosts, there are some tick-borne pathogens that are more restricted in host range. *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) is a tick-transmitted, obligate intracellular bacterium that infects erythrocytes (13,77-81). *Anaplasma marginale* is phylogenetically related to many other tick-borne pathogens (80,82), including *Rickettsii*

rickettsia (Rickettsiales: Rickettsiaceae), the etiological agent of Rocky Mountain Spotted Fever. Interestingly, the order *Rickettsiales* is that from which the ancestors of mitochondria arose (82).

The disease caused by this organism in cattle is called simply Anaplasmosis and it is a reportable disease (81) in Canada under the federal government's Health of Animals Act. Care must be taken not to confuse Anaplasmosis with the infection of a human or dog by *A. phagocytophilum*, which I will discuss very briefly further on. Anaplasmosis can occur in other ruminants such as bison, but the infection in cattle is of greater concern (for mainly economic reasons). *A. marginale* infection of cattle is less severe if the animal is under two years of age (78,79,81) and cattle under six months old may not even become ill. For cattle over two years old, anaplasmosis causes death in 29% to 49% of clinical cases of infection (81). Infected cattle remain so for the rest of their lives, acting as a reservoir. Immediately following introduction of *A. marginale,* red blood cells are infected and the bacteria replicates inside these, with up to 10^9 red blood cells per milliliter of blood becoming infected (13). After a prepatent period averaging 28 days, clinical symptoms that may occur include anemia, arising from the destruction of red blood cells infected with *A. marginale*. Other clinical signs may include weight loss, fever, lethargy, weakness and respiratory problems (78,81). Dairy cattle may also have milk production impacted by an *A. marginale* infection. Once an animal has recovered and becomes persistently infected there tend to be no lingering clinical symptoms (79). The proportion of erythrocytes infected once the animal has recovered does vary but never reaches the levels obtained during the initial infection (13). This lack of symptoms despite the persistence of the pathogen in the host is why cattle movement is thought to

be the major route in the geographic spread of *A. marginale* (13), introducing *A. marginale* to susceptible tick populations (43,81). This is the opposite of what is being seen with *B. burgdorferi*, where infected ticks are moving into a geographic area and introducing *B. burgdorferi* to susceptible hosts.

While mechanical transmission (by biting flies, needles, surgical instruments, etc.) and transplacental transmission of *A. marginale* can occur, transmission by ticks is the major route of infection (83) because the bacteria can replicate within the tick. Over 20 tick species have been implicated as vectors (78), but the major vectors in North America are *D. andersoni* and *D. variabilis*. While *A. marginale* has been recovered from *D. albipictus*, the competency of that species as a vector is in need of more research (84). *Dermacentor andersoni* is the main vector in the northwestern USA (13). *Anaplasma marginale* has been reported from every state in the USA. Within the tick, *A. marginale* replicates first inside cells lining the gut and eventually spreads throughout the tick. As with *B. burgdorferi*, the pathogen becomes established in the salivary glands. Transovarial transmission does not occur, so each new generation of tick must reacquire the pathogen.

Anaplasma marginale has been reported from all states in the USA and has been found on a few occasions in Canada (13,78,85). Worldwide, Anaplasmosis is a very common disease, especially in tropical and sub-tropical regions. But at present, Canadian cattle are considered disease free. Canadian *D. variabilis* and *D. andersoni* have been shown to be competent at *A. marginale* transmission (43,85), but for unknown reasons this pathogen has not established in Canada. While my research will not attempt to

address this directly, I will show where in Alberta the two main vectors of *A. marginale* are located.

1.3.3. *Anaplasma phagocytophilum*

Previously named *Ehrlichia phagocytophilum* (80), this bacterium is the etiological agent of human granulocytic anaplasmosis (HGA; also called human granulocytic ehrlichiosis, HGE), although this pathogen can also infect other mammals, including dogs. This pathogen is often found concurrently in *I. scapularis* with *B. burgdorferi*. *Anaplasma phagocytophilum* is not a major public health concern like *B. burgdorferi* or an economic concern like *A. marginale*. But it is still a pathogen that can potentially be acquired from ticks in Canada. The first human case of infection with *A. phagocytophilum* in Alberta occurred in 2008 (86). In Canadian dogs, the seroprevalence of *A. phagocytophilum* is estimated to be 0.19% nationally (74).

1.4. Species modeling

I have made several references to the geographic range of ticks and tick-borne pathogens, and I now will discuss that issue directly and give a brief background on methods for modeling a species distribution. The method I used for my research (Chapter Three) is called Maxent (87), but before discussing that I will give a brief introduction to modeling and some of the concepts underpinning such work.

1.4.1. Modeling basics

Modeling a species distribution most often refers to determination of a mathematical framework that can predict whether conditions are suitable for a species to maintain its life cycle. This does not always have to be directly

tied to geographic space (88). For example, Ogden et al. (89) created a dynamic population model *a priori* for the life cycle of *I. scapularis*. This model was created based on foreknowledge of the crucial variables at each point in the life cycle. While this model was then applied to geographic space, it does demonstrate that modeling can be done only in variable space. Most models attempt to relate the survival and persistence of an organism to environmental variables (temperature, humidity, altitude, precipitation, soil pH, etc.) and determine the ecological niche for that species within those variables. An important distinction must be recognized between the realized niche (that which we observe "in the wild") and the potential niche (all sites matching the conditions determined as minimally/maximally permissible for life cycle maintenance) (90). If a model is created beforehand and then applied to the real world, as Ogden et al. did (89), it is essentially a projection of the estimated fundamental niche. A model constructed using field samples, which is my approach, is essentially an estimate of the realized niche. Both approaches have positives and negative attributes; consequently, one must simply be aware of what is being modeled. An important consideration when constructing a model from field data is so-called absence data (91,92). This is information on where the species of interest is not located, which is different than simply not collecting a sample from a site. Where the species is not can be just as informative as where it is. Absence data could provide information as to why a species is not found at a site that falls within the predicted fundamental niche. The difficulty with absence data is that obtaining it requires extra time and resources to rigorously sample a location before it can be identified as an absence site.

1.4.2. Modeling methods

Work done on the distribution of ticks in Alberta previously made use of hand-drawn maps (32,34,36,37). It was known that the distribution of ticks was directly related to environmental factors (37), but the researchers of the time were limited by the technology available to them. Despite this, as will be seen in Chapter Three, some of these early assessments of tick distributions are still accurate. In the years since the earlier work in Alberta, geographic information systems (GIS) have emerged alongside powerful computers and vast databases of locational data for factors such as temperature, precipitation, etc. As a result, our ability to quickly and accurately model environmental conditions has improved. In almost all cases, the geographic space is divided into a lattice of cells (squares or, alternatively, hexagons). All values for an individual variable within the area of the cell are combined so that the cell counts as a single value (or range) for the variable.

Some modeling approaches involve first creating a model, then using GIS software to project the output of the model onto a geographic space. This was the approach used in the Ogden paper (89) where a population model was created and then projected. This was also the case in the work by Eisen et al. (93), in which a regression model for tick abundance was first created separately and the output projected based on a grid of environmental variables to give a map of where ticks were suspected of being most abundant. A direct modeling approach is more common and involves incorporating GIS environmental data directly with sampling information. For example, this data could be used to calculate a habitat suitability index value for each cell (94) which is then mapped to show suitability over the entire

area. While the calculation of the index value per cell varies between them, several software packages have been developed to facilitate direct modeling in a GIS environment (87,94-96). Among these is the Maxent software package, which I used for my research.

1.4.3. Maxent

Maxent (87,95,97,98) is a software package that models the distribution of a species using environmental data and presence records. This software has been shown (95,97,98) to work well with small sample sizes and was designed to work with presence data only (no absence data is required). Maxent uses a machine learning method to generate a value per map cell indicating how closely the conditions in match those in cells where the species of interest was recovered, relative to all cells under consideration. The authors have refined and improved the software since its creation in 2006 and have done extensive testing (99) to demonstrate the strength of this method. There is also an active community using this software and publishing using it. And, the Maxent software is publically available for free. I will provide more details about this software in Chapter 3.

1.5. Conclusion

To conclude, I will give a brief summary of the structure of my thesis.

Chapter Two is titled "Surveillance for ticks (Arachnida: Acari) on companion animals in Alberta, Canada.". This chapter is a report on the "raw" data gathered for my research. I will describe the findings of the surveillance for ticks and place them in the context of tick species composition in Alberta. I will also provide information that characterizes the hosts from which the

ticks were recovered. Results of testing ticks for the presence of *B. burgdorferi* will also be provided.

Chapter Three is titled "The geographic distribution of the five most common tick species found in Alberta". This chapter will model the distributions of the most common ticks from the surveillance and provide a province-wide picture of where each of these species is distributed. This includes the first distribution map of *I. scapularis* for Alberta. The environmental data used to generate these models are also displayed, as are data on the models themselves. I will then place these distributions in the context of previous research on tick distributions in Alberta.

In the final chapter I will provide some final synthesis on this research as a whole and some directions for future research.

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Table 1.1 Ticks recovered in Alberta

Tick species	Common name(s)	Reference
Amblyomma americanum (Linnaeus 1758)	Lone-star tick	(25,100)
Dermacentor albipictus (Packard 1869)	Moose tick; Winter tick	(32,34,36,37,100,101)
Dermacentor andersoni (Stiles 1908)	Rocky Mountain Wood tick; Spotted fever tick	(32-34,36,37,100-102)
Dermacentor variabilis (Say 1821)	American dog tick	(34) (author states there was associated travel out of Alberta)
Haemaphysalis chordeilis (Packard 1869)	Bird Tick	(32,100)
Haemaphysalis leporispalustris (Packard 1869)	Rabbit tick	(25,32-34,36,100,101)
Ixodes angustus (Neumann 1899)		(23,32,36,100,101)
Ixodes kingi (Bishopp 1911)	The rotund tick	(32,34,36,100,101)
Ixodes marmotae (Cooley and Kohls 1938)		(34) (author states this was most likely *I. kingi*)
Ixodes pacificus (Cooley and Kohls 1943)	Western Blacklegged tick	(22)
Ixodes scapularis (Say 1821)	Deer tick ; Black legged tick	(22,25)
Ixodes sculptus (Neumann 1904)		(32,34,36,100,101,103)
Ixodes spinipalpis (Hadwen and Nuttall 1916)		(32,36,100,101,104)

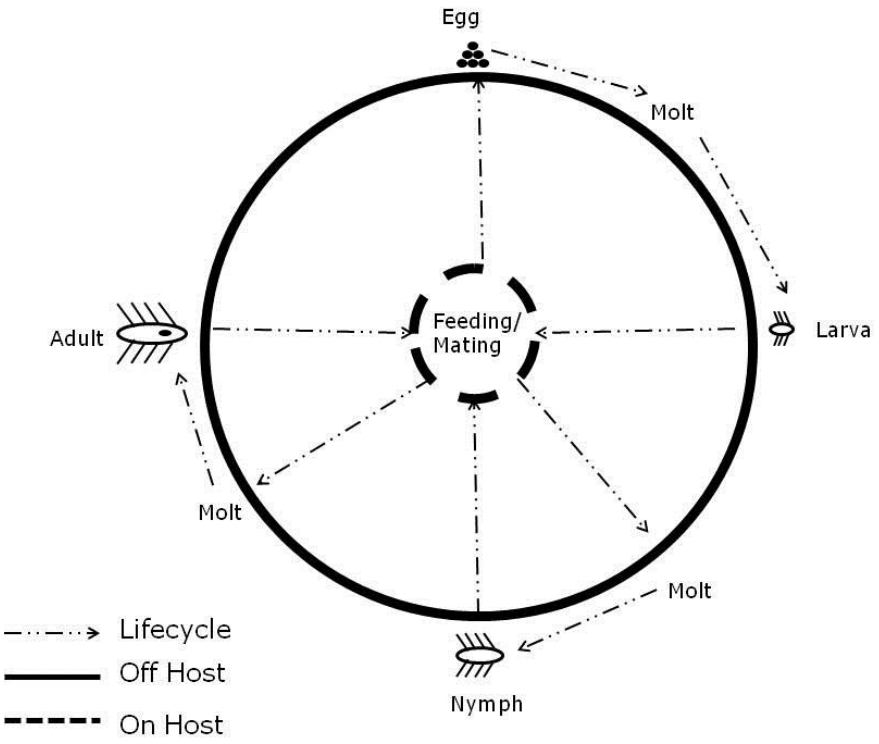

Figure 1.1 General life cycle of a three-host ixodid tick

Chapter 2. Surveillance for ticks (Arachnida: Acari) on companion animals in Alberta, Canada.

2.1. Introduction

Arthropod-borne diseases are a growing concern in North America and around the world (1-5). Ticks are responsible for the transmission of several pathogens in North America, and are increasingly on the public health radar as both nuisance pests and disease vectors. In Canada, Lyme disease is becoming a public health issue in areas of the country where it was not previously a concern. Certain tick-borne pathogens, especially Lyme disease, affect both human and companion animal populations, and are a concern to both human public health and veterinary communities. There are also difficulties associated with determining the true status of Lyme disease across Canada (6) which are compounded by Lyme disease not being on the list of Nationally Notifiable Diseases. In contrast, Lyme disease is a notifiable disease in Alberta for both humans and animals. In 2008, under the animal Health Act, the discovery of an *Ixodes* spp. tick on animal was made notifiable.

In 2007, Murray Kennedy and I identified several ticks, recovered in Alberta from hosts with no history of out-of-province travel, as *Ixodes* spp. Two of these ticks tested positive (testing done in Winnipeg by the Public Health Agency of Canada (PHAC)) for *Borrelia burgdorferi* (Johnson) sensu stricto, the bacterium responsible for causing Lyme disease. According to previous research on the tick fauna of Alberta (7-9) no tick species that vectors Lyme disease is currently thought to be resident in the province. Previous

recoveries of Lyme disease vector species (10, 11) have been attributed to transport by migratory birds. Most of the province-wide research on ticks (7-9, 31) was conducted decades ago and may no longer reflect the current tick species composition in this region. More recent research in Alberta has focused on biology of individual species (12-17) rather than species diversity.

In response to the recovery of *B. burgdorferi*-positive ticks in Alberta and the lack of a current description of the composition of the tick population in Alberta, Murray Kennedy (my former supervisor) and I devised a passive surveillance for ticks on companion animals. To achieve our goal of obtaining ticks from as many locations in Alberta as possible, we were confronted with the reality that conducting sampling directly for ticks over an area as large and as sparsely populated as Alberta requires a large input of resources and personnel. Enlisting the help of veterinary clinics into a passive surveillance program was an efficient solution that has been effective elsewhere in North America (18). The use of companion animals as sentinel species for ticks can provide an early warning to human public health workers of potential disease vectors and provide veterinarians and researchers with an updated description of the tick species composition.

In this chapter I describe the ticks received at our laboratory during three and a half years of surveillance for ticks on companion animals. I compare the composition of tick species I identified to what has been reported in the past in Alberta. I report on the results of testing for the presence of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in select ticks. The ticks involved in my analysis were recovered from companion animals (94% dogs,

2% cats, 2% horses, 2% other host type) by veterinarians and submitted with a history sheet. I use the data from the history sheets to characterize the hosts from which ticks were recovered, to draw associations between different tick species and host travel history and to summarize the scenarios that led to the recovery of a tick from different hosts. This chapter provides several key findings: updated information on composition of the tick species in Alberta, the discovery of several ticks in which *Borrelia burgdorferi* was detected, correlations between the species of tick on a host and the travel history of the host, a characterization of hosts from which ticks were recovered and finally the typical scenarios which led to a tick being recovered by a veterinarian.

2.2. Materials and Methods

In July 2007 Murray Kennedy and I, with the assistance of the Office of the Chief Provincial Veterinarian, the Alberta Veterinary Medical Association and staff at Alberta Agriculture and Rural Development, initiated a passive surveillance for ticks at the Food Safety and Animal Health Division of Alberta Agriculture and Rural Development (19). Veterinarians in Alberta could submit any ticks they recovered from companion animals. The passive surveillance accepted tick submissions any time of year and was still active at time of writing. Data used in the current study were from ticks received between July 1, 2007 and December 31, 2010.

I designed a one page history sheet (see Appendix 1 and also http://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/afs12714) that veterinarians were required to complete and include with each tick submission. One history sheet per host was required, and all ticks found on

an individual host were to be shipped together in one container. Ticks were not to be fixed, frozen or otherwise preserved. The history sheet gathered the following information: veterinary clinic contact information and location, host animal home location (city/town, address), name of the owner of the host animal, host animal name, type of host animal (dog, cat, etc.), host animal age, type of other companion animals present in home, date ticks arrived at the laboratory, whether or not the host animal was out of town/off farm, whether or not the host animal was out of Alberta in the past two months, location of travel (if any), where the host animal exercises (specific parks, ravines, backyard, etc.), the reason why the pet was presented to clinic, and other relevant details. One additional question was added to the history sheet midway through the time period analyzed: whether or not the tick(s) were attached to the host animal's skin. Attachment to the skin was added because the PHAC laboratory requested that information along with submissions to their laboratory. All information was kept confidential and the analysis presented does not reveal any information that could identify an individual host, owner, clinic or veterinarian.

Veterinary clinics were asked to record the main reason the host was presented to the clinic. The reasons given for presentation to the clinic were grouped into categories and the recovery of a tick was determined to be either the primary reason for presentation or a coincidental finding. Coincidental findings were of interest as these ticks would not have been noticed by the pet owner. "Groomer" refers to any tick found while the host was being groomed professionally. Groomer-found ticks were recorded as coincidental findings even if the host was presented to the veterinary clinic subsequent to the groomer finding the tick(s). This was because the

discovery of the tick required a more intensive interaction with the host. Mobility issues include all cases where the host was described as "having trouble getting around", "temporary hind end paralysis" or other difficulties related to walking.

The analysis I performed for this chapter was done for using either the host or the ticks as the unit of analysis. The difference was that at the host (submission) level, all ticks of the same species (regardless of life cycle stage or sex) were counted as one occurrence of that tick species. For example, if ten *Dermacentor albipictus* (Packard) ticks (five females, three males, two nymphs) were collected from the same dog, that would count as one occurrence of *D. albipictus.* But at the tick level this would be ten individual ticks each with the same host history. If more than one species of tick were found on a host, each species was counted as a separate occurrence of a tick on a dog at the level of both the host and tick. So, for example, a dog with one *D. albipictus* and one *D. variabilis* (Say) would count as two hosts in the host (submission) analysis and as two ticks at the tick level.

Due to the large amount of data resulting from the high number of *D. albipictus* ticks from individual horses, beginning in 2008 a representative subsample of five ticks was selected for submissions when large numbers of ticks were found from the same animal. Only data for the five representative ticks (and the corresponding history) were included in this study. This was deemed acceptable as an estimation of tick density on the host was not part of my original experimental design.

I identified ticks to the level of species and assigned a valid species name (20) and life cycle stage (adult/female, adult/male, nymph or larva). Midway through the time period I am reporting on, I began recording the engorgement status of the ticks (fully, partial, not engorged)[1]. Identifications were made using the dichotomous keys of Keirans and Clifford (21), Keirans and Litwak (22) and Yunker et al (23), on the basis of tick morphology. Quite often ticks were damaged and/or degraded to the point where identification was only possible to genus. Several soft ticks were identifiable only as such and were classified to the level of family (Argasidae). Many samples were too degraded for various reasons (mould, fungal growth, etc.) or critical features were absent, in which case the ticks were identified as 'Unable to identify tick species' or 'Unknown' (for life cycle stage). All ticks tested for *Borrelia burgdorferi* by the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC) had the species identification we assigned confirmed by the NML prior to testing. Several of the *Dermacentor* ticks were sent to Douglas Colwell of the University of Calgary for confirmation that we had identified them correctly. Representative voucher specimens preserved in 70% ethanol will be deposited with the E.H. Strickland Museum at the University of Alberta. The author will attempt to deposit specimens of each species, sex and life cycle for which intact specimens were received and maintained in good condition.

[1]This was done as the engorgement status of *Borrelia burgdorferi* positive ticks was a trait requested by various team members, which they used as a rough estimator the likely occurrence of pathogen transfer. Transmission of *B. burgdorferi* from tick to host is estimated to require 48 to 72 hours of attachment. A fully engorged, *Borrelia*-positive tick is more likely to have transmitted the pathogen than an un-engorged *Borrelia*-positive tick.

An odds ratio (OR) was calculated per species (or genus) to compare how likely that species of tick was to be identified from a host that had left Alberta versus a host that had not left Alberta. The OR and corresponding confidence intervals were calculated using the STATA (version 10) software. The formula used to calculate the OR was: OR= (A*D) / (C*B), where A=# submissions of species of interest from hosts that had left Alberta, B=# submissions of species of interest from hosts that did not leave Alberta, C= [(total # submissions (all species) from hosts that had left Alberta)-(A)], D= [(total # submissions (all species) from hosts that did not leave Alberta)-(B)].Testing for the presence of the Lyme disease pathogen *B. burgdorferi* was carried out on ticks identified as either *Ixodes scapularis* (Say), *I. pacificus* (Cooley and Kohls), or *Ixodes* spp. Ticks identified as *I. kingi* (Bishopp) were sent for testing until mid-2008, after which *I. kingi* ticks were not tested. Testing was carried out in Winnipeg, Canada at the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC) as part of the passive surveillance for black legged ticks carried out by the Field Studies – Zoonotic Diseases and Special Pathogens unit. Ticks that were decomposing, mouldy or very desiccated were not sent for *B. burgdorferi* testing. Testing was carried out using a real-time polymerase chain reaction (rtPCR) on DNA extracted from whole ticks. The reaction primers and reactions conditions used are based on the published methods (24). The rtPCR used was a multiplex reaction that also detects the presence of *Anaplasma phagocytophilum*.

2.3. Results

For the 3.5-year period between July 1, 2007 and December 31, 2010 a total of 1195 ticks collected from 814 host animals were submitted (Table 2.1) to the surveillance program. Sixteen tick species were identified, fifteen of which were Ixodidae (hard ticks). The other identified species (*Otobius megnini* (Duges)) was a member of the Argasidae (soft ticks). Five ticks were members of Argasidae but were too degraded to identify further. Five genera of Ixodidae were identified (*Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes* and *Rhipicephalus*). No identification was assigned to 22 ticks (2% of total) due to their poor physical condition. The most common problems preventing identification to the level of species were the absence of the gnathosoma and degradation. The majority of ticks were alive upon arrival at the laboratory. Many ticks that arrived dead were desiccated, allowing for species identification but not for testing for the presence of *B. burgdorferi*.

Adult females were the most common sex received, making up 73.0% of all specimens. Males comprised 20.2% of specimens, nymphs 5.3% and larvae 0.5%. Eleven ticks (1.0% of specimens) were not assigned a life cycle stage or sex, six of which were too degraded to identify and five of which were identified as members of Argasidae but no determination of life cycle stage, species or genus was possible. Only two of the six ticks identified as being larvae were identified (both were *Dermacentor albipictus*). Sixty-four nymphs encompassing six species and five genera were identified.

Of the 16 tick species encountered, *D. variabilis* was the most common, comprising 36.5% of submissions (297/814) and 36.8% of all ticks (440/1195). *Ixodes kingi* was next most common species at 13.6% of

submissions (111/814). Combined with *D. albipictus* (9.2%, 75/814), *D. andersoni* (9.1%, 74/814), *Rhipicephalus sanguineus* Latreille (8.6%, 70/814), *I. scapularis* (7.5%, 61/814) and *Ixodes* spp. (6.4%, 52/814), these seven species made up approximately 90.9% of all submissions. Of the remaining tick species, only *Haemaphysalis leporispalustris* Packard (1.6%), *Amblyomma americanum* Linnaeus (1.2 %) and the unidentified ticks (2.2%) made up more than 1% of submissions.

Ticks were recovered from six different types of hosts (Table 2.1). Dogs made up the majority of hosts (94.5%). Cats and horses were the next most common followed by rabbits, a cow and a weasel. Each tick species was found on at least one dog host. Horses mostly had *D. albipictus* and tended to have a larger number of ticks per animal than other host types. Cats had a wider variety of ticks, with *D. albipictus* and *Ixodes* spp. being more common. The few rabbits from which samples were received had mostly *H. leporispalustris*. The small number of production animals and the small number of larger animals in general was most likely because most veterinary clinics that participated in the surveillance were small animal practices; very few mixed (large/small) animal practices participated. No avian hosts were involved in the surveillance. The only wildlife involved was one weasel and at least two rabbits. None of the rabbits were identified as hares.

Of the 678 ticks for which engorgement state was recorded, 70.2% were fully or partially engorged. Of the 527 ticks whose attachment state was noted, 93.7% were attached to the host.

2.3.1. Submission timing

The highest rate of tick submissions was observed in late spring through to summer (May through August) (Figure 2.1), and there was a slight increase during the fall (October/November). The date of sample arrival at the laboratory was used for analysis. The date of collection of the tick(s) from the host was not used in the analysis as there were many omissions. The time between tick collection and reception at the laboratory was typically within one week.

The largest number of submissions received in a single year (2010) was 346 (Figure 2.1). There were only six months of data for 2007 because the surveillance was not initiated until July. By comparison, the period from July to December had 90 submissions in 2007, 72 in 2008, 88 in 2009, and 158 in 2010. The months in which the most ticks were received were May, June and July. The busiest single month was June 2010 when 99 submissions were received.

For the seven most common tick species received, different patterns were observed for the collection date (Figure 2.3). *Dermacentor andersoni* (n=74) and *D. variabilis* (n=297) were received mainly between May and August. *Dermacentor albipictus* (n=75) was received at a near constant rate throughout the year, with an increase in fall and none during the summer. *Ixodes kingi* (n=111) peaked in spring to early summer. There was no apparent pattern to the *R. sanguineus* submissions (n=70) but there was an increase in November. *Ixodes scapularis* (n=61) submissions peaked in the spring then declined, with a second smaller peak in the fall and early winter. The ticks identified only as *Ixodes* spp. (n=52) were similar to the pattern

for *I. scapularis* in submission timing except the increase in fall was larger and of shorter duration.

The observed timings of tick submission can be explained by the tick life cycle (Figure 1.1) and how winter forces the ticks to go into a dormant phase. The major peak in the early spring to mid-summer represents mainly two groups: those ticks which overwintered as adults and then attached to a host in the spring and those ticks which overwintered as fed nymphs which then molted to adults and attached to hosts in the spring. The smaller increase in the fall represents those ticks which molted into adults and attached to hosts, instead of overwintering as fed nymphs. The timing of *D. albipictus* is more constant throughout the year. This is most likely due to *D. albipictus* being a one-host tick, which only seeks out a new host once in its' lifecycle. As immature ticks were not noticed on the hosts as often, it could be these ticks were on the host and escaped notice until they molted into the larger, adult stage. Also, as staying on the host does shelter the ticks from the extremes of winter, the lifecycle of *D. albipictus* could be less influenced by winter compared to three-host ticks. The lack of a pattern to the timing of *R. sanguineus* submissions is most likely due to that tick being brought into the province by hosts that had travelled. As *R. sanguineus* is not completing its' lifecycle in Alberta, winter has less effect on the timing of submissions and a different pattern is observed compared to other three-host ticks.

2.3.2. Host travel

Travel out of Alberta affected the species of tick most frequently found on a host (Table 2.2, Figure 2.2). The seven tick species that accounted for 90.9% of submissions (Table 2.1) were also the most common species from

hosts that remained in Alberta (Table 2.2). The most common tick genera recovered from a host that remained in Alberta for two months preceding their collection were *Ixodes* or *Dermacentor.* These two genera combined comprised 89.4% of ticks recovered from hosts that stayed in Alberta. The high number of ticks from *Ixodes* was due to the high number of *I. kingi* recovered, which was the most common species recovered from hosts that remained in Alberta (21.8%) (Figure 2.2). The other most common species from hosts in Alberta were *D. variabilis* (17.0% of submissions), *D. albipictus* (15.4%), *D. andersoni* (11.4%), *I. scapularis* (10.9%), *Ixodes* spp. (9.9%) and *R. sanguineus* (4.3%).

The ticks found on hosts that left Alberta had a different composition. Of the genera recovered, 68.6% were *Dermacentor*, 14.2% were *Rhipicephalus* and 13.3% were *Ixodes*. *Dermacentor variabilis* was the most common tick species (Figure 2.2), making up 60.9% of the ticks recovered from dogs that had left Alberta. *Rhipicephalus sanguineus* was the next most frequent species (13.6%). The remaining most common species encountered were *D. andersoni* (5.9%), *I. scapularis* (4.0%), *I. kingi* (3.7%), *Ixodes* spp. (3.1%) and *D. albipictus* (1.1%). Nearly all hosts that travelled out of the province were dogs (98.9%). The only other host types that left Alberta were two cats and two horses. Overall hosts were split between those that remained in Alberta (48.5%) versus having left Alberta (43.4%) in the two months preceding tick recovery, with 8.1% having unknown travel histories.

The odds ratios (OR) (Table 2.2) compared the potential for a species of tick to be recovered from a host if the host had left Alberta in the two month period preceding collection to the potential for being recovered from a host

that remained in Alberta. At the level of genus, a recovered tick was more likely to be *Ixodes* if the host had remained in Alberta than if the host had travelled out of Alberta (OR=0.19). The genera *Rhipicephalus* (OR=3.67) and *Dermacentor* (OR=2.74) were more likely to be identified from hosts that had left Alberta compared to hosts that remained in Alberta. Since *D. variabilis* made up a large majority of the out-of-Alberta ticks, separating that species from the other *Dermacentor* spp. reveals that only *D. variabilis* (OR=7.63) was more likely to be recovered from hosts that had travelled out-of-province. *Dermacentor albipictus* (OR=0.06) and *D. andersoni* (OR=0.49) were more likely to be recovered from hosts that remained in Alberta. Of the remaining seven most common species received, only *R. sanguineus* (OR=3.5) was more likely to be recovered from a host that had left Alberta. *Ixodes kingi* (OR=0.14), *I. scapularis* (OR=0.34) and *Ixodes* spp. (OR=0.29), were all more likely to be recovered from hosts that had remained in Alberta.

Out-of-province travel destinations of hosts were wide ranging (Table 2.3). Host travel was reported to six nations, seven Canadian provinces and 11 states in the United States of America. The most common travel destination was Saskatchewan (111/353 hosts). British Columbia and Manitoba were next most common (37 and 39 hosts, respectively). Many hosts travelled through multiple states in the USA and were recorded as 'Multiple Locations'. Montana was the state visited most frequently (7 hosts). Other states near to Alberta were travel destinations, but North Dakota was not. Almost every region of North America was visited by at least one host, with the major exceptions being the northern territories of Canada and the Atlantic coast of the USA. There were multiple hosts (33) that had travelled from Mexico (no

Mexican state was specified) to Alberta. These were dogs transported by a dog rescue organization and were undergoing veterinary examinations before being adopted. Ontario was visited 21 times. Mode of transport was not recorded. Of the 106 dogs less than one year old, 62 (58%) travelled out of province. No dog less than one month old left the province.

2.3.3. Clinic participation

Over the 3.5 years of the passive surveillance, 199 veterinary clinics submitted samples. Eighty-four clinics submitted samples in only one year, 57 clinics submitted samples in two years and 38 clinics submitted samples in three years. Only 20 clinics participated in all 3.5 years of the surveillance. There was an observed increase in submissions when clinics were notified that the surveillance was still active, and if there was local media coverage of tick-related issues. Regardless of how many years an individual clinic was active in the surveillance, the number of submissions that individual clinics made was usually greater than one (Figure 2.4). Approximately one-half of all clinics submitted ticks five times or less. Fifty-six clinics made only one submission (7% of submissions). Only 17 clinics submitted ticks nine times or more, but when combined, these clinics accounted for approximately 24% of all submissions. Of these, one clinic made 28 submissions and there were five clinics that submitted 13 times. In general, the ticks received came from clinics that participated multiple times over two years or less.

2.3.4. Host interaction with veterinary clinics

The hosts involved in the surveillance were presented to veterinary clinics for a variety of reasons (Table 2.4). Most often ticks were noticed prior to

visiting the veterinary clinic (55.3%). Tick removal prior to interacting with the veterinary clinic occurred in 13.8% of submissions.

Ticks were a coincidental finding on 282 hosts (38.5%). Among these, hosts interacted with the clinic because of vaccinations (9.1%), general exam/check-up (8.1%), lump exams (5.5%), ticks discovered by a groomer (3.7%), and ticks found during neuter/spay of the host (2.1%). Overall, most ticks were noticed on the host before interacting with the veterinary clinic but over one-third of ticks required closer scrutiny of the host for detection.

Host age varied, with more ticks recovered from younger hosts than from older hosts (Figure 2.5). Not all host ages were reported or known. Relatively younger hosts were more common with two-thirds being less than five years old. Dog hosts younger than one year accounted for 13.0% (106/814) of all hosts and were the age group with the highest number of submissions (14.8%, 106/716). Ticks were recovered from three dogs less than one month old. The number of submissions decreased as the host age increased. Dog hosts had a maximum age of 15 years. Hosts older than 15 years consisted of three horses and one cat.

Since dogs comprised 94.5% of hosts (Table 2.1), a closer examination of that host type revealed more meaningful findings while still covering the majority of the surveillance results. Stratifying the results in Table 2.4 by those in Figure 2.5 indicated that the discovery of ticks from a dog occurred for different reasons depending on the age of the dog (Figure 2.6). In total, 656 dogs (80.6% of all hosts, 85.3% of dog hosts) had an age and a reason for presentation recorded on the history sheet. The ticks submitted from

hosts when the tick was the primary presentation reason follows the same pattern as the overall population shown in Figure 2.5. The ticks found coincidentally were encountered more often on younger dogs, especially dogs less than one year old. The ticks found coincidentally became relatively less frequent through the older age groups.

Vaccinations and general exams were responsible for a large majority of the host presentations that led to a tick being found on younger dogs (Figure 2.7), and were the main reason a tick was discovered on dogs less than one year of age. No ticks were submitted after being discovered by groomers on dogs less than one year of age and only five dogs older than one year had ticks discovered while being neutered or spayed.

Overall, the discovery of a tick was usually made before the animal was presented to the veterinarian. Coincidental findings of ticks were less frequent and occurred more often on younger animals presented to a veterinary clinic for vaccinations or a general exam. Host animals of all ages, even one month old, were found to have ticks.

2.3.5. *Borrelia burgdorferi* testing of ticks

Testing for the presence of *B. burgdorferi* in ticks was conducted on 108 ticks (Table 2.5), 22 of which tested positive (all females). The majority of positive ticks were engorged and were attached to the skin of the host. The *B. burgdorferi*-positive ticks were nearly all recovered from dog hosts with the exception of one from a cat. *Ixodes scapularis* accounted for 15 of the 22 *B. burgdorferi*-positive ticks. Of the *I. scapularis* tested, approximately 27.8% harboured *B. burgdorferi*. The next most common *B. burgdorferi*-positive host was *Ixodes* spp. *Ixodes kingi* ticks were submitted for *B.*

burgdorferi testing but after receiving all negative results and finding no support in the literature for *I. kingi* as a vector for *B. burgdorferi*, *I. kingi* were no longer tested in 2008. Overall, *B. burgdorferi*-positive ticks were found on 2.7% of all hosts.

Of the 22 *B. burgdorferi*-positive ticks, 16 were collected from hosts that had not left Alberta in the previous two months. Of the four *B. burgdorferi* ticks that tested positive from hosts that had left Alberta, two (one *I. jellisoni* and one *I. scapularis*) travelled to British Columbia, Canada and two (one *I. scapularis* and one *Ixodes* spp.) travelled to Ontario, Canada.

The timing of submission of *B. burgdorferi*-positive ticks followed the same pattern as the general tick population (Figure 2.8). The most common time for a *B. burgdorferi*-positive tick to be submitted was between April and July with a smaller increase in the fall (October/November). The greatest number of *B. burgdorferi*-positive submissions was received in 2010. The two original *B. burgdorferi*-positive ticks that initiated this passive surveillance were not included in the analysis. The average time between tick collection and arrival at our laboratory was 4 days for *B. burgdorferi*- negative and *B. burgdorferi*-positive ticks. Two specimens of *I. scapularis* tested positive for the presence of *A. phagocytophilum.* Both ticks also tested positive for *B. burgdorferi*, and were from hosts that had not travelled out of Alberta.

2.4. Discussion

2.4.1. Tick-borne pathogens in Alberta

The discovery of two ticks with a concurrent infection of *A. phagocytophilum* and *B. burgdorferi* is notable as *A. phagocytophilum* was

only recently identified for the first time in a human in Alberta. In 2009 the first human case of anaplasmosis (25) in Canada was reported in Calgary, Alberta. This person had no travel history out of the province. The two *A. phagocytophilum*-positive ticks identified by our surveillance were submitted in 2010, one in April and the other in October. These ticks were recovered from hosts living in smaller communities around Edmonton, Alberta and neither tick host had travelled out-of-province. The tick (*I. scapularis)* recovered from the Human Granulocytic Anaplasmosis (HGA)-positive human tested negative for *A. phagocytophilum*. Testing was done at the same PHAC laboratory that tested all of the surveillance ticks. The testing done on the human host was carried out at a different laboratory (Mayo Medical Laboratories in Rochester, Minnesota, USA). Although the vector in the human case was not recovered, our results documenting that this pathogen was detected three times in Alberta in a little over one year, all from hosts with no travel history out of Alberta, provides strong circumstantial evidence that both the pathogen and its vector are resident in Alberta. This is cause to examine the status of this pathogen in Alberta more closely as other research (3,26,27) has found that the range of *I. scapularis* (and *B. burgdorferi*) will increase throughout Canada. Our passive surveillance is well suited for detection of ticks carrying *A. phagocytophilum*, which can also infect dogs.

The 22 *B. burgdorferi*-positive ticks are a large number to find in an area of the world where the Lyme disease pathogen is not considered endemic and is rarely reported (26). That 16 of these ticks were from hosts that did not leave the province is noteworthy. These 16 ticks may have been brought into Alberta by migratory birds. Ogden et al (27) estimated a prevalence of 2.2%

for *I. scapularis* on migratory birds, leading to an estimate of 50 to 175 million ticks dispersed across Canada. But this was based on birds migrating along the east coast of North America. The numbers and type of tick dispersed in Alberta by the birds migrating through this part of North America is not necessarily the same. The timing of submission of *B. burgdorferi*-positive ticks was similar to that of *I. scapularis*, with an increase in spring and a smaller increase in fall. The spring submissions could be explained as originating from infected nymphs that fell from a migratory bird, molted, and then attached to a dog. This is the current hypothesis of how *I. scapularis* is dispersed (10, 27) to non-endemic regions. The timing of increased *I. scapularis* submissions in the spring supports this, as infected nymphs could have detached from migratory birds recently arrived in Alberta, then molted to adults and subsequently attached to dogs.

The fall submissions of *B. burgdorferi*-positive adult *I. scapularis* require more examination. October is too late in the year for the adult *I. scapularis* to have been recently molted from a nymph that dropped off a migratory bird. It could be that a larval tick dropped from a migratory bird in the spring, molted through a nymph to an adult by the fall, and attached to a dog in October. Based on current assumptions of the *B. burgdorferi* status in Alberta, this would require the larva to acquire *B. burgdorferi* from the migrating bird it was attached to, as *B. burgdorferi* cannot be transmitted transovarially. This is unlikely, as Ogden et al. (27) found no infected larvae after examining approximately 39,000 birds in eastern Canada. The examination of infected ticks on migratory birds in Canada has been based mainly on research in eastern Canada. Very little of the research in Canada on migratory birds dispersing ticks has considered Alberta; whether the

results from eastern Canada are applicable to Alberta remains uncertain. Both Scott (11) and Morshed (10) examined relatively small numbers of birds from a combined total of three locations in Alberta and did not identify any infected larvae. The theory of infected larvae being distributed to Alberta by migratory birds has not been proven to date. Such a scenario, in order to accommodate the surveillance findings, implies that if an *I. scapularis* larva is deposited in Alberta, conditions are suitable for it to progress through its life cycle to become an adult. If female *I. scapularis* recovered in Alberta can then be shown to produce viable fertilized eggs in Alberta from which viable larvae emerge, then the possibility that *I. scapularis* can establish in Alberta exists, regardless of whether or not it is introduced.

Other possible explanations exist for the recovery of *B. burgdorferi*-positive adult *I. scapularis* in October in Alberta. There may already be a host reservoir of *B. burgdorferi* in Alberta that is maintaining the pathogen and transmitting it to transplanted *I. scapularis* regardless of whether or not these ticks are established in Alberta. If there is a host reservoir of *B. burgdorferi* in Alberta, this bacterial population should be more closely related to other populations in nearby regions compared to the east coast of North America. The *B. burgdorferi* found in nine of the ticks collected in early 2007 that prompted initiation of the surveillance was genotyped (28) and reported to be part of a cluster of genotypes characteristic of the mid-western USA, distinct from the east coast genotypes. The 22 *B. burgdorferi* positives in this analysis have not been genotyped; however, such investigation would be beneficial. Another possibility is that in addition to a host reservoir of *B. burgdorferi*, a population of *I. scapularis* is already established in Alberta and is acquiring *B. burgdorferi* locally. More research

on host populations in Alberta that could act as reservoirs, and on ticks recovered in Alberta, is required to validate this hypothesis. Overall, my findings should indicate to veterinary and public health practitioners that Lyme disease-infected ticks are present in Alberta and that *I. scapularis* is commonly found on hosts in Alberta.

2.4.2. Ticks of Alberta

The results presented in this chapter represent the largest report of tick species recovered in Alberta, Canada (7-9, 29-31). The most common species encountered in our surveillance have been reported previously from Alberta, but have not been recovered previously by a single collection or surveillance of ticks. The discovery that *D. andersoni* and *I. kingi* were among the most frequently recovered ticks from hosts that remained in Alberta supports previous research indicating that these ticks are resident in the province. Although *I. kingi* was the tick most frequently found on hosts that remained in Alberta, it was recovered from only 3.7% of hosts that travelled outside of Alberta. Since this species is considered to occur throughout western North America (32), this large discrepancy may suggest that *I. kingi* plays a more prominent role in Alberta than in other parts of western North America. As indicated by Salkeld (32), the role *I. kingi* plays in pathogen transmission is in need of more study. As our data showed *I. kingi* to be the most common tick found on dogs in Alberta, there is particular incentive in this province to further investigate the role, if any, that *I. kingi* may play in disease transmission cycles.

The proportion of submissions that comprised *D. variabilis* was larger than expected, as this tick was not considered to be resident much further west

than Saskatchewan. It was expected that *D. variabilis* would comprise a smaller percentage of submissions than *D. andersoni,* which is considered established in Alberta, but the opposite occurred. The number of *D. variabilis* encountered may be due to dogs, considered to be the major host for *D. variabilis*. *Dermacentor variabilis*, commonly called the American dog tick, is routinely found on dogs elsewhere in North America. Finding it on dogs relatively close to an area (Saskatchewan) where it has been known to exist for a long period of time (7) is not too surprising. Despite the number of *D. variabilis* encountered on dogs in Alberta, dogs were still more likely to acquire *D. variabilis* outside of the province.

The moose tick (*D. albipictus*; the winter tick) is so-named since it is the species most commonly found on moose (*Alces alces* L.) (13,33). It is considered to feed mainly on cervids and has a one-host life cycle, dropping from its host only to lay eggs. Our discovery that *D. albipictus* was the most common species recovered from horses is understandable as they are relatively closer in size to moose than the other hosts in the surveillance. Our discovery of so many *D. albipictus* on dogs (9.2% of submissions) indicate that the range of hosts associated with *D. albipictus* is not restricted to hosts of comparable size to moose. It may be that moose are the most suitable hosts and dogs are not as effective for propagation of *D. albipictus*, similar to what Welch and Samuel found (33) when comparing moose to other cervids. Results of our surveillance show that winter ticks do attach to dogs and dogs should be considered among the potential hosts of *D. albipictus*, regardless of how using dogs as a host may affect the fecundity of *D. albipictus*.

Rhipicephalus sanguineus was found on a small number of hosts that had not left the province, but was more likely to be recovered from hosts that had left the province. As the most cosmopolitan tick in the world (34), not finding this species on dogs would have been exceptional. It is worth noting that dogs were more likely to serve as hosts for *R. sanguineus* if they travelled outside Alberta. Combined with the *I. kingi* and *D. variabilis* discoveries, our results suggest that the tick fauna in Alberta may differ in composition compared to the rest of western North America. The current research cannot provide a causal explanation, but it does suggest a future area of research interest.

Some tick species have been recovered in Alberta that were not encountered through the surveillance. *Ixodes sculptus* Neumann was not recovered although it was previously reported in Alberta (9), and has been recovered from dogs in other regions (32). The surveillance was not able to recover many soft tick species and tick species targeted to other host types (especially lizards and birds). Ticks still in the immature life cycle stages (larvae, nymphs) were not encountered frequently regardless of species. The absence of certain tick species or life cycle stages is probably the result of the limited host range in this surveillance. A more encompassing array of host types and sizes would increase the ability to detect these other species and stages.

The discovery of so many *I. scapularis* on dogs that had not left the province is noteworthy because of the role *I. scapularis* occupies in the transmission of tick-borne pathogens, especially *B. burgdorferi* (Lyme disease) and *A. phagocytophilum* (HGA). This species is the major vector of tick-borne

disease on the east coast of North America (26, 35), and was reported previously in Alberta (10, 11). The report of an *I. scapularis* in Alberta by Scott (11) refers to a tick recovered in May 1998, nine years before our tick surveillance encountered its first *B. burgdorferi*-positive adult. By the end of 2010, 22 positive ticks had been recovered and *I. scapularis* comprised 10.9% of the ticks recovered from dogs that had not left Alberta. Whether the *I. scapularis* population was simply undetected previously or is in fact a new occurrence in Alberta is unknown.

Many dogs involved in this study travelled considerable distances, across North America and even across oceans. The surveillance showed that dogs can and do act as vectors for ticks, carrying ticks from questing locations back home. With nearly half the dogs that participated in the study travelling out of province, the likelihood of a dog acquiring a certain tick species must not be based solely on the local fauna. The results of this surveillance indicate that dogs, in addition to birds, are a potential route for dispersal of tick species.

2.4.3. Passive surveillance involving veterinarians

This passive surveillance program demonstrated the value of partnering with a network of veterinarians. Compared to more historical methods of tick collection, having a passive surveillance program available to veterinarians greatly increased the efficiency of recovery. The existence of the surveillance itself raised awareness of ticks on dogs amongst veterinarians, staff, clients and the public, which is desirable goal. There is a risk that increased awareness of the surveillance and its' findings may bias future submissions. This may be because veterinarians start searching more diligently for ticks

on all clients presented or become more selective and submit only *Ixodes* spp. Veterinary pharmaceutical companies may increase their promotion of anti-tick treatments based on my findings. Lyme disease and ticks may become a greater concern among the public. There are many possible unintentional consequences of releasing these findings. But overall it is better to have this information publically available to allow individuals and institutions to make informed decisions about tick safety.

Veterinary examinations proved to be important in the detection of ticks. Over 30% of ticks would have gone unnoticed if the animal had not been examined by a veterinarian. Veterinary exams for ticks played a larger role in the discovery of ticks on hosts less than one year old. This may be due to animals of this age being examined more frequently compared to older animals. The examination for ticks when a dog was presented for a vaccination was the largest cause of finding ticks on younger hosts. Discovery by a groomer was also important but applied more evenly across all ages. Veterinary clinics were not directed to submit hard ticks only, so the small number of soft tick species must reflect species noticed. The small number of immature life cycle stages recovered also reflects what was noticed on the dog. The immature stages are much smaller in size and do not engorge to the extent of adult females, so it is understandable that even a close inspection by a veterinarian could miss these smaller ticks. The results do not indicate that dogs are more likely to have ticks than other potential host types. No information was received concerning what proportion of dogs interacting with a veterinary clinic did or did not have ticks. Only a subsample of all dogs in Alberta was involved, no information about the overall dog population in Alberta was gathered, and results only

indicate which species of tick were most likely to be found on a dog that had ticks, and interacted with a veterinary clinic that was participating in the surveillance program.

Using companion animals, especially dogs, as a sentinel species for ticks is not a novel approach (18, 36-41), although many of these previous studies focused on serological testing of dogs for evidence of exposure to a tick-borne pathogen. Many of these programs aim to use dogs as an early warning, sentinel system for potential threats to the health of the human population. Such a warning would allow time to implement tick control measures, public education and other disease prevention and mitigation strategies. Our surveillance differed in several ways. There was no measure of pathogen exposure in dogs and ticks recovered from humans were not accepted. Any testing done by the veterinary clinic on the host animals was not reported to the surveillance. Some reasons for presentation to a veterinary clinic, such as mobility issues, lethargy and lameness (2.7% of hosts) have been associated with symptoms of tick-related illness or tick paralysis. Our data cannot verify that any of these symptoms were attributable to *B. burgdorferi*-positive ticks. Finally, there were many participating clinics (199) and these were spread over a large geographic area, which was not common among the other surveys. The results of this surveillance indicate that more research is needed in Alberta to gain a better understanding of the ticks and pathogens they vector.

2.5. References

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Table 2.1 The species, life cycle stage, engorgement and attachment status, and host animals for ticks collected in a 3.5-year passive surveillance program of companion animals in Alberta, Canada[1].

Tick species	Tick life cycle stage						Tick Engorged					Tick Attached?				Host tick(s) recovered from							
	Female	Male	Nymph	Larva	Unknown	Total	Yes	No	Partially	Unknown	Total	Yes	No	Unknown	Total	Dog	Cat	Horse	Rabbit	Cow	Weasel	Unknown	Total
Amblyomma americanum	13	1				14	8		1	5	14	8		6	14	9	1						10
Amblyomma maculatum	1					1				1	1			1	1	1							1
Dermacentor albipictus	76	55	39	2		172	24	26		122	172	28	3	141	172	55	5	14		1			75
Dermacentor andersoni	66	17	1			84	30	13	2	39	84	26	2	56	84	71	1	1				1	74
Dermacentor occidentalis	1		5			6				6	6			6	6	1		1					2
Dermacentor spp.	2					2				2	2			2	2	2							2
Dermacentor variabilis	337	103				440	149	93	34	164	440	191	10	239	440	296						1	297
Family Argasidae					5	5				5	5			5	5	1							1
Haemaphysalis leporispalustris	13	5	1			19	10	6		3	19	11	5	3	19	6	1		6				13
Haemaphysalis spp.	1		1			2	1			1	2	1		1	2	1			1				2
Ixodes cookei	5					5				5	5			5	5	4	1						5
Ixodes jellisoni	3					3				3	3			3	3	3							3
Ixodes kingi	150	16				166	92	5	1	68	166	80	3	83	166	109	1				1		111
Ixodes ochotonae	3					3	2		1	0	3	3		0	3	2	1						3
Ixodes pacificus	7					7	3			4	7	1		6	7	7							7
Ixodes scapularis	62	1				63	39	2	1	21	63	38	1	24	63	60	1						61
Ixodes spp.	51		1			52	13	2	4	33	52	16	1	35	52	49	3						52
Ixodes woodi	3					3	3			0	3	3		0	3	3							3
Otobius megnini			10			10	6		4	0	10	10		0	10	1	1						2
Rhipicephalus sanguineus	66	43	5			114	26	53	8	27	114	68	4	42	114	70							70
Rhipicephalus spp.	2					2				2	2			2	2	2							2
Unable to identify	10	1	1	4	6	22	11	2	3	6	22	10	4	8	22	16	1	1					18
Overall	872	242	64	6	11	1195	417	202	59	517	1195	494	33	668	1195	769	17	17	7	1	1	2	814

[1] Ticks were collected from hosts by veterinarians practicing in Alberta, Canada between July 1, 2007 and December 31, 2010. Ticks were identified to species using dichotomous keys (21, 22, 23). If a tick was too damaged for species identification then a genus or family identification was made. Tick engorgement was based on the size of the tick relative to its size when not fed.

Table 2.2 Comparison of tick species encountered on a host by host travel history in the preceding two months[1].

	Host stayed in Alberta n	Host left Alberta n	Host travel history unknown n	Total n	Odds Ratio (95% Confidence interval)
By genus					
Ixodes	178	47	20	245	0.19 (0.13,0.27)
Dermacentor	175	242	33	450	2.74 (2.03, 3.70)
Rhipicephalus	17	50	5	72	3.67 (2.09, 6.45)
Haemaphysalis	9	1	5	15	0.12 (0,0.75)
Amblyomma	4	7	0	11	1.98 (0.61, 6.38)
Not identified	11	5	3	19	0.50 (0.18, 1.40)
By species					
Ixodes kingi	86	13	12	111	0.14 (0.08, 0.25)
Dermacentor variabilis	67	215	15	297	7.63 (5.44,10.70)
Dermacentor albipictus	61	4	10	75	0.06 (0.02, 0.17)
Dermacentor andersoni	45	21	8	74	0.49 (0.29, 0.84)
Ixodes scapularis	43	14	4	61	0.34 (0.18, 0.62)
Ixodes spp.	39	11	2	52	0.29 (0.15, 0.58)
Rhipicephalus sanguineus	17	48	5	70	3.50 (1.98, 6.17)
Unknown	10	5	3	18	0.55 (0.20, 1.56)
Other tick species	27	22	7	56	0.91 (0.51, 1.61)
All tick species	395	353	66	814	
By host type					
Dog	363	349	57	769	
Cat	14	2	1	17	
Horse	14	2	1	17	
Rabbit	3		4	7	
Unknown			2	2	
Cow	1			1	
Weasel			1	1	
All hosts	395	353	66	814	

[1] The odds ratio (OR) was calculated per species (or genus) to compare how likely that species of tick was to be identified on a host that left Alberta versus a host that had not left Alberta. The OR and corresponding confidence intervals were calculated using the STATA (version 10) software. The formula used to calculate the OR was: OR= (A*D) / (C*B), where A=# submissions of species of interest from hosts that had left Alberta, B=# submissions of species of interest from hosts that did not leave Alberta, C= [(total # submissions (all species) from hosts that had left Alberta)-(A)], D= [(total # submissions (all species) from hosts that did not leave Alberta)-(B)].

Table 2.3 Host travel destinations outside of Alberta by species of tick recovered[1].

Tick species	Canada							USA												Other Nations				Multiple locations	Unknown	Total
	British Columbia	Manitoba	New Brunswick	Nova Scotia	Ontario	Prince Edward Island	Saskatchewan	Alabama	Arizona	Arkansas	California	Idaho	Indiana	Montana	State not specified	Oregon	Texas	Washington	Wisconsin	Costa Rica	Dominican Republic	Mexico	Turkey			
Amblyomma americanum										1				1	3							1		1		7
Dermacentor albipictus	3						1																			4
Dermacentor andersoni	11						1							2	3							1		3		21
Dermacentor occidentalis																								1		1
Dermacentor spp.							1																			1
Dermacentor variabilis	6	37	1	1	15		102				1	1	1	3	13			1	1		1	28	1	31	1	215
Haemaphysalis leporispalustris														1	1											1
Ixodes cookei	1																									1
Ixodes jellisoni	1																									1
Ixodes kingi	4	1			1		1							1	1									4		13
Ixodes ochotonae																								1		1
Ixodes pacificus	2								1															1		4
Ixodes scapularis	2				4	1	3	1	1	1							1									14
Ixodes spp.	2	1			1		2				1				1	1								2		11
Ixodes woodi	2																									2
Otobius megnini																								1		1
Rhipicephalus sanguineus	1									1	1				6					3	1	28	1	6		48
Rhipicephalus spp.	1																					1				2
Unable to identify	1							1							1							1		1		5
Total	37	39	1	1	21	1	111	2	2	3	3	1	1	7	28	1	1	1	1	3	1	33	1	52	1	353

[1] Travel location was recorded based on province or state (Canada or United States of America) or by Nation (e.g. Mexico, Turkey). Hosts were categorized as visiting multiple locations only if the destinations were named specifically.

Table 2.4 Reasons tick hosts were presented to veterinary clinics.

Reason for presentation of tick host veterinary clinic	Host travel history			Overall
	Left Alberta in last 2 months	Did not leave Alberta in last 2 months	Unknown	
Tick was primary reason				
Host present	154	160	24	338
Host absent	45	61	6	112
Total	199	221	30	450
Tick was coincidental finding				
Vaccinations	37	29	8	74
General exam	42	19	5	66
Lump exam	16	26	3	45
Groomer	10	15	5	30
Neuter/Spay	6	8	3	17
Lethargy	2	6		8
Mobility issues	3	5		8
Other	2	3	2	7
Dental	2	4		6
Lameness	3	3		6
Porcupine quills		4	1	5
Blood work	2	1		3
Eye exam		2	1	3
Surgery	1	2		3
Declaw	1			1
Total	127	127	28	282
Not Stated				
Total	27	47	8	82
Overall	353	395	66	814

Host absent refers to when the pet owner removed the tick elsewhere and brought only the tick into the veterinary clinic. A coincidental finding was when the host interacted for a different reason and a tick was found un-expectantly.

Table 2.5 Attributes of ticks tested for *Borrelia burgdorferi.*

	Borrelia burgdorferi test result			
	Positive	Negative	Not tested	Overall
Tick species				
Ixodes scapularis	15	39	9	63
Ixodes spp.	5	20	27	52
Ixodes cookei	1	2	2	5
Ixodes jellisoni	1	1	1	3
Ixodes kingi		18	148	166
Ixodes pacificus		4	3	7
Ixodes woodi		2	1	3
Other			896	896
Total	22	86	1087	1195
Out of Alberta in previous two months?				
No	16	57	322	395
Yes	4	23	326	353
Unknown	2	5	59	66
Total	22	85	707	814
Year				
2007	1	10	96	107
2008	6	31	293	330
2009	2	14	233	249
2010	13	31	465	509
Total	22	86	1087	1195
Average number of days from tick collection to arrival at laboratory	4	4	6	6
Host type				
Dog	21	80	668	769
Cat	1	5	11	17
Other hosts			28	28
Total	22	85	707	814
Life cycle stage				
Female	22	86	764	872
Male			242	242
Nymph			64	64
Larva			6	6
Not identified			11	11
Total	22	86	1087	1195
Tick Attached				
No		1	32	33
Unknown	9	49	610	668
Yes	13	36	445	494
Total	22	86	1087	1195
Tick Engorged				
No	1	2	199	202
Partially		4	55	59
Unknown	7	46	464	517
Yes	14	34	369	417
Total	22	86	1087	1195

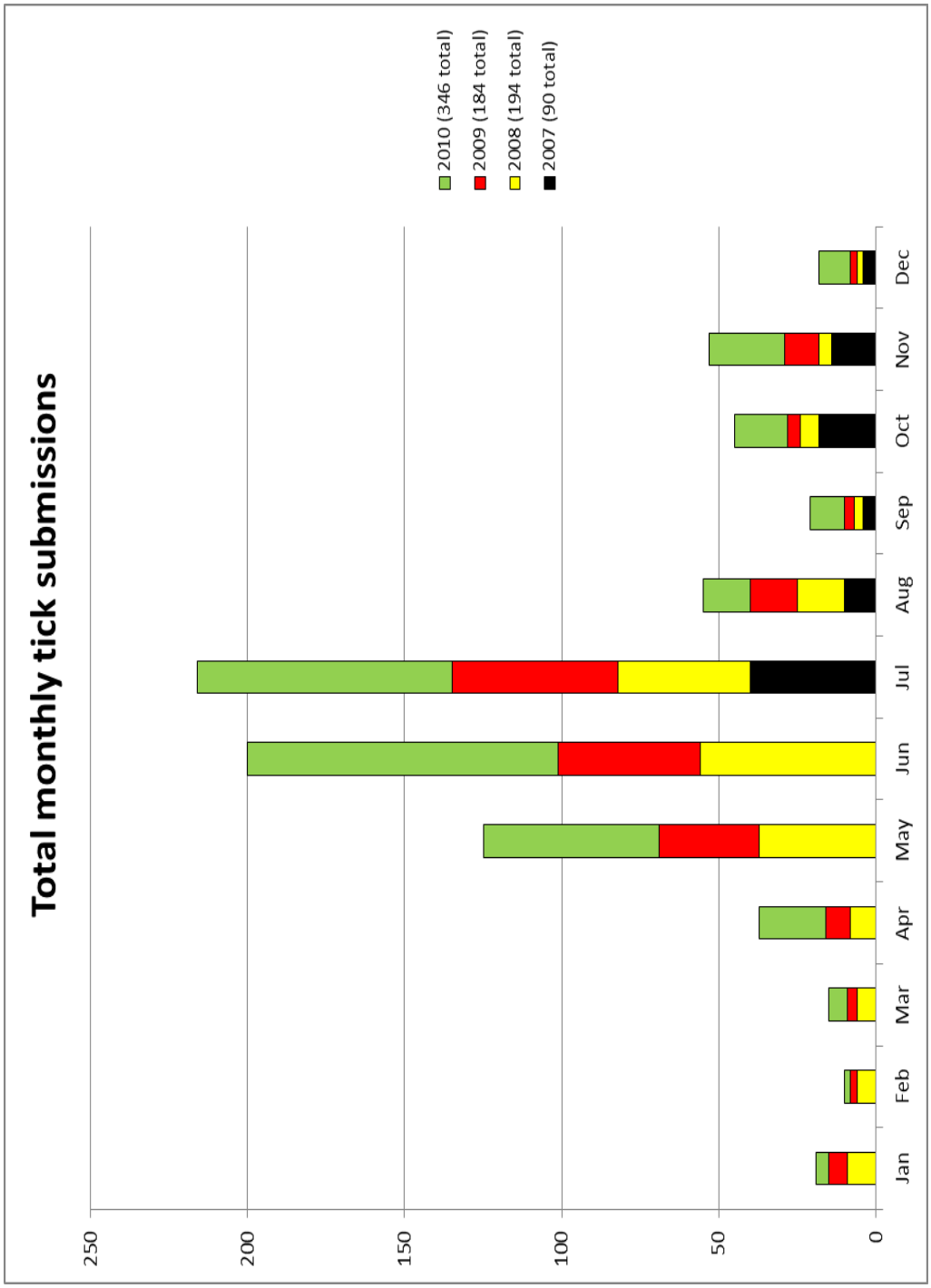

Figure 2.1 Submissions to the tick surveillance program by month over 3.5 years.

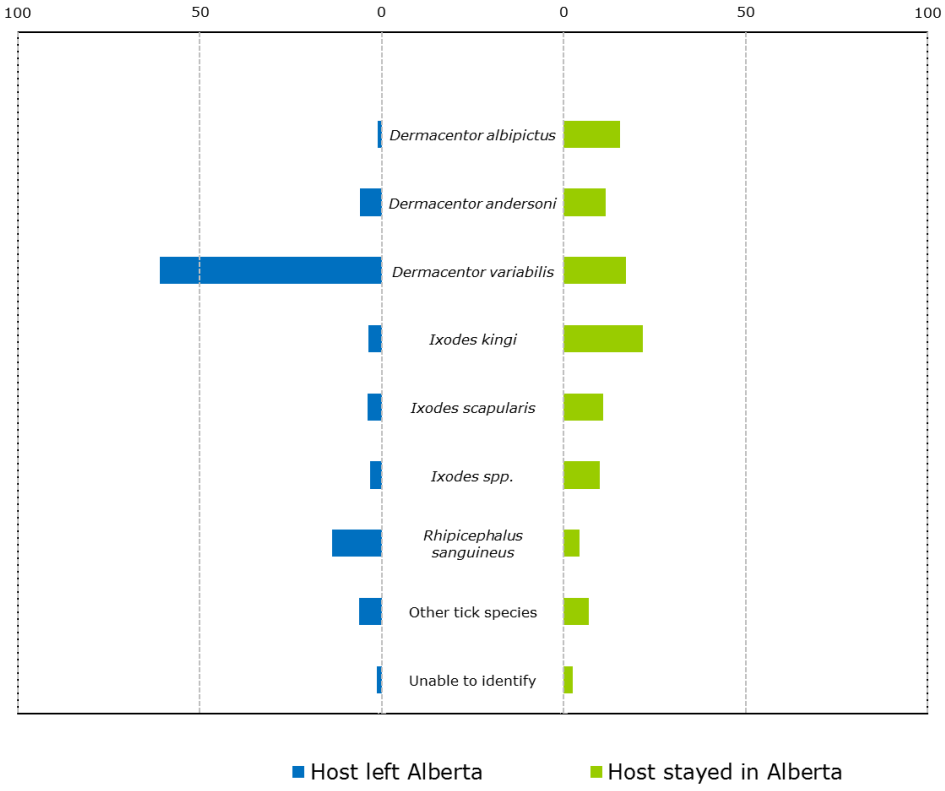

Figure 2.2 The proportion of all submissions represented by the most common species of tick received compared between ticks from hosts that had left Alberta in the last two months and ticks from hosts that stayed in Alberta.

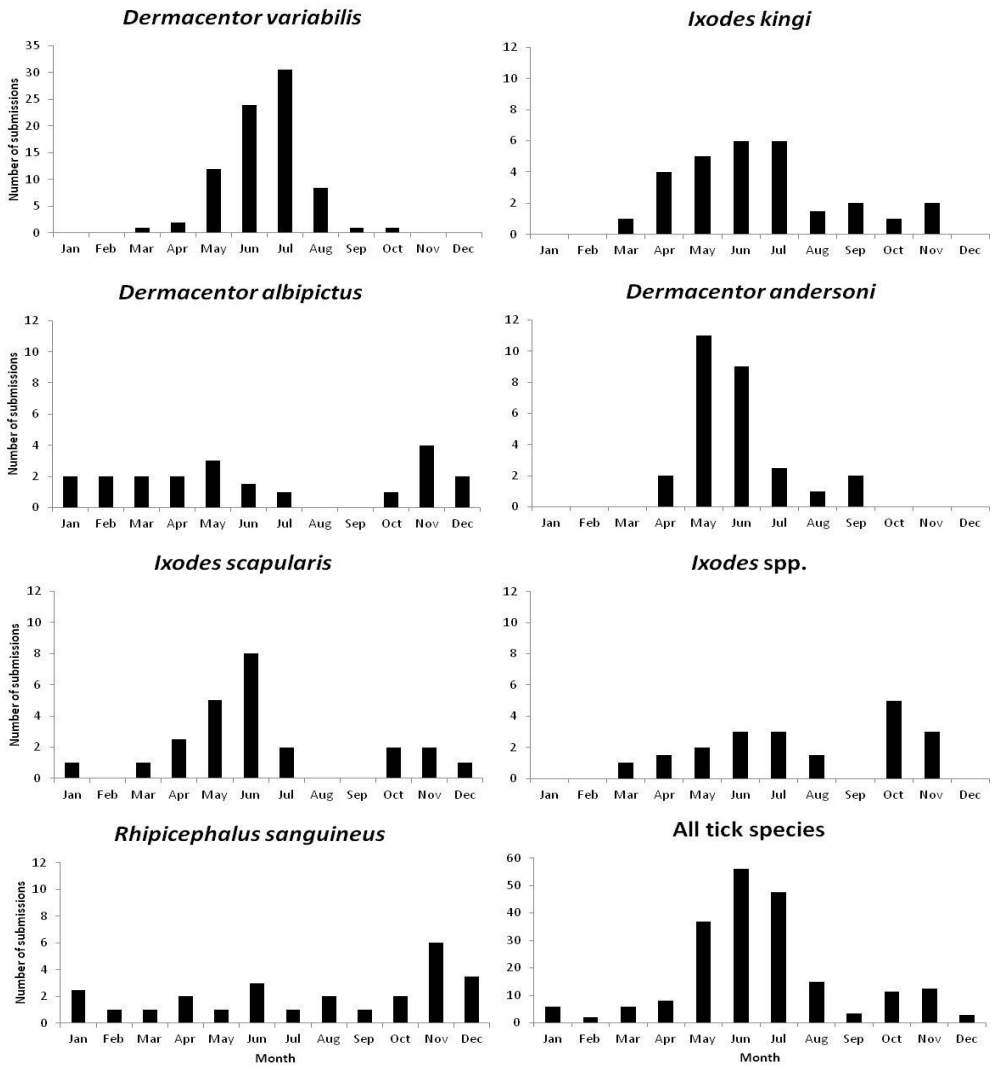

Figure 2.3 The number of submissions per month for the seven most common species received from dog hosts that did not leave Alberta and the median number per month for all tick species from three and half years of surveillance.

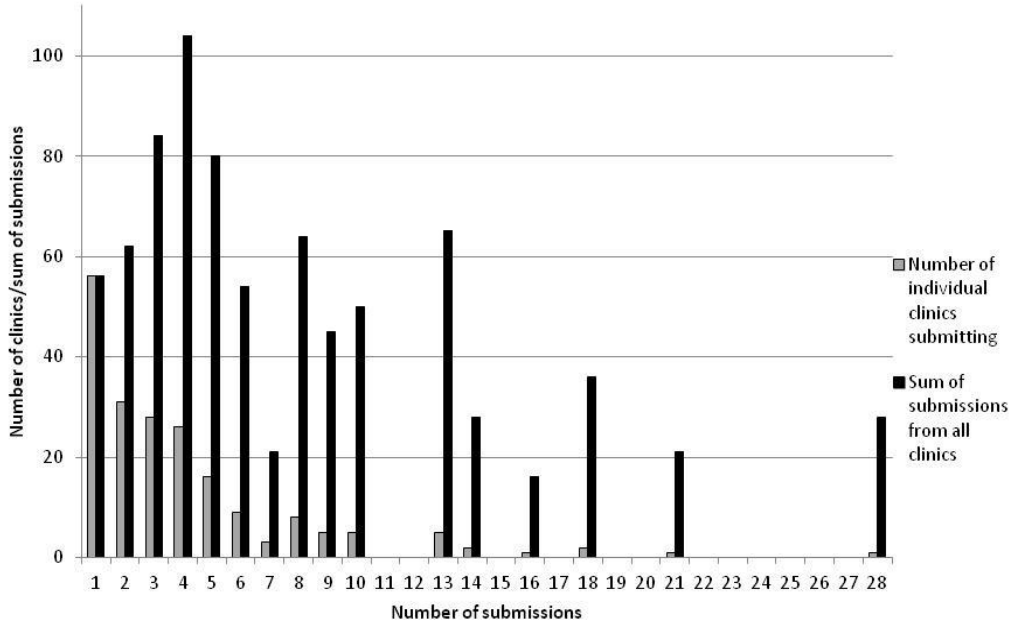

Figure 2.4 Levels of veterinary clinic participation in surveillance for ticks over 3.5 years. Veterinary clinics were grouped by the total number of submissions each clinic made to the surveillance over the course of three and a half years. The sum of submissions by all the clinics in each group was then determined.

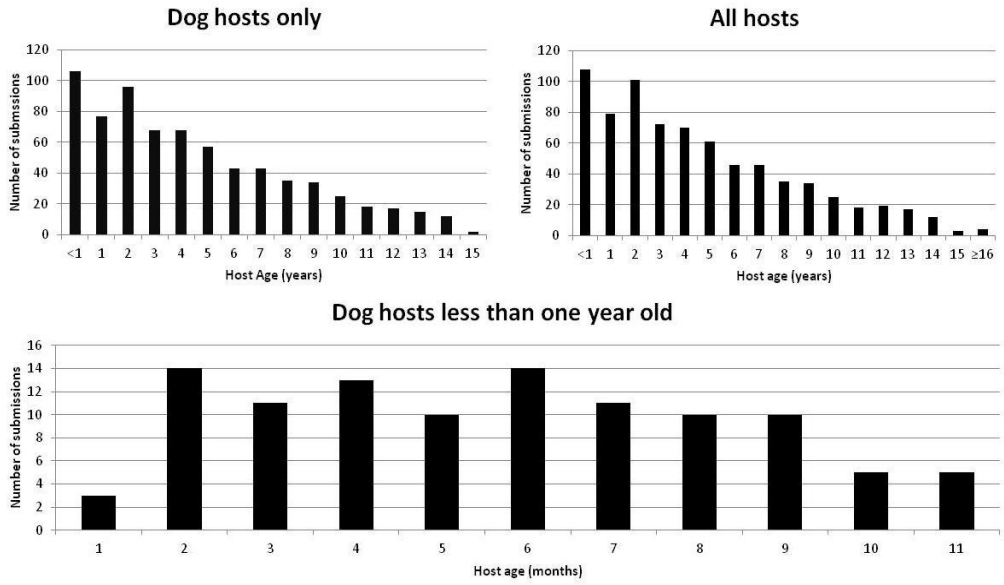

Figure 2.5 Comparison of tick submissions from hosts of different ages. Host ages were grouped by year. All hosts n=750. Dog hosts only n=716. Dogs less than one year n=106. Other host types less than one year comprised one horse and one cat. There were 64 hosts with unknown ages.

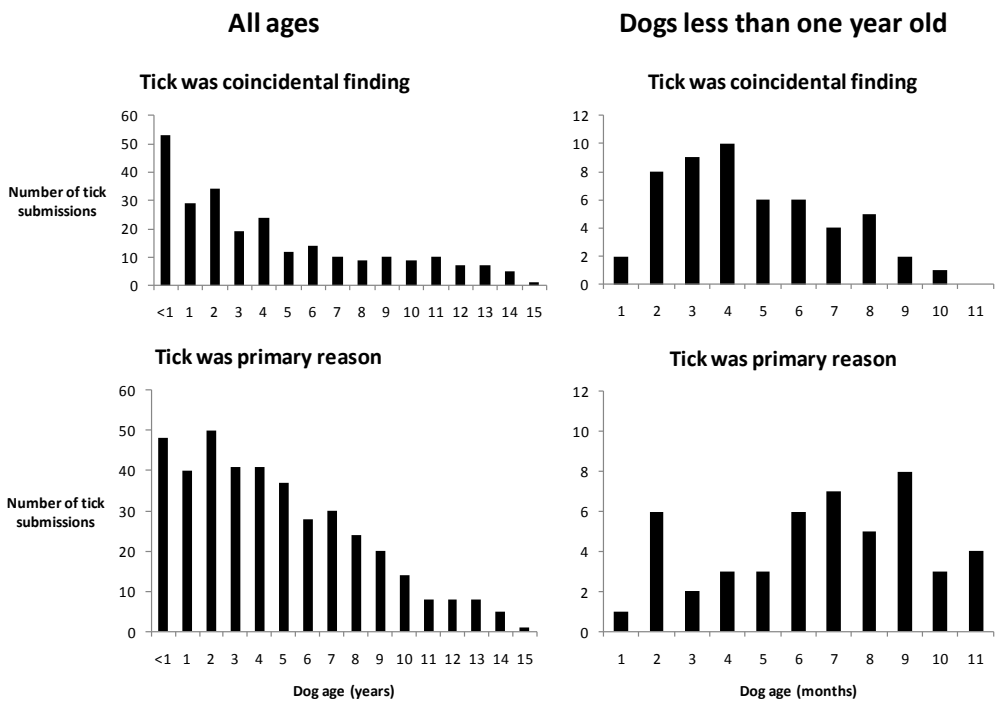

Figure 2.6 Number of tick submissions by dog host age and whether or not a tick was the primary reason for dog presentation to a veterinary clinic. ”Tick was primary reason” refers to those dogs that interacted with a veterinary clinic because a tick was observed on the dog beforehand. “Tick was coincidental finding” refers to all dogs from which a tick was recovered when the dog was at the clinic for some other reason.

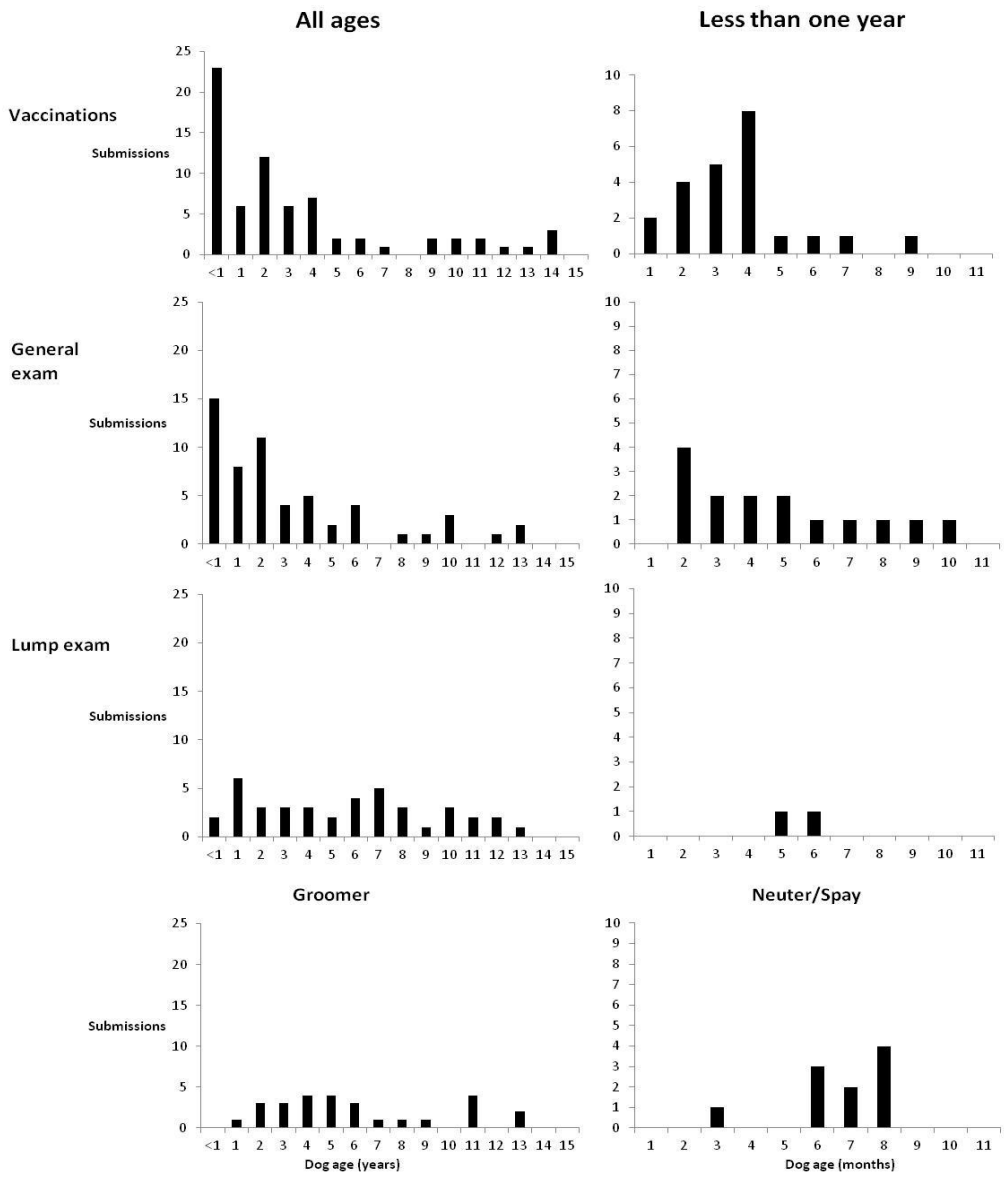

Figure 2.7 The most common reasons for the coincidental discovery of a tick by dog host age. There were no ticks submitted from dogs less than one year of age in the 'Groomer' category and only five dogs in the 'Neuter/Spay' category that were older than 1 year from which ticks were submitted (data not shown).

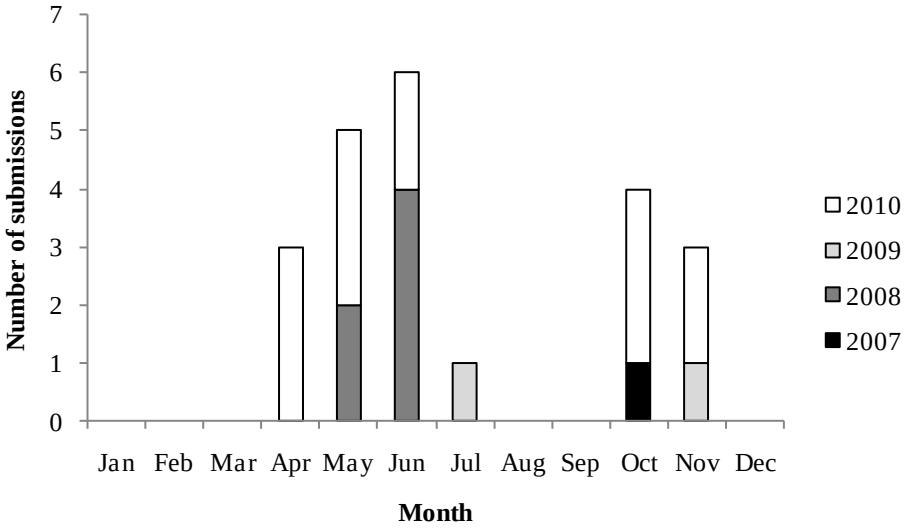

Figure 2.8 Monthly submissions of ticks that tested positive for the presence of *Borrelia burgdorferi*.

Chapter 3. The geographical distributions of the five most common tick species found in Alberta.

3.1. Introduction

In Chapter 2, I detailed the species composition of ticks found during the tick surveillance program. In this chapter, a key focus is to determine the geographical distributions of the most common tick species found Alberta. The range of a tick species is important knowledge as some species may act as vectors of pathogens. Knowing which vectors are present in an area can then aid in forecasting which pathogens co-occur in the same area. This allows resources in veterinary medicine and public health to be directed more efficiently to higher risk areas. Field studies searching for tick-borne pathogens can then focus limited resources and personnel on those areas more likely to give useful results.

Estimations of the ranges of different tick species in Alberta has been undertaken previously (1-3). However, the last province-wide determination of tick distributions was completed nearly four decades ago by Wilkinson (3) and, as I will show, the distribution and species composition of ticks now present in Alberta has changed. There has also been evidence presented (4,5) that the distribution of some species may be changing as the climate changes. An updated understanding of the distributions of tick species in Alberta is needed.

Methods now exist for modeling the species distributions that were not frequently used when earlier distributions in Alberta were determined. The Maxent method (6-8) developed by Phillips is capable of generating a species

distribution map over geographic space using presence only records in combination with environmental variables. The method is capable of using relatively small sample sizes (<100) and has parameters that can be adjusted for sampling bias, both of which are limitations of my dataset.

In this chapter I use the Maxent technology to generate updated distributions for five tick species along with binary maps of these distributions for use in future field studies. I show that the mountain ranges are not the main area of tick activity as is popularly envisioned. I map the environmental variables used in modeling and discuss which variables were the most important for the distribution of each species. I demonstrate that the north to south temperature gradient and the arid conditions in the southeast of Alberta are major factors determining species distribution patterns. Finally, I provide the first estimation of the range of *I. scapularis* in Alberta.

3.2. Materials and Methods

Details on the tick surveillance program are described in Chapter 2. The analysis in this chapter was performed using the address given for the dog host as a point location for the tick species recovered from that host. Host animals that had been out of Alberta in the two months preceding tick recovery were excluded from this analysis. If an address was not provided and the host lived in the same city or town as the submitting veterinary clinic, the address of the veterinary clinic was used instead. If multiple ticks of the same species were recovered from a dog host, all of those ticks would count as only one occurrence of that species at that point. If a host had more than one species on it, then one single occurrence of each tick species

was recorded at that point. The given addresses for the host dogs were entered into Google Earth to obtain latitude and longitude point values in decimal degrees. Provincial base maps of Alberta including borders, municipalities, parks, rivers, lakes and military bases were provided as shapefiles by Brett Oliver-Lyons of Alberta Agriculture and Rural Development. Table 3.1 lists the number of samples available for all species reported in Chapter 2.

Environmental variables used in modeling were downloaded as raster layers from the WorlClim.org website. These layers were 16 bioclimatic values generated by Hijmans et al. (9) from monthly temperature and precipitation surfaces and are listed in Table 3.2. The monthly temperature and precipitation surfaces used to calculate the bioclimatic layers were generated from average monthly climate readings from the period of 1950 to 2000 from a large number of weather stations around the planet. These climate readings were then interpolated (see Hijmans et al. 2005, (9)) to generate high resolution (approximately 1 km^2) global layers. For more information about the calculation of the 16 bioclimatic variables and the creation of the temperature and precipitation surfaces, see (9) and www.worldclim.org . The creators of these layers multiplied temperature values by 10 to decrease the storage size of the layers. For example, a temperature range of -1.2^oC to +5.8^oC appears as -12^oC to +58^oC. I used an Alberta provincial border polygon shape file in ArcGIS 10 to select the area of the environmental layers covered by the province and extract just the data relevant to Alberta. Point maps of tick species were generated using ArcGIS 10. The 'raster' package in R was used to generate the summary statistics for the bioclimatic variables and generate all the plots of the bioclimatic variables and the

Maxent models. A map of Alberta's natural regions and subregions (10) was used for comparison with the generated Maxent models to identify the type of natural region that may be associated with the species modeled.

The Maxent software (6) version 3.3.3k was downloaded from http://www.cs.princeton.edu/~schapire/maxent/ . A brief description of how Maxent works will be given; more detailed explanations concerning the Maxent software itself, refinements of the software and the machine learning theory behind Maxent are available elsewhere (6-8,11-14). Maxent models species distributions in geographic space using environmental variables (temperature, precipitation, etc.) in combination with presence only records. The strength of the Maxent method is that it works well on small sample sizes and does not require absence records. This makes it well suited to use with sample records not collected using more rigorous sampling designs (e.g., a randomly selected, statistically significant number of quadrats representative of the larger sampling area), such as museum collections and the surveillance data such as I have for the ticks. Maxent is a machine learning method that works through multiple iterations to create a model (an exponential formula in this case) that best fits (predicts) the sample sites according to the features (environmental data) provided, with the restriction that the features must match their empirical average. In the words of the creators of the software, Maxent attempts:

> "...to estimate a target probability distribution by finding the probability distribution of maximum entropy (i.e., that is most spread out, or closest to uniform), subject to a set of constraints that represent our incomplete information about the target distribution." (6) .

The geographic space across which the features and samples are located is divided into cells (squares of equal area), with each cell having a location in geographic space along with a single value of each feature per cell. Cells in which the species of interest is present are identified as sample points and all other cells are considered background points. Maxent randomly extracts a subset of all cells without samples for use as background points in the analysis. The random selection of background points results in a different set of background points every time the model is run. This leads to slightly different models with each run even when using the same samples and feature layers. Doing multiple runs (replicates) of Maxent and then reporting on the average results of the replicates is one way to account for this variation.

Maxent produces several outputs, the main one being a probability distribution across the area on which the model was calculated. This logistic model assigns a value between zero and one to each cell, with values closer to one indicating that a cell is more likely to contain conditions for the species of interest. Maxent reports on the contribution and importance of individual features, the overall performance of the model, features of the model itself, model predictions at the background points and the calculation of various threshold values. Maxent also calculates the area under (AUC) the receiver operator curve (ROC). The AUC statistic is a measure of how different the model is at accurately predicting the sample points compared to a random prediction. The AUC value was the main statistic I used to evaluate model performance, with values closer to one considered to be better than those closer to 0.5 (a random model). To generate the ROC plot, Maxent

creates a plot of sensitivity (1-Omission Rate) against 1-specificity (represents the fractional predicted area / commission error).

The following changes from the Maxent default settings were made. Product and threshold features were not used. A jackknife of variable importance was carried out. The randomseed option was activated, instructing the software to randomly use a different subset of the background points per run. The background points were chosen from a pool including the sample points. Cross-validation (with either five or ten replicates) was used, which allowed the software to create an ROC plot and calculate the corresponding AUC. Cross validation is a way of dividing the sample points that allows all samples to be used to both create and test the model. The samples are divided into *n* number of roughly equal groups and the model is then created (trained) using *n*-1 of those groups. The group not included is then used to test the accuracy of the model. The process is subsequently repeated so that each group is used to test the model.

The samples used to model each species were spatially biased, concentrated in more densely populated areas of the province. Two actions were taken to compensate for this: adjusting the regularization value and inclusion of a sample bias file. Maxent can use a species-specific sampling bias layer to adjust for higher intensity of sampling in some areas relative to others. I created a bias layer for each species separately which consisted of a raster layer aligned with the environmental raster layers with a value of 100 in each cell that had a tick present and a value of 1 where a tick was absent.

Model outputs for each species were first examined to see if any of the bioclimatic variables could be dropped. Average results from five cross-

validated runs of the model were used to rank the variables according to which contributed the most individually (the AUC value with only that variable in the model) and which weakened the model the most by its absence (the AUC value without that variable in the model). The AUC value with only that variable in the model measures how strong that variable is on its own at predicting the presence of the species being modeled. The AUC value without that variable in the model measures how unique the information that variable provides is to modeling the species compared to the information given by the other variables. The variable rankings from models with and without that variable were combined and the lowest ranking variable was then dropped. The model was then re-run with the remaining bioclimatic variables.

If the AUC of the average of five replicate runs of the reduced model was larger than the previous AUC, the remaining variables were then ranked according to the new model outputs and the new lowest ranking variable was dropped. If dropping the lowest ranked variable lead to the AUC value decreasing, that variable was restored and the second lowest variable was dropped instead, and the model was rerun. This process was repeated until the AUC value was not increased by dropping any of the remaining variables.

Next, the regularization parameter was optimized. This value, also called a beta multiplier, is a value the software user sets (default=1). The software multiplies this to all regularization parameters in the model. Higher values of this give a more spread-out model. I used this to help account for the sampling bias in the dataset as a more spread-out model will be less tightly fitted to heavily sampled sites. To determine which value above the default

to use, I ran ten cross-validated runs of each species for every value of 0.25 above 1.00 (1.25, 1.50, 1.75, 2.00....5.00). I then examined the average output of the model for each regularization setting and ranked according to which setting had the highest AUC value with the smallest standard deviation. The top-ranked value was then the one used for the final model.

The binary maps were generated using the Maxent-generated grid of the average logistic output of 10 cross-validated runs of the model along with a threshold value. The threshold value is used to convert the logistic output of Maxent (which will be a value between 0 and 1) to a binary (1 or 0) present/absent prediction, with the threshold value being the point at which an absent prediction becomes a present prediction. Maxent calculates and includes in its output several commonly used threshold values. The determination of which threshold value to use is crucial. As my goal is to predict a range as accurately as possible, both sensitivity and specificity are important. Liu et al. (15) examined several approaches for determining a threshold value and found five methods that performed better than others. Among those that performed well in the work of Liu et al. (15) and what is calculated by Maxent, I decided to use the 'Equal test sensitivity and specificity' threshold value. I chose this over the 'Maximum test sensitivity plus specificity' since Liu et al. (15) found that approach to be more sensitive to the prevalence of data used to construct the model. The 'raster' package in R was used to convert the average logistic output file from Maxent to the final binary grid.

3.3. Results

3.3.1. Distribution of tick species and veterinary clinics

Ticks were submitted to the surveillance program after being recovered from hosts residing in all parts of Alberta (Figure 3.1). As the surveillance targeted companion animals, the host locations reflect the distribution of the human population in Alberta. Very few ticks were submitted from the northern third of the province, reflecting the smaller human population in that area compared to the central and southern regions. The major cities (Calgary and Edmonton) both represent metropolitan areas of over 1 million humans (two thirds of the human population in Alberta) and stand out as large groupings of tick submissions (Figure 3.2). In Edmonton there appeared to be more ticks from locations closer to the river valley than other parts of the city. No ticks were submitted from within the borders of any of the national or provincial parks or military bases. The locations of veterinary clinics that participated in the surveillance are shown in Figure 3.3. The distribution of clinics also reflects the concentration of the human population.

The distribution of ticks that tested positive for *B. burgdorferi* is plotted in Figure 3.4. The majority of these ticks were from hosts located in the major cities (Figure 3.5). Comparing the major cities, there were four positive ticks from the Calgary area and 14 from the Edmonton area. The remaining four positive ticks were from the central and northern regions of the province. Figure 3.6 shows how the *B. burgdorferi*-positive ticks are distributed among the cities and municipal districts of Alberta. No adjustment for host travel within Alberta was made due to poor response on the history sheets accompanying the ticks. In general, more positive ticks appeared in samples from central Alberta.

As stated in Chapter 2, 16 tick species were identified but seven species comprised 90.9% of all submissions. Table 3.1 shows that only five of the 16 species were sufficiently abundant to be included in modeling. Of the seven species that were the focus of Chapter 2, two were not modeled. *Rhipicephalus sanguineus* had only 17 specimens recovered from hosts that had not left Alberta and was therefore not mapped. As the ticks grouped as *Ixodes* spp. in Chapter 2 could be not be assigned to distinct species, this group was not mapped either. Figures 3.7 to 3.12 show the distribution in the province of where the five modeled species were recovered. The five species all appear to be clustered around larger human population centres, indicating a sampling bias in the data. *Dermacentor variabilis* was the most widely distributed of the five whereas *D. albipictus* was recovered primarily toward the central region of the province. *Dermacentor andersoni* and *I. kingi* were found more frequently in the southern third of the province. *Ixodes scapularis* was recovered most often in the central part of the province around and east of Edmonton, except for a notable cluster in Calgary. Overall, the distribution of recovery locations seemed to indicate that the different species do have different distribution patterns within Alberta.

3.3.2. Environmental variables

The 16 environmental variables used in Maxent to model the tick distributions are summarized in Table 3.2 in general groupings according to whether the variable represents an annual trend, a quarterly trend or an extreme value. I also graphed each layer (Figures 3.12 to 3.15) to visualize how the range of each variable is distributed over the province. There is sharp north-south distribution of bio06 (minimum temperature of the coldest

month) (Figure 3.15). In almost all layers, the Rocky Mountains are clearly distinguished from trends in the rest of the province. The portion of the province covered by prairie (SE corner) stands out as warm and dry relative to other parts of the province. The central third of the province has temperature values closer to the lower third/prairie area but with larger precipitation values.

3.3.3. Maxent models

The main characteristics of the Maxent models generated are shown in Table 3.3. Using the AUC statistic as the main indicator of model performance, the five models range from an AUC = 0.8707(*D. albipictus*) to an AUC = 0.9555 (*D. variabilis*). All models were able to predict distributions above a random prediction (AUC = 0.5). I was able to increase the regularization multiplier for all five species from the default value of one. The number of variables used ranged from 11 (five dropped) to 16 (none dropped). Table 3.4 lists which variables were used for each species model and the relative ranking of importance of a variable to a species model. Across all models, three temperature-related variables were the most important: the mean annual temperature (bio01), the minimum temperature of the coldest month (bio06) and the mean temperature of the coldest quarter (bio11). All three variables exhibited a north to south, colder to warmer gradient (Figures 3.12, 3.13, 3.15). Only seven variables were common to all five models, with the precipitation of the driest month (bio14) being the least used of the 16 variables. In general, the Maxent models created were better than a random prediction of each species distribution and relied mainly on temperature values to make the predictions.

The models for each of the five species are shown in Figures 3.16 to 3.20. Each model was run 10 times per species and the average logistic output of the 10 runs is shown. For illustration I assigned a color gradient to the range of values present over the area of each model. For all species modeled, the Rocky Mountains were not included in the predicted ranges, with the exception of a few valleys.

Of the five species modeled, *Dermacentor albipictus* was the most widely distributed tick across the province with the main part of its distribution centred on Edmonton in the central region of the province. The range covers most of the parkland and foothills natural regions found in the province, along with the southern parts of the boreal forest. The grasslands in the southeast region of the province were not predicted as suitable for *D. albipictus*. Four of the five most important variables to predict the *D. albipictus* distribution (Table 3.4, Figures 3.12, 3.13, 3.15) were temperature variables. These variables indicate that the colder temperatures present in the north and the warmer temperatures in the southeast of the province act to restrict the distribution of *D. albipictus* to the relatively moderate temperature areas of the province.

Dermacentor andersoni had a very different distribution, focused mainly on Calgary and areas south and south east of the city. The area predicted as suitable for *D. andersoni* consists mostly of grassland natural regions with some parts of the foothills and parkland regions included. Both temperature- and precipitation-related variables were important to the distribution of *D. andersoni*, with the top five variables (Table 3.4, Figures 3.12, 3.13, 3.14,

3.15) indicating that *D. andersoni* is limited by very cold temperatures but can be found in more arid regions than *D. albipictus*.

Dermacentor variabilis was predicted to have a range that covers most of the parkland region of the province. Similar to *D. albipictus*, the range of *D. variabilis* was predicted to include the area around Edmonton and east to the Saskatchewan border and south to Calgary and the Montana border. Unlike *D. albipictus*, the range of *D. variabilis* does not extend substantially to the west or northwest of Edmonton into the boreal forest region of the province. The foothills regions were also not included in the range of *D. variabilis* and the grasslands in the southwest were also indicated as less suitable. Assessment of the variables most important to *D. variabilis* indicates that the mild conditions in the parkland areas of the province are the most appropriate for *D. variabilis*.

Ixodes kingi was predicted to occupy the extreme south of the province. The distribution of *I. kingi* is restricted almost exclusively to the grasslands area that comprises the southern portion of Alberta. The five most important variables for this species indicate that *I. kingi* is found in warmer temperatures and drier conditions than exist elsewhere in the province.

Ixodes scapularis has a range focused on the area east of Edmonton to the Saskatchewan border. Like *D. variabilis*, there is also an extension of the range running south to Calgary. The predicted range covers an area mostly identified as parkland with some of the grasslands included in the south as well as the southern edge of the boreal forest in the central area of the province. Assessment of the most important variables governing its

distribution indicates that predicted areas are not close to areas of relatively extreme temperature and precipitation values.

The logistic thresholds generated by Maxent are listed in Table 3.3 for the modeled species. These thresholds were used to convert the raw logistic outputs modeled in Figures 3.16 to 3.20 into the binary Figures 3.21 to 3.25, respectively. The binary graphs show where in the province each tick species is found, according to the Maxent models I created. These figures were generated to serve as guides to direct future field studies that seek to capture one of the five tick species. The ranges follow the same basic shapes as the raw logistic outputs. Only *I. kingi* and *D. andersoni* are located in the prairie grasslands of the southeast. No species was predicted to be present in the Rocky Mountains. Calgary and the area of parkland surrounding Highway 2 north to Red Deer are predicted to have all five tick species. Edmonton and area east to the Saskatchewan border is predicted to harbour *D. albipictus*, *D. variabilis* and *I. scapularis*. The boreal forest region that covers the northern half of Alberta is predicted to have *D. albipictus* on its southern edge and *D. variabilis* and *I. scapularis* on its southeastern edge.

3.4. Discussion

All models created have AUC values that indicate they are better able to predict the location of the modeled tick species compared to a random prediction. The distributions generated from those models represent the first province-wide assessment of tick species distributions in over four decades (1-3). The models I have presented confirm that differences exist in the geographical distributions among tick species within the province. I found these distributions to be influenced primarily by the north to south

temperature gradient and by the difference in precipitation between the southeast grassland region and the rest of the province.

Dermacentor albipictus had the largest range of the five modeled species (Figure 3.16). The range encompasses most of the parkland and portions of the foothills and boreal forest regions. The range of *D. albipictus* extends the furthest north of the species modeled and also is the range that encompasses more of the boreal forest than the other species. Previous studies estimating the range of *D. albipictus* (3,16,17) extended the range further north than the range I determined. Samuel (16) stated that a lack of samples in northern areas hampered earlier estimates of the northern extent of the range. I suspect that my data have the same limitations, as Zarnke et al. (17) found that conditions in Alaska are suitable for *D. albipictus* to complete its life cycle. The range I determined is based on a larger sample size than that used by Wilkinson (3) but I did not have samples from further north than between 54^{o} and 56^{o} latitude whereas Wilkinson was able to include two samples north of 60^{o} latitude. Consequently, although I had a larger sample size, the lack of samples from the far north of the province affected the distribution predicted by my model of *D. albipictus.* I suspect that the true northern limit of the range of *D. albipictus* lies somewhere between the northern limit I predicted and that predicted by Wilkinson. The southern extension of the range aligns with records presented by Bishopp and Trembley (18) for *D. albipictus* in Montana.

Wilkinson (3) indicated that *D. variabilis* is found in Canada to the east of Alberta but not within the province. Gregson (2) reported that the nearest records for *D. variabilis* were located on the Saskatchewan side of the border

with Manitoba. Brown and Kohls (1) did not report any *D. variabilis* in Alberta, but Bishopp and Trembley (18) reported *D. variabilis* from Montana. My results show that *D. variabilis* occurs in Alberta in many areas south of 54^{o} latitude except in the foothills and Rocky Mountains (Figure 3.18). The projection of the range directed east from Calgary is probably an artefact. As stated by Wilkinson (3), *D. variabilis* is adapted for areas with relatively high precipitation levels. This eastward extension places the range in the driest part of the province which does not seem appropriate. Also this extension mirrors a major highway leading from Calgary to the city of Medicine Hat, suggesting there is an effect of sampling bias towards human population centres.

The westward expansion of the *D. variabilis* range occurred sometime between the work of Wilkinson (3) in 1967 and the start of this surveillance in 2007. The route the tick followed into Alberta from Saskatchewan could have been westward along the southern edge of the boreal forest and parkland regions and then south through the parkland region toward Montana. Such a route would have relatively warmer temperatures than further north while avoiding the more arid grasslands to the south.

The distribution of *D. andersoni* that I determined using Maxent (Figure 3.17) aligns with the range predicted by earlier works (2,3,18,19). My results confirm these earlier estimations that *D. andersoni* is better adapted to the grasslands region than *D. variabilis*. The sampling bias along the Trans-Canada highway that I deemed present in the range of *D. variabilis* does not appear to occur in the predicted range of *D. andersoni*. There are small areas in the west of the province at the northern extent of the Rocky

Mountains where *D. andersoni* is predicted to occur. These areas are a combination of foothills, montane and subalpine regions that do not fit with the general view of the species as one that prefers grasslands; therefore I predict that these areas are not suitable for *D. andersoni*. James et al. (20) determined the range of *D. andersoni* in the United States, and found that Montana comprised the largest portion of the range of *D. andersoni* of all the U.S.A. These results align with early determinations of the range of *D. andersoni* in the US made by Bishopp and Trembley (18). The range that I determined for *D. andersoni* aligns with the northern portion of the ranges in Montana indicated by James et al. (20) and with the early prediction by Bishopp and Trembley (18).

Gregson (2,21) predicted a distribution of *I. kingi* that closely matches the range I determined. The characterisation of *I. kingi* as a grasslands species seems appropriate and fits with the range I predicted. As also noted by Salkeld et al. (22), I found that *I. kingi* can occasionally occur closer to forested regions (such as the Edmonton area) but the majority of the samples come from grasslands.

The determination of the range for *I. scapularis* in Alberta is novel. Although the tick has been recovered previously in the province, there has never been an attempt to determine the range of this species in Alberta. Both Gregson (2) and Brown and Kohls (1) did not report any locations for this species in Alberta. Bishopp and Trembley (18) only mention a few specimens recovered from Ontario. The more recent research by Ogden et al. (23) in 2005 does include a small area of suitable habitat for *I. scapularis* in southeastern Alberta at the northern limits of their range predictions, which they attribute

to ticks from migratory birds. Subsequent work by Ogden et al. in 2006 (4) predicted that large parts of Alberta would become suitable for *I. scapularis* habitation due to the effects of climate change. In 2009, Ogden et al. (5) stated that the range of *I. scapularis* is increasing in Canada from the few initial sites of establishment in Ontario. In this context, where the potential exists for establishment in more northern areas, the range I determined for *I. scapularis* is appropriate. I suspect that there may be the same sampling bias in this range as in the range I determined for *D. variabilis*. The same extension along the Trans-Canada Highway from Calgary to Medicine Hat is visible in the *I. scapularis* range as in that of *D. variabilis*. As with *D. variabilis*, *I. scapularis* is found in more humid areas than exist in southeastern of Alberta. If this range extension along the Trans-Canada Highway is ignored, *I. scapularis* would then have a range in Alberta centred mainly in the parkland regions around Edmonton and east to the Saskatchewan border with a southern extension ending around Calgary. Such a range also corresponds to the areas where most of the *B. burgdorferi*-positive ticks were recovered. Figure 3.6 does indicate that *B. burgdorferi*-positive ticks were recovered from the city of Calgary (and two municipal districts bordering it) and the city of Fort McMurray, areas far outside of this range where I suspect *B. burgdorferi*-positive ticks are located. I suspect they represent hosts that had travelled to the parkland regions around Edmonton and acquired *B. burgdorferi*-positive ticks while in that region before returning home.

The northern third of the province is not well represented in the data. This is a result of the bias in the samples towards more densely populated centres, which are all located south of 54^{o} latitude with the exception of Fort

McMurray. It could also be that there are generally fewer ticks in this region. Gregson (2) indicated that few tick specimens were recovered north of 54^{o} and my results and distribution models support such a statement. With the exception of *D. albipictus*, most of the previous work in Canada indicates that ticks are more commonly found in the south of the country. Gathering more data on ticks in northern Alberta is needed to determine if ticks truly are less common in this region.

There were several tick species that had previously been recovered (1,2) in Alberta that I did not identify among my specimens, such as *I. sculptus* and *I. spinipalpis*. This is most likely due to the host type in this surveillance being mostly dogs. A larger variety of hosts combined with rodent trapping and flagging for ticks may be needed to recover the other species recovered by Brown and Kohls (1) and Gregson (2). There is also the potential that there are other tick species in Alberta that have not yet been reported which may require a more varied sampling strategy to recover.

3.5. References

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Table 3.1 Number of points available for modeling of tick species.

	Total	Recovered from a host that had not left Alberta in the previous two months	Co-ordinates available?	Reduced to one point per grid cell
Ixodes kingi	111	86	83	82
Dermacentor variabilis	297	67	65	65
Dermacentor albipictus	75	61	58	57
Dermacentor andersoni	74	45	45	45
Ixodes scapularis	61	43	42	41
Amblyomma americanum	10	3	3	na
Amblyomma maculatum	1	1	1	na
Dermacentor occidentalis	2	1	1	na
Dermacentor spp.	2	1	1	na
Family Argasidae	1	1	1	na
Haemaphysalis leporispalustris	13	7	7	na
Haemaphysalis spp.	2	2	2	na
Ixodes cookei	5	3	3	na
Ixodes jellisoni	3	2	2	na
Ixodes ochotonae	3	2	2	na
Ixodes pacificus	7	3	3	na
Ixodes spp.	52	39	38	na
Ixodes woodi	3	0	na	na
Otobius megnini	2	1	1	na
Rhipicephalus sanguineus	70	17	17	na
Rhipicephalus spp.	2	0	na	na
Unable to identify	18	10	10	na
Totals	814	395	385	290

Table 3.2 The 16 bioclimatic variables used to model tick distributions in Maxent.

	Shorthand name	Bioclimatic variable	Unit	Mean	Min	Max	SD
Annual values	bio01	Annual mean temperature.	°C	0.30	-8.60	6.20	2.17
	bio12	Annual precipitation.	mm	453.66	270.00	836.00	78.76
	bio07	Annual temperature range (bio05-bio06).	°C	45.71	27.90	53.00	4.71
	bio02	Mean diurnal range (mean of (monthly max temp - monthly min temp)).	°C	12.22	8.20	15.30	1.03
Quarterly precipitation	bio16	Precipitation of the wettest quarter.	mm	205.85	128.00	309.00	37.45
	bio17	Precipitation of driest quarter.	mm	61.23	28.00	174.00	17.56
	bio18	Precipitation of warmest quarter.	mm	205.08	118.00	309.00	37.90
	bio19	Precipitation of coldest quarter.	mm	69.49	28.00	214.00	21.73
Quarterly temperature	bio08	Mean temperature of the wettest quarter.	°C	13.95	-7.40	18.60	2.14
	bio09	Mean temperature of the driest quarter.	°C	-6.97	-21.30	12.20	3.54
	bio10	Mean temperature of the warmest quarter.	°C	14.17	0.30	18.70	1.91
	bio11	Mean temperature of the coldest quarter.	°C	-15.35	-23.90	-5.50	4.30
Extreme values	bio05	Maximum temperature of the warmest month.	°C	22.28	7.30	28.80	2.13
	bio06	Minimum temperature of the coldest month.	°C	-23.42	-31.70	-12.00	4.42
	bio13	Precipitation of the wettest month.	mm	77.15	52.00	116.00	13.27
	bio14	Precipitation of the driest month.	mm	18.29	7.00	47.00	5.18

The bioclimatic raster layer variables summarized in this table are only for the geographic area encompassed by Alberta and were extracted from the larger global layers available at www.worldclim.org. Temperature values were converted (divided by 10) to the actual values for display in this table only. Min = Minimum, Max = Maximum, SD = Standard deviation. A quarter refers to a period of three months.

Table 3.3 Overall characteristics of generated Maxent models for five species of ticks.

	Dermacentor albipictus	*Dermacentor andersoni*	*Dermacentor variabilis*	*Ixodes kingi*	*Ixodes scapularis*
Sample points	57	45	65	82	41
Number of variables used	12	11	11	15	16
Regularization multiplier	1.25	2	1.5	1.5	2
AUC	0.8707	0.9226	0.9555	0.9515	0.9402
AUC Standard Deviation	0.0426	0.0386	0.0178	0.0148	0.0317
Logistic threshold	0.5311	0.5076	0.563	0.5223	0.5196
Most important variable to build the model	Annual Mean Temperature	Mean temperature of the coldest quarter	Minimum temperature of the coldest month	Minimum temperature of the coldest month	Minimum temperature of the coldest month
Variable with the most unique information	Annual Mean Temperature	Annual Mean Temperature	Annual precipitation	Mean temperature of the coldest quarter	Mean temperature of the wettest quarter
Variable most capable of predicting the species	Annual Mean Temperature	Mean temperature of the coldest quarter	Annual Mean Temperature	Annual Mean Temperature	Annual Mean Temperature

Values were obtained from outputs generated from running the Maxent software for each species.

Table 3.4 Relative rankings of environmental variable importance to each species model.

Variable	*Dermacentor albipictus*	*Dermacentor andersoni*	*Dermacentor variabilis*	*Ixodes kingi*	*Ixodes scapularis*
Annual mean temperature	1	1	2	9	4
Mean diurnal range (mean of (monthly max temp - monthly min temp))	9	5	na	2	15
Maximum temperature of the warmest month	4	7	8	5	9
Minimum temperature of the coldest month	3	6	7	3	2
Annual temperature range (bio05-bio06)	na	2	na	12	7
Mean temperature of the wettest quarter	6	na	9	11	1
Mean temperature of the driest quarter	7	na	na	7	16
Mean temperature of the warmest quarter	2	na	5	10	6
Mean temperature of the coldest quarter	8	8	3	1	13
Annual precipitation	10	11	1	14	5
Precipitation of the wettest month	5	4	10	8	3
Precipitation of the driest month	na	na	4	na	8
Precipitation of the wettest quarter	na	3	11	15	11
Precipitation of driest quarter	na	10	na	13	14
Precipitation of warmest quarter	11	na	na	6	10
Precipitation of coldest quarter	12	9	6	4	12

Each environmental variable per Maxent species model was ranked relative to all the other environmental variables used to create that species model. Ranks were based on the AUC value of the Maxent model if only that environmental variable was used in the model (larger AUC value ranked higher) and on the AUC value if that variable was not used in the model (smaller AUC value ranked higher). If two environmental variables were tied, the variable with the lower AUC value when that variable was excluded from the model was ranked ahead of the other. Variables not used in the model for a species are listed as 'na'.

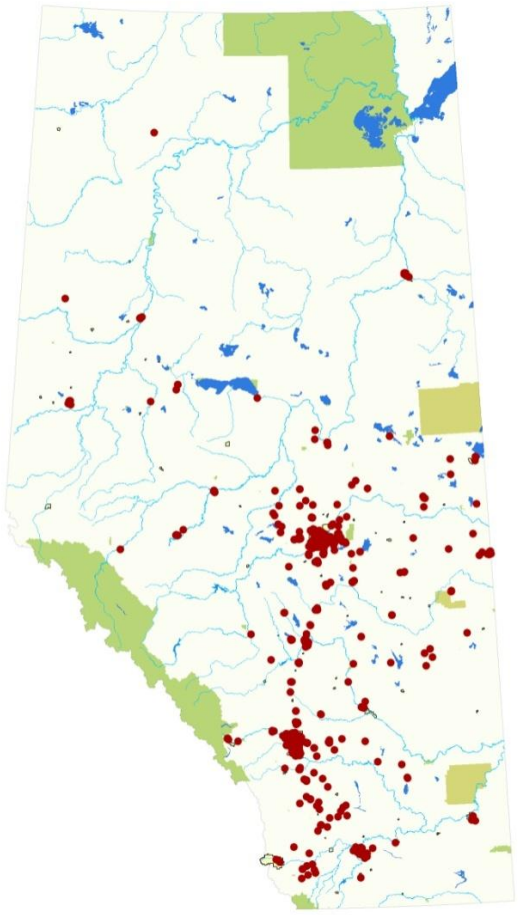

Figure 3.1 Locations in Alberta from which ticks were recovered and submitted to the surveillance program between 2007-07-01 and 2010-12-31. Red dots represent locations where ticks were acquired. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

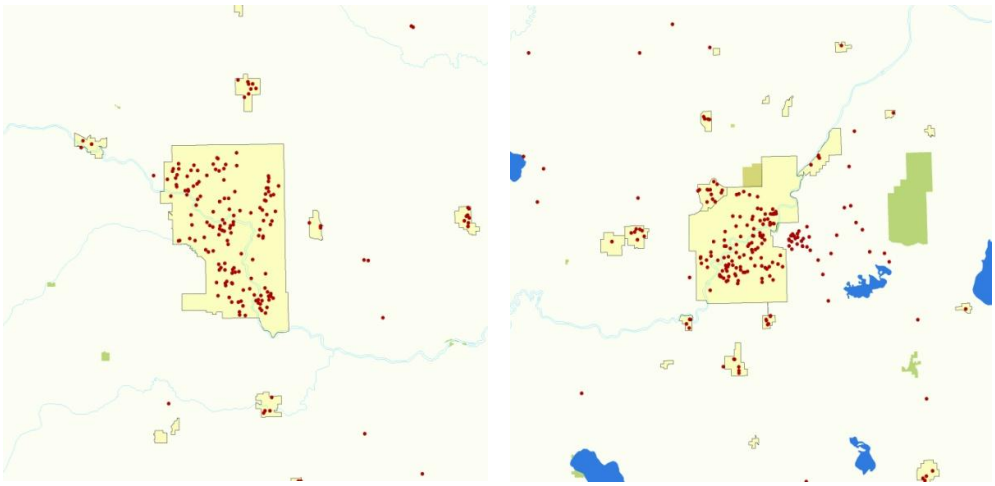

Figure 3.2 Locations in the major cities from which ticks were recovered between 2007-07-01 and 2010-12-31. Tick locations are represented by red dots in the city of Calgary (left) and city of Edmonton (right). Cities and towns are coloured yellow. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

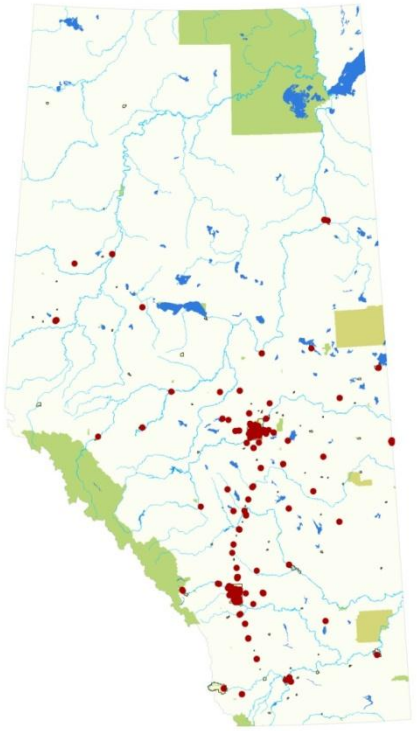

Figure 3.3 Locations of veterinary clinics in Alberta that participated in the tick surveillance program between 2007-07-01 and 2012-12-31. Red dots represent veterinary clinic locations. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

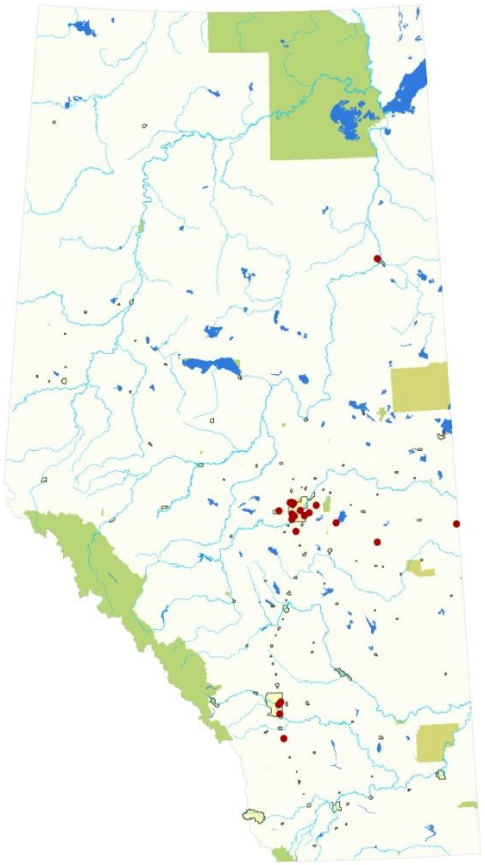

Figure 3.4 Locations of *Borrelia burgdorferi*-positive ticks collected in Alberta between 2007-07-01 and 2010-12-31. Red dots represent locations from which *B. burgdorferi*-positive ticks were recovered. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

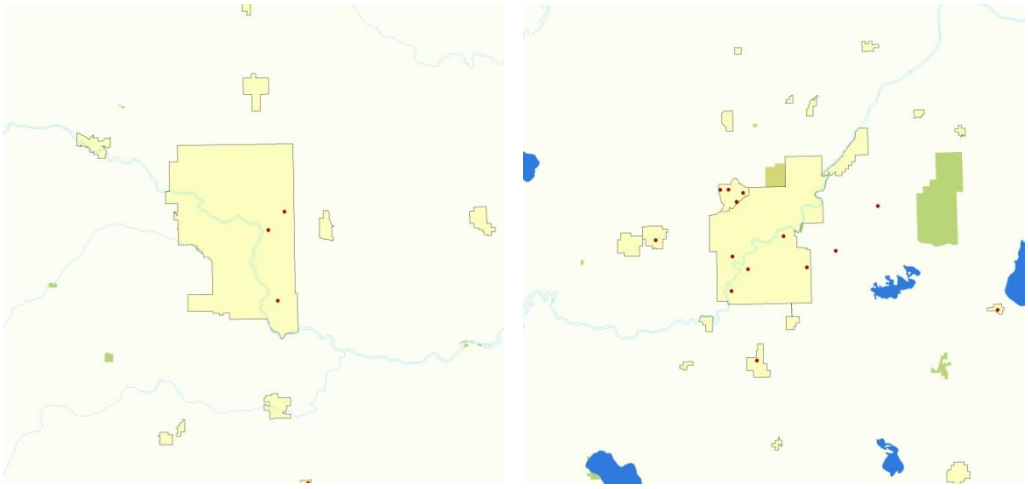

Figure 3.5 Locations of *Borrelia burgdorferi*-positive ticks recovered within the major cities of Alberta between 2007-07-01 and 2010-12-31. Red dots represent locations for ticks that tested positive for *B. burgdorferi*. City of Calgary (left), city of Edmonton (right). Cities and towns are coloured yellow. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

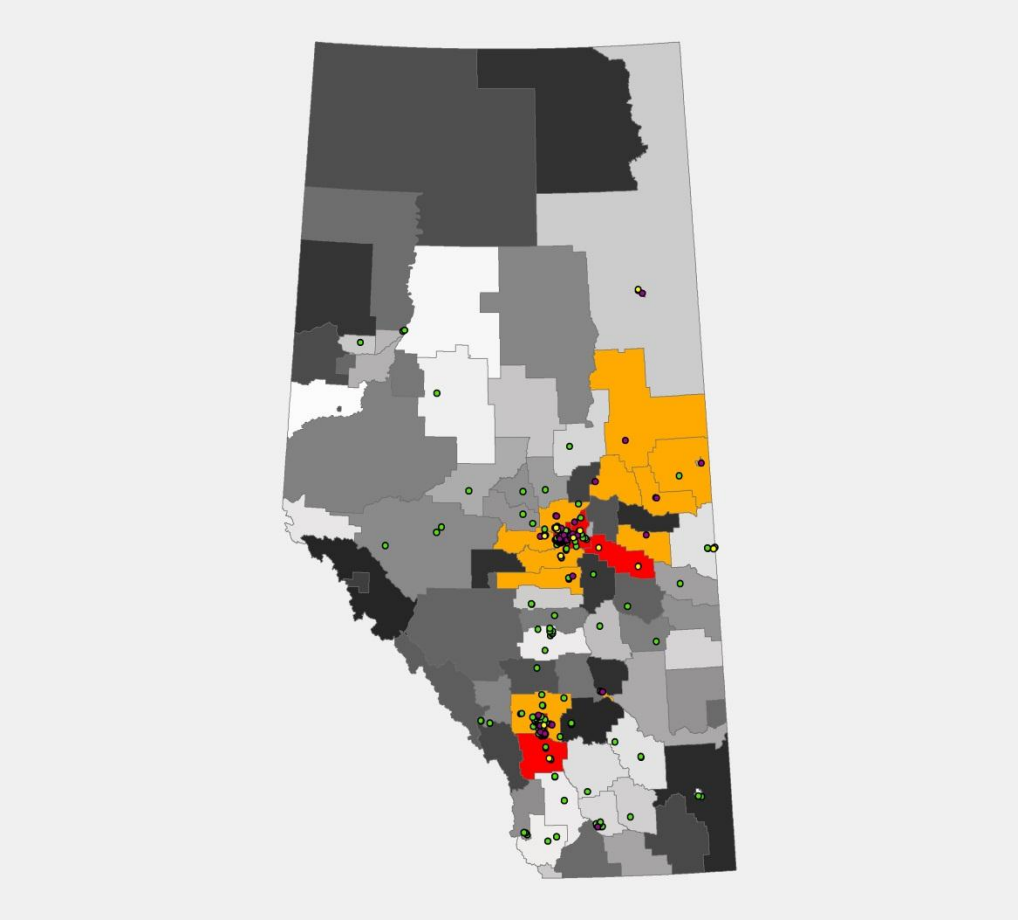

Figure 3.6 The municipal districts and major cities where *B. burgdorferi*-positive ticks were recovered between 2007-07-01 and 2010-12-31. Municipal districts and major cities are shaded in tones of grey, white or black. Municipal districts or cities where at least one *I. scapularis* was recovered are shaded orange. Municipal districts or cities where at least one *B. burgdorferi*-positive tick was recovered are shaded red. Purple dots indicate *I. scapularis*. Yellow dots indicate a *B. burgdorferi*-positive tick. Green dots indicate all other ticks submitted. All points are from hosts that had not left Alberta.

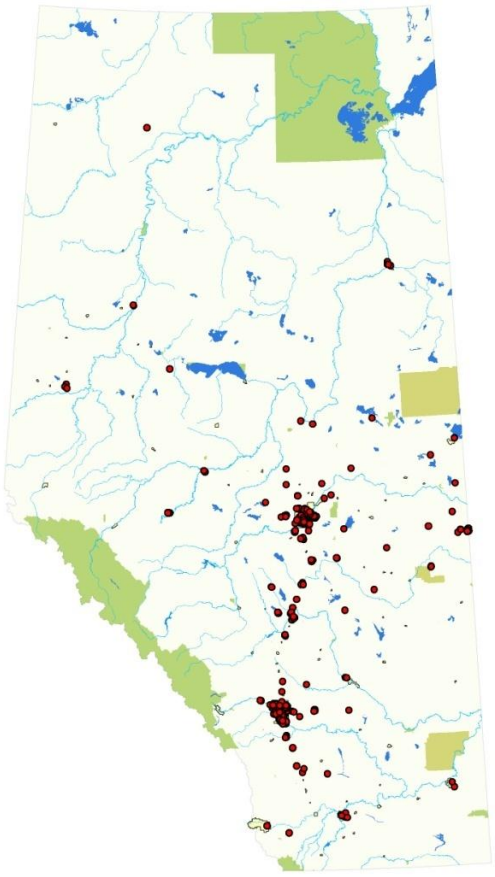

Figure 3.7 Locations where *Dermacentor variabilis* was recovered between 2007-07-01 and 2010-12-31. Red dots represent locations where ticks were recovered. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

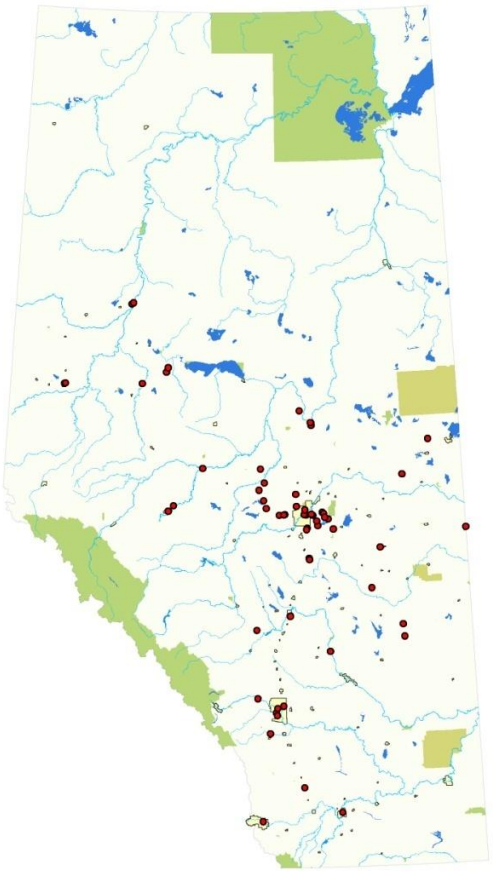

Figure 3.8 Locations where *Dermacentor albipictus* was recovered between 2007-07-01 and 2010-12-31. Red dots represent locations where ticks were recovered. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

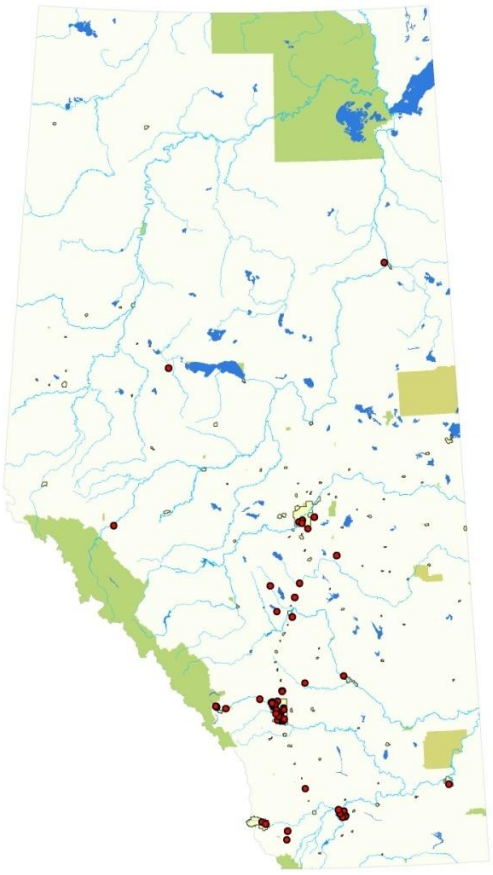

Figure 3.9 Locations where *Dermacentor andersoni* was recovered between 2007-07-01 and 2010-12-31. Red dots represent locations where ticks were recovered. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

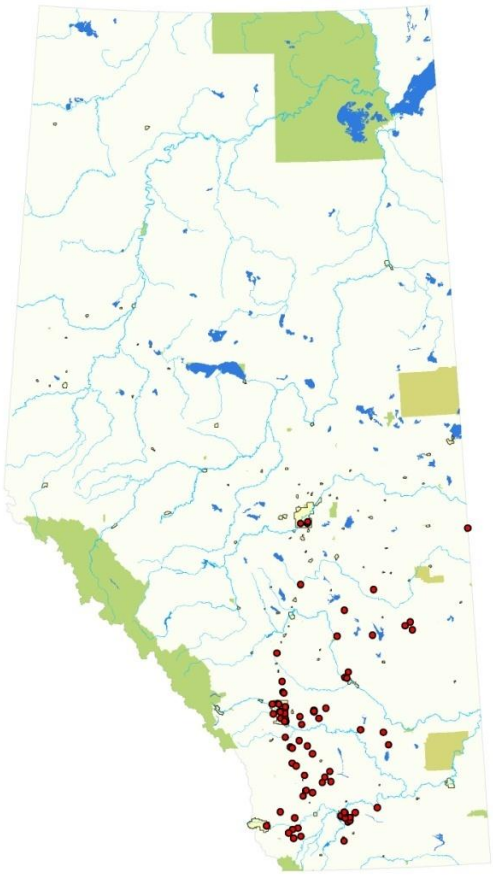

Figure 3.10 Locations where *Ixodes kingi* was recovered between 2007-07-01 and 2010-12-31. Red dots represent locations where ticks were recovered. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

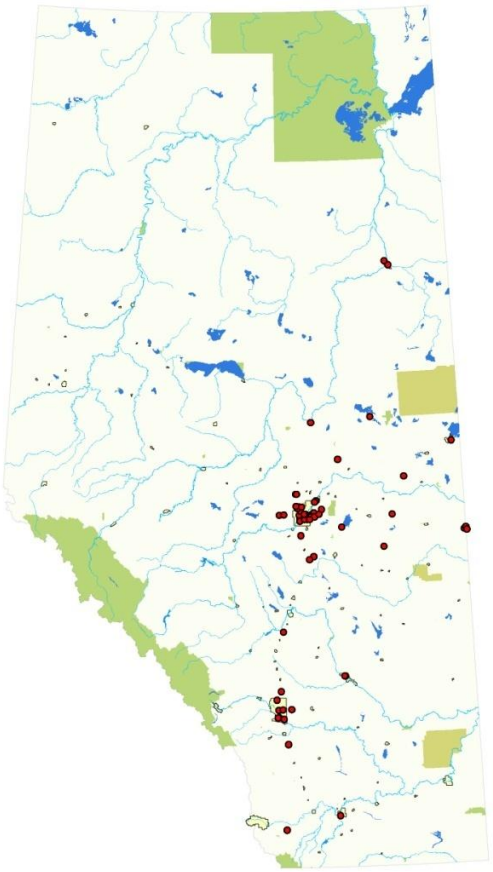

Figure 3.11 Locations where *Ixodes scapularis* was recovered between 2007-07-01 and 2010-12-31. Red dots represent locations where ticks were recovered. National and provincial parks are coloured green. Military bases are coloured olive. Major rivers and lakes are coloured blue.

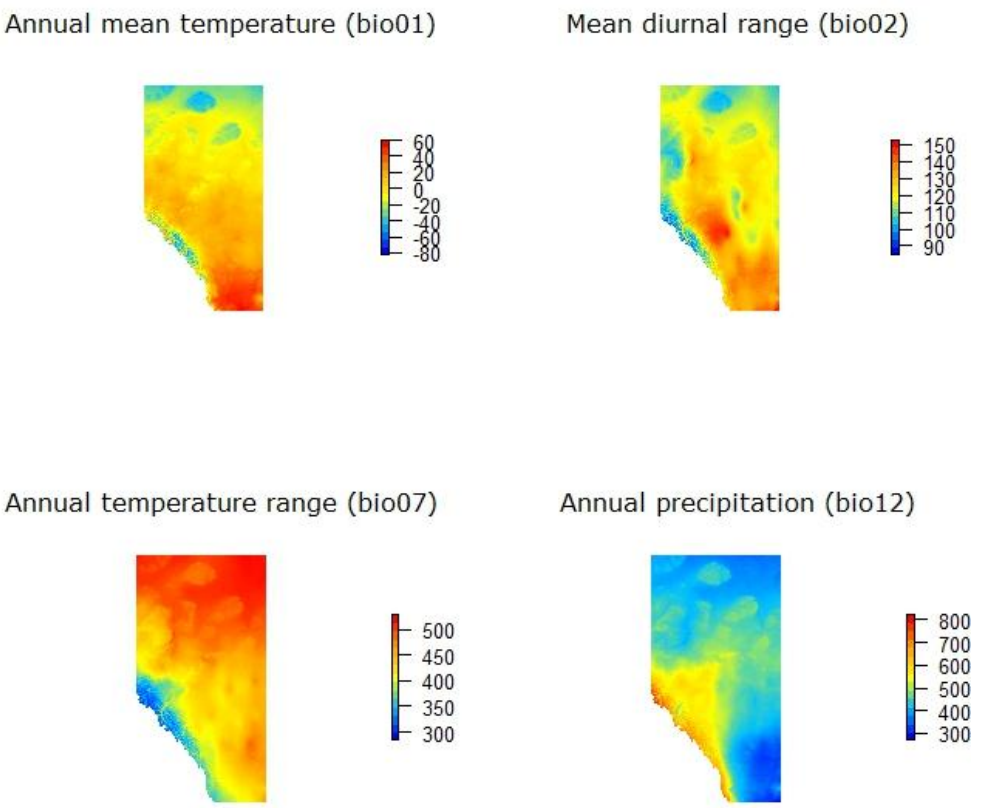

Figure 3.12 Environmental variables dealing with annual trends.Temperature values are in ^{o}C and precipitation values are in mm. Temperature values were multiplied by 10. Values depicted for the geographic area covered by Alberta were extracted from the larger global layers available at www.worldclim.org. Data represents average values for the time period 1950-2000 (9).

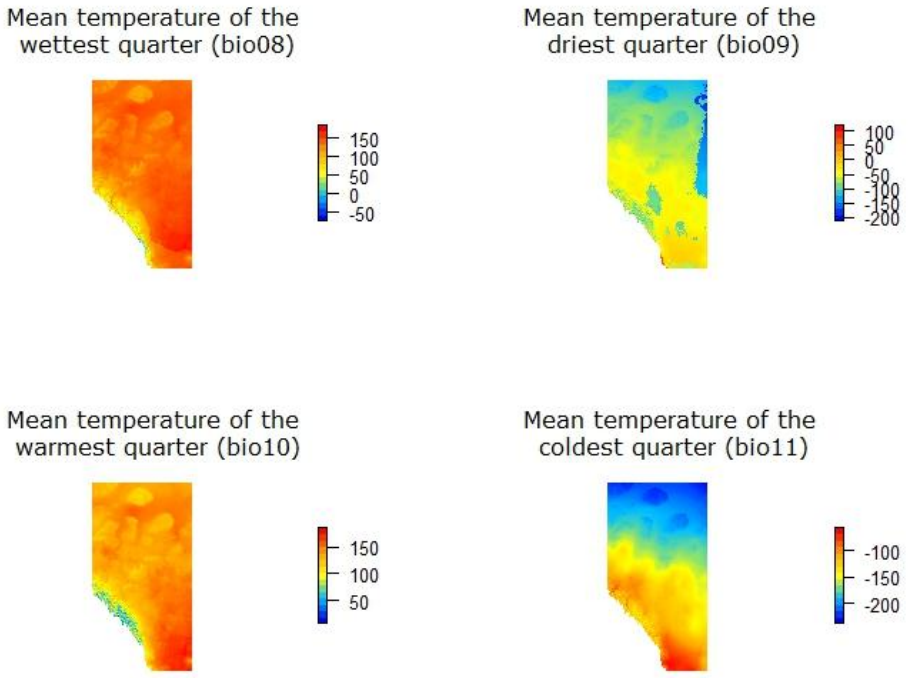

Figure 3.13 Environmental variables dealing with quarterly temperature trends. Temperature values are in ^{o}C and precipitation values are in mm. Temperature values were multiplied by 10. A quarter refers to a time period of three months. Values depicted for the geographic area covered by Alberta were extracted from the larger global layers available at www.worldclim.org. Data represents average values for the time period 1950-2000 (9).

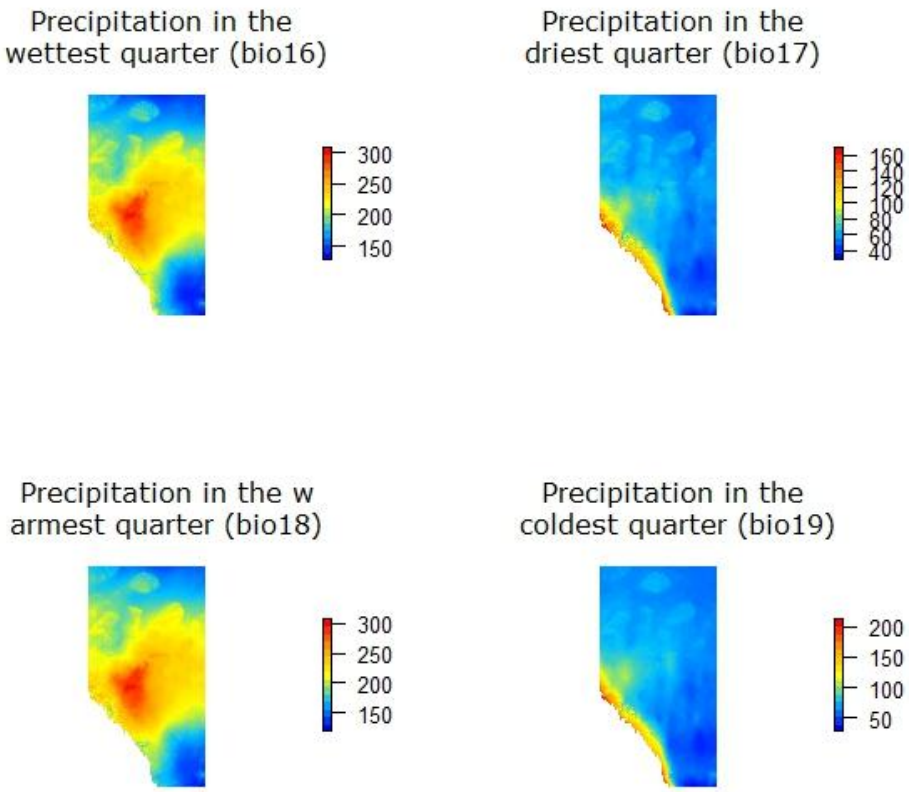

Figure 3.14 Environmental variables dealing with quarterly precipitation trends. Temperature values are in ^{o}C and precipitation values are in mm. Temperature values were multiplied by 10. A quarter refers to a time period of three months. Values depicted for the geographic area covered by Alberta were extracted from the larger global layers available at www.worldclim.org. Data represents average values for the time period 1950-2000 (9).

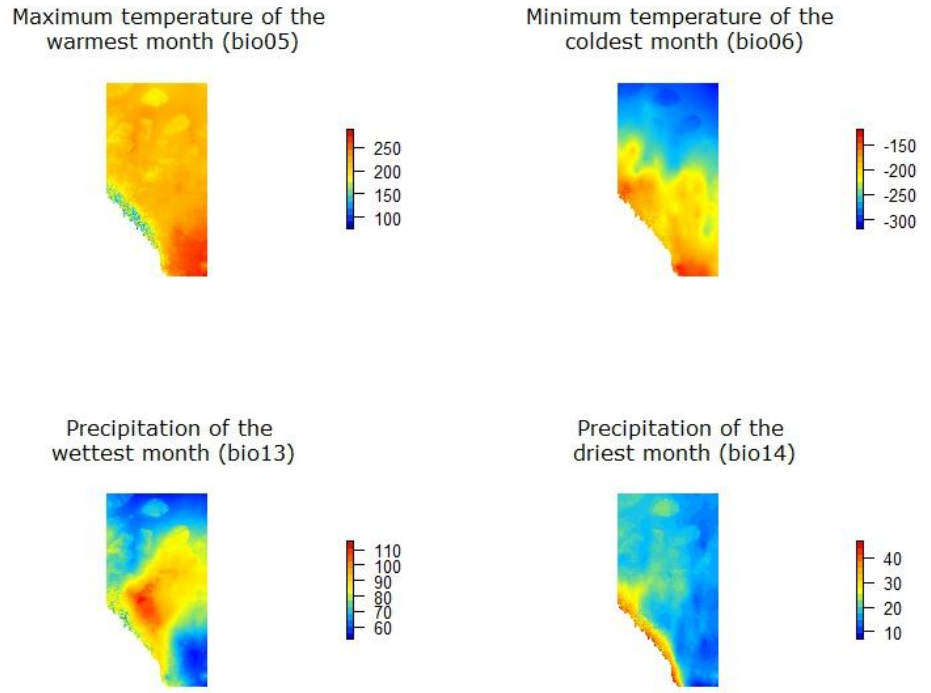

Figure 3.15 Environmental variables dealing with trends in extreme temperature or precipitation values.Temperature values are in ^{o}C and precipitation values are in mm. Temperature values were multiplied by 10. Values depicted for the geographic area covered by Alberta were extracted from the larger global layers available at www.worldclim.org. Data represents average values for the time period 1950-2000 (9).

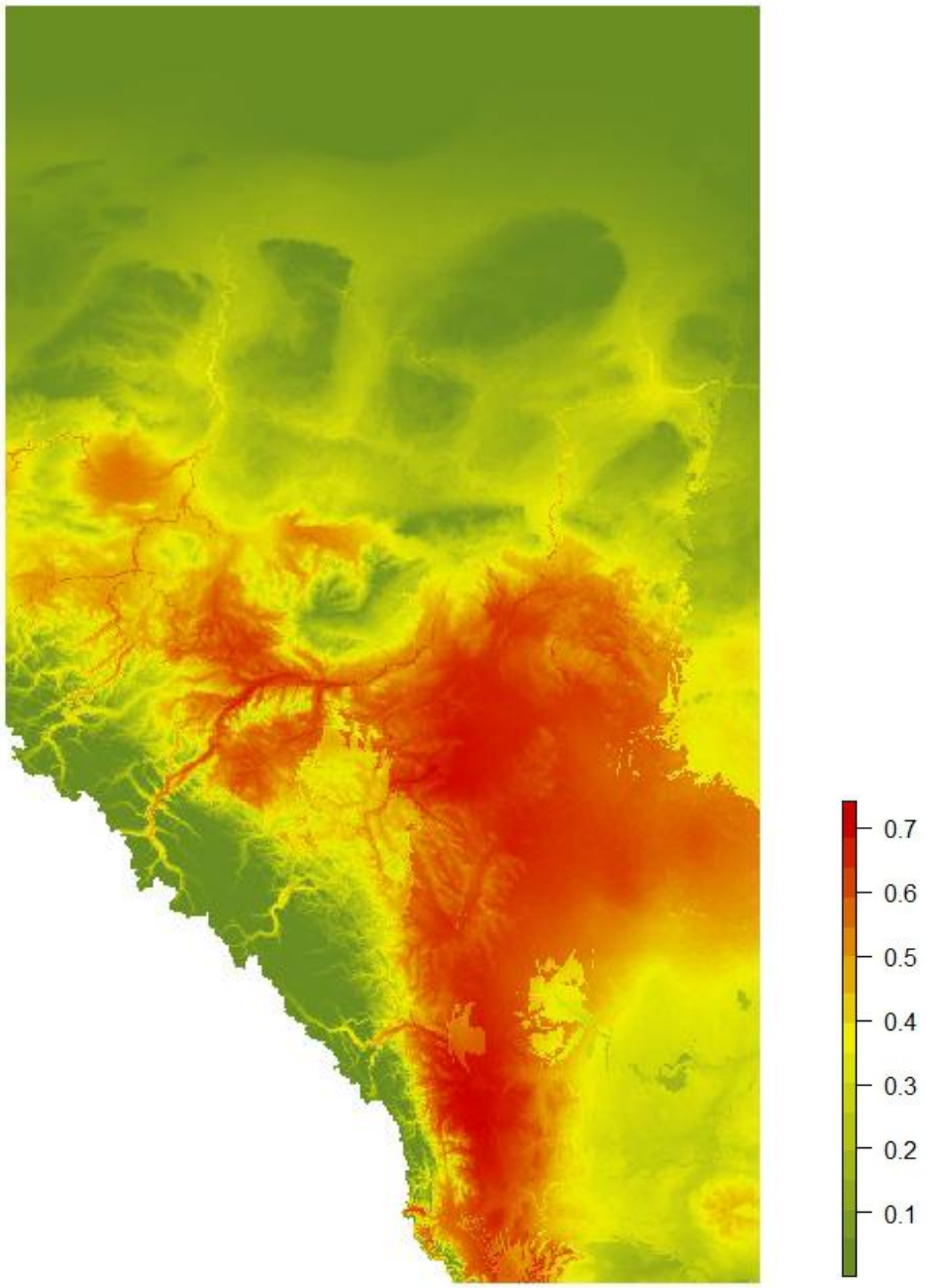

Figure 3.16 The range of *Dermacentor albipictus* in Alberta.The distribution of the species was determined from 57 specimen records and bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded

using Google Earth (www.google.com/earth/index.html) to obtain latitude and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1, coloured green to red) per grid cell from ten runs of Maxent is displayed. Larger values (coloured more red) of the Maxent output indicate areas more likely to have environmental conditions suitable for the tick species to be present while smaller values (coloured more green) represents the opposite.

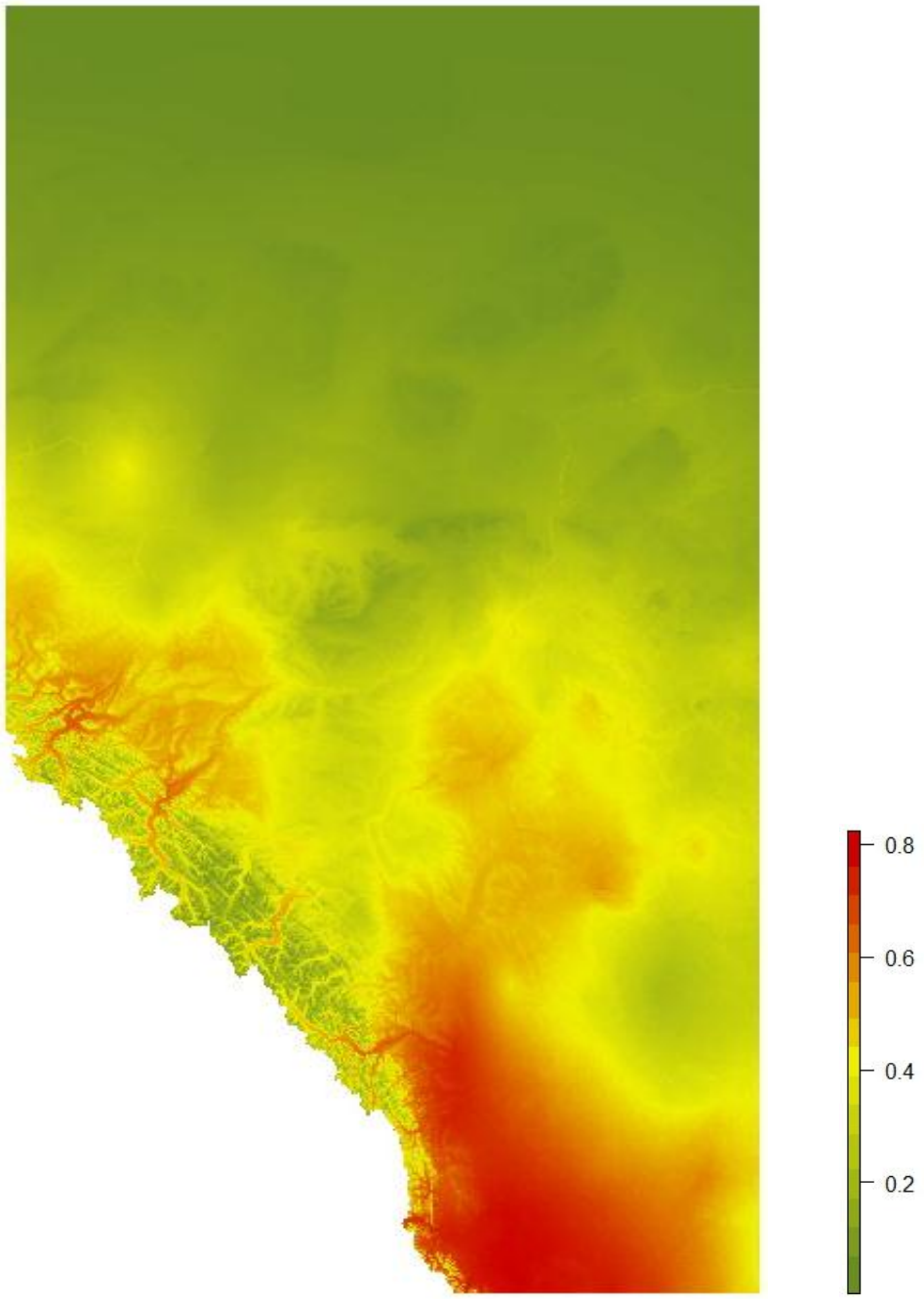

Figure 3.17 The range of *Dermacentor andersoni* in Alberta. The distribution of the species was determined from 45 specimen records and bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded using Google Earth (www.google.com/earth/index.html) to obtain latitude

and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1, coloured green to red) per grid cell from ten runs of Maxent is displayed. Larger values (coloured more red) of the Maxent output indicate areas more likely to have environmental conditions suitable for the tick species to be present while smaller values (coloured more green) represents the opposite.

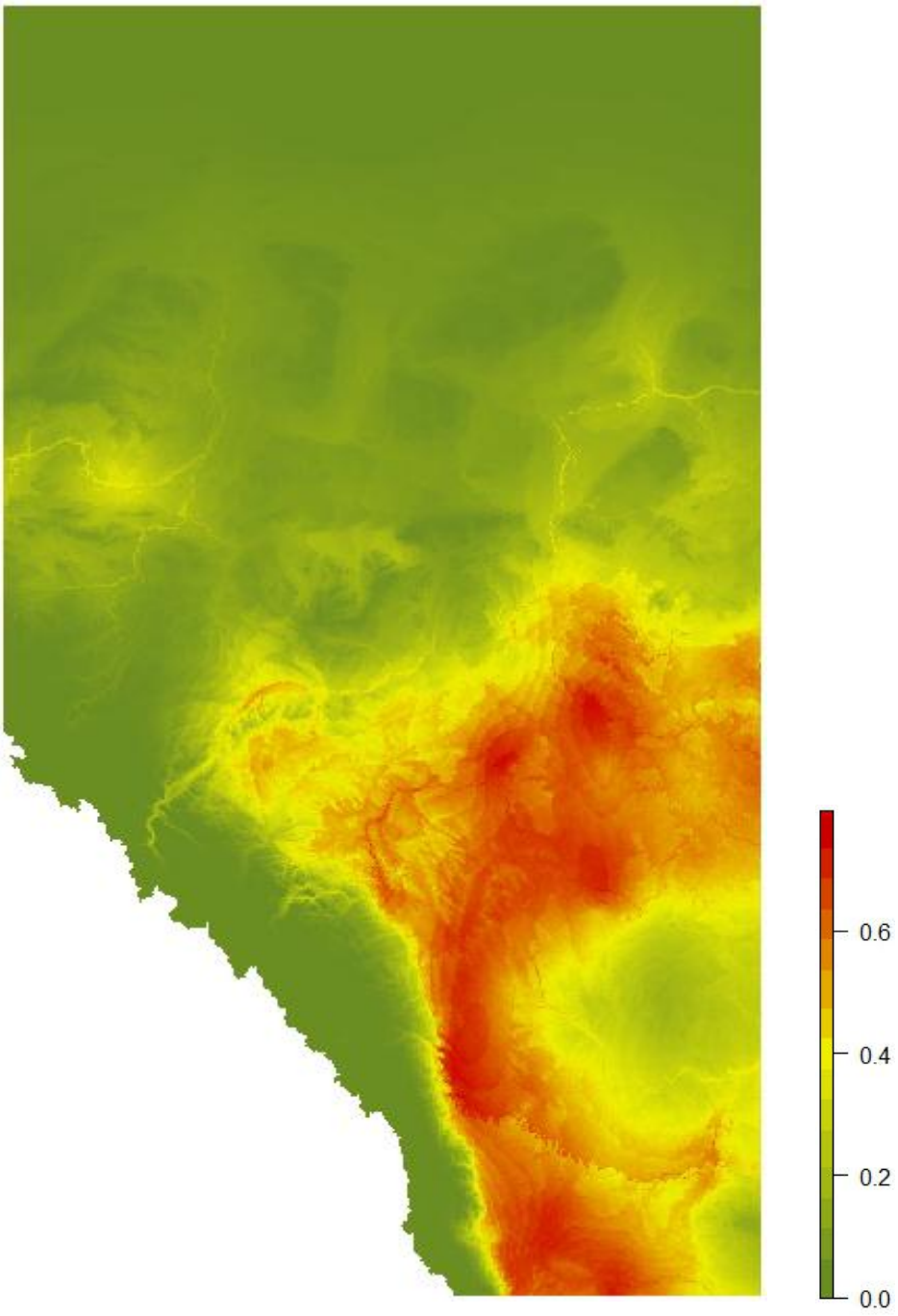

Figure 3.18 The range of *Dermacentor variabilis* in Alberta. The distribution of the species was determined from 65 specimen records and bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded using Google Earth (www.google.com/earth/index.html) to obtain latitude

and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1, coloured green to red) per grid cell from ten runs of Maxent is displayed. Larger values (coloured more red) of the Maxent output indicate areas more likely to have environmental conditions suitable for the tick species to be present while smaller values (coloured more green) represents the opposite.

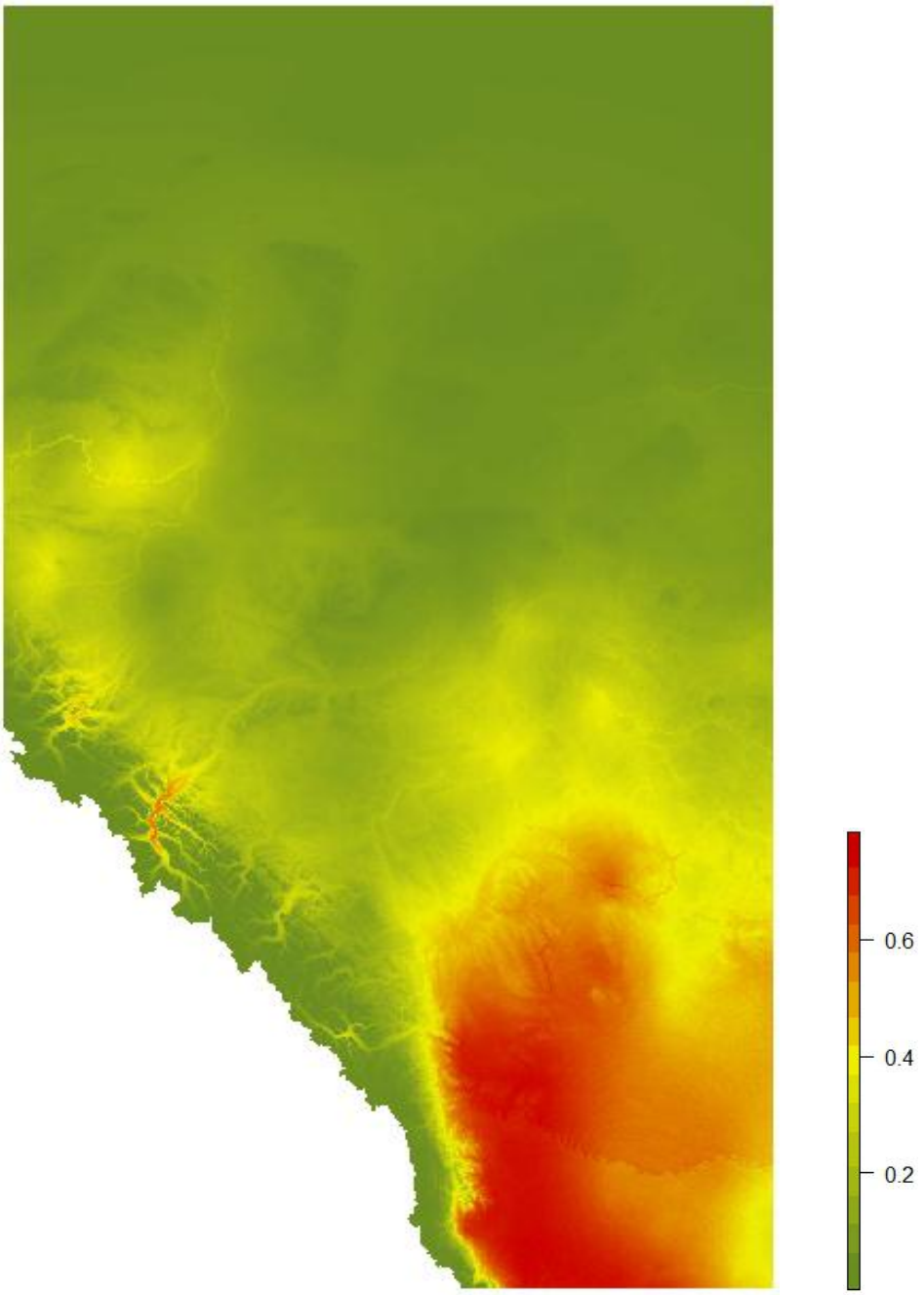

Figure 3.19 The range of *Ixodes kingi* in Alberta. The distribution of the species was determined from 82 specimen records and bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded using Google Earth (www.google.com/earth/index.html) to obtain latitude

and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1, coloured green to red) per grid cell from ten runs of Maxent is displayed. Larger values (coloured more red) of the Maxent output indicate areas more likely to have environmental conditions suitable for the tick species to be present while smaller values (coloured more green) represents the opposite.

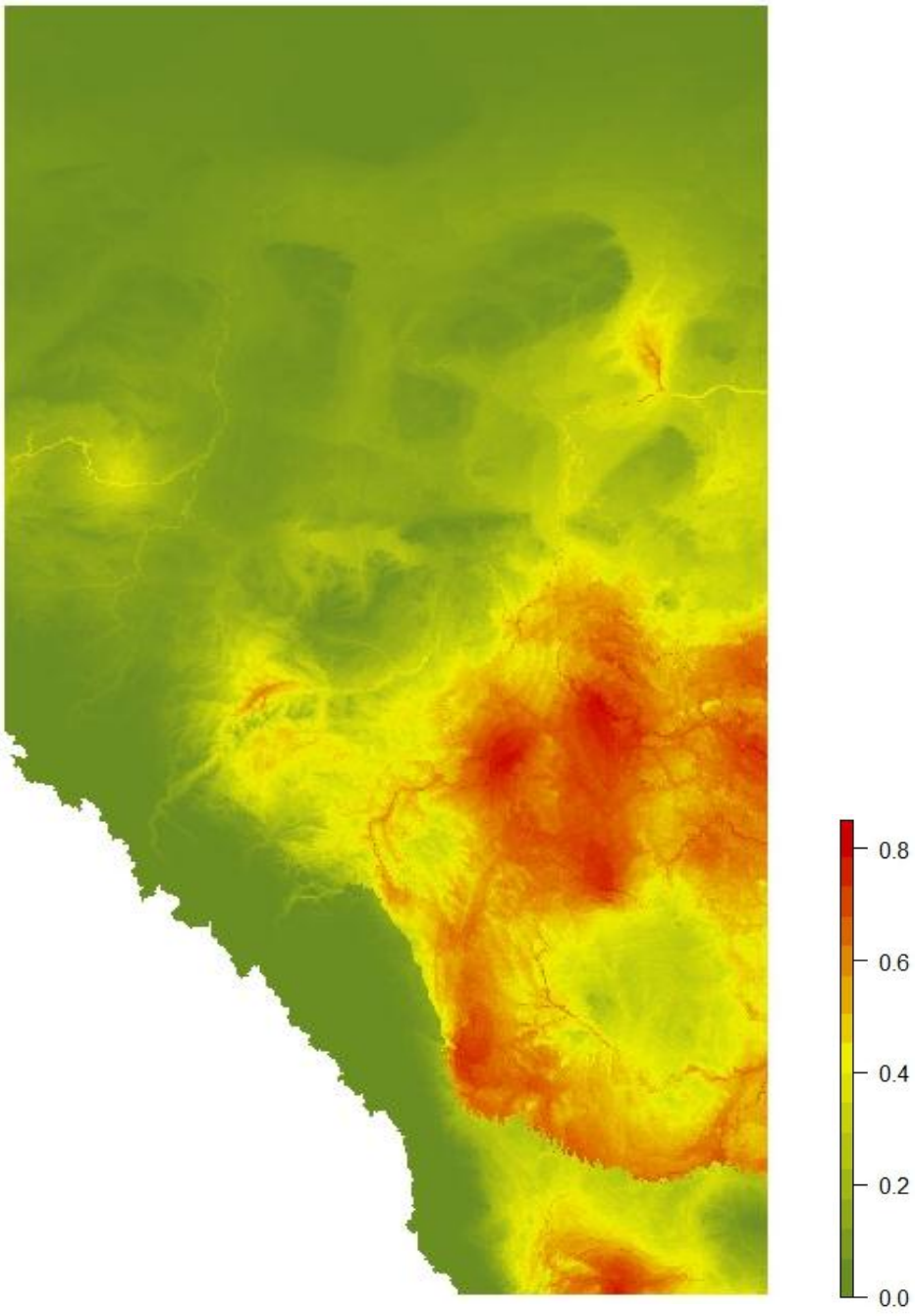

Figure 3.20 The range of *Ixodes scapularis* in Alberta. The distribution of the species was determined from 41 specimen records and bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded using Google Earth (www.google.com/earth/index.html) to obtain latitude

and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1, coloured green to red) per grid cell from ten runs of Maxent is displayed. Larger values (coloured more red) of the Maxent output indicate areas more likely to have environmental conditions suitable for the tick species to be present while smaller values (coloured more green) represents the opposite.

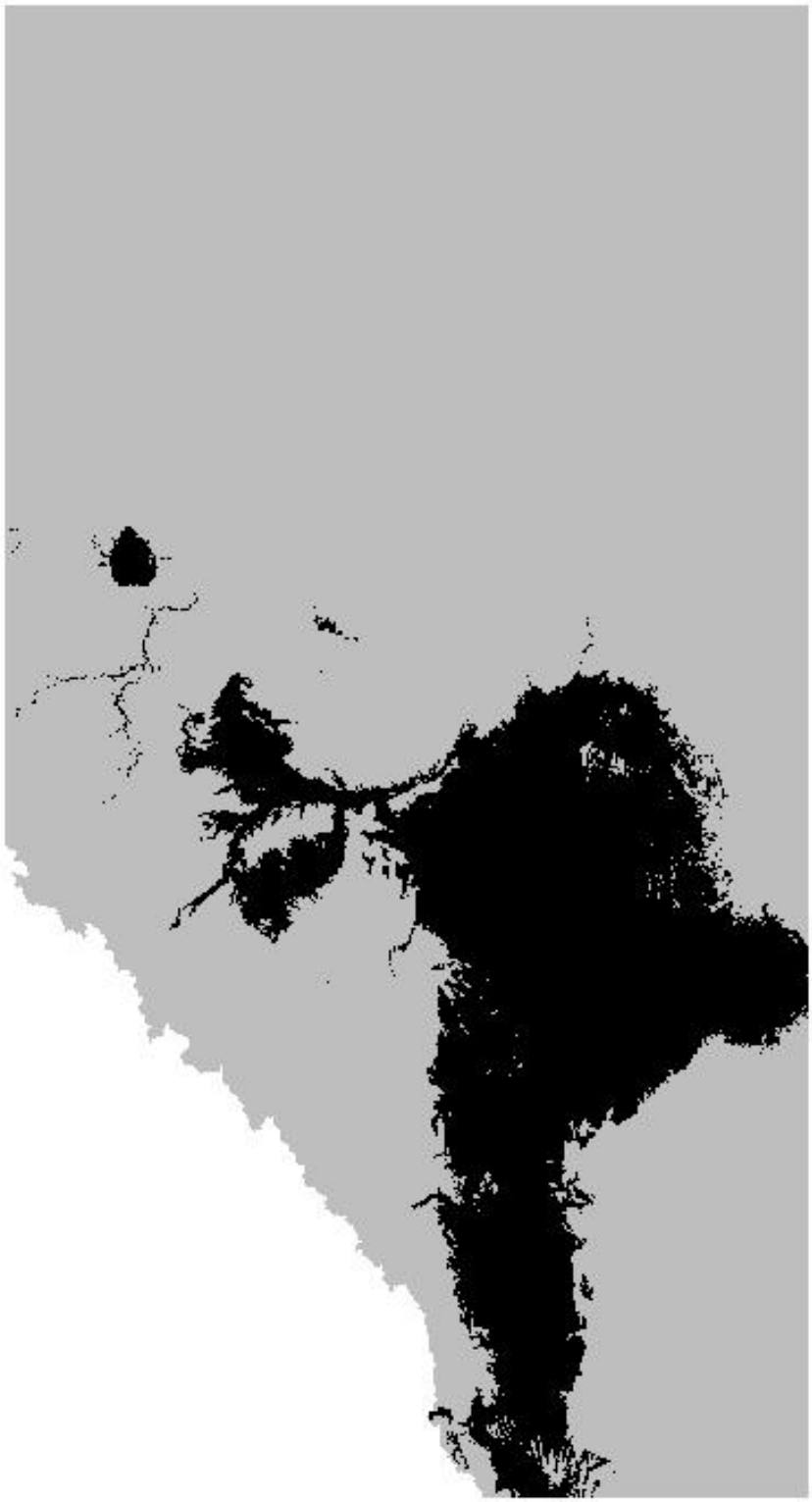

Figure 3.21 A binary map of the range of *Dermacentor albipictus* in Alberta. The distribution of the species was determined from 57 specimen records

and bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded using Google Earth (www.google.com/earth/index.html) to obtain latitude and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1) per grid cell from ten runs of Maxent was calculated. A threshold value of 0.5311 was calculated for the average of ten models using the "Equal test sensitivity and specificity" setting of Maxent. The threshold value was used within the raster package for R version 2.10 (www.r-project.org/) to convert the logistic output of Maxent (which will be a value between 0 and 1) to a binary (1 or 0) present/absent prediction, with the threshold value being the point at which an absent prediction becomes a present prediction. All cells which are predicted to have the species present are coloured black and those without are coloured grey.

Figure 3.22 A binary map of the range of *Dermacentor andersoni* in Alberta.The distribution of the species was determined from 45 specimen

records and bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded using Google Earth (www.google.com/earth/index.html) to obtain latitude and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1) per grid cell from ten runs of Maxent was calculated. A threshold value of 0.5076 was calculated for the average of ten models using the "Equal test sensitivity and specificity" setting of Maxent. The threshold value was used within the raster package for R version 2.10 (www.r-project.org/) to convert the logistic output of Maxent (which will be a value between 0 and 1) to a binary (1 or 0) present/absent prediction, with the threshold value being the point at which an absent prediction becomes a present prediction. All cells which are predicted to have the species present are coloured black and those without are coloured grey.

Figure 3.23 A binary map of the range of *Dermacentor variabilis* in Alberta. The distribution of the species was determined from 65 specimen records

and bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded using Google Earth (www.google.com/earth/index.html) to obtain latitude and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1) per grid cell from ten runs of Maxent was calculated. A threshold value of 0.563 was calculated for the average of ten models using the "Equal test sensitivity and specificity" setting of Maxent. The threshold value was used within the raster package for R version 2.10 (www.r-project.org/) to convert the logistic output of Maxent (which will be a value between 0 and 1) to a binary (1 or 0) present/absent prediction, with the threshold value being the point at which an absent prediction becomes a present prediction. All cells which are predicted to have the species present are coloured black and those without are coloured grey.

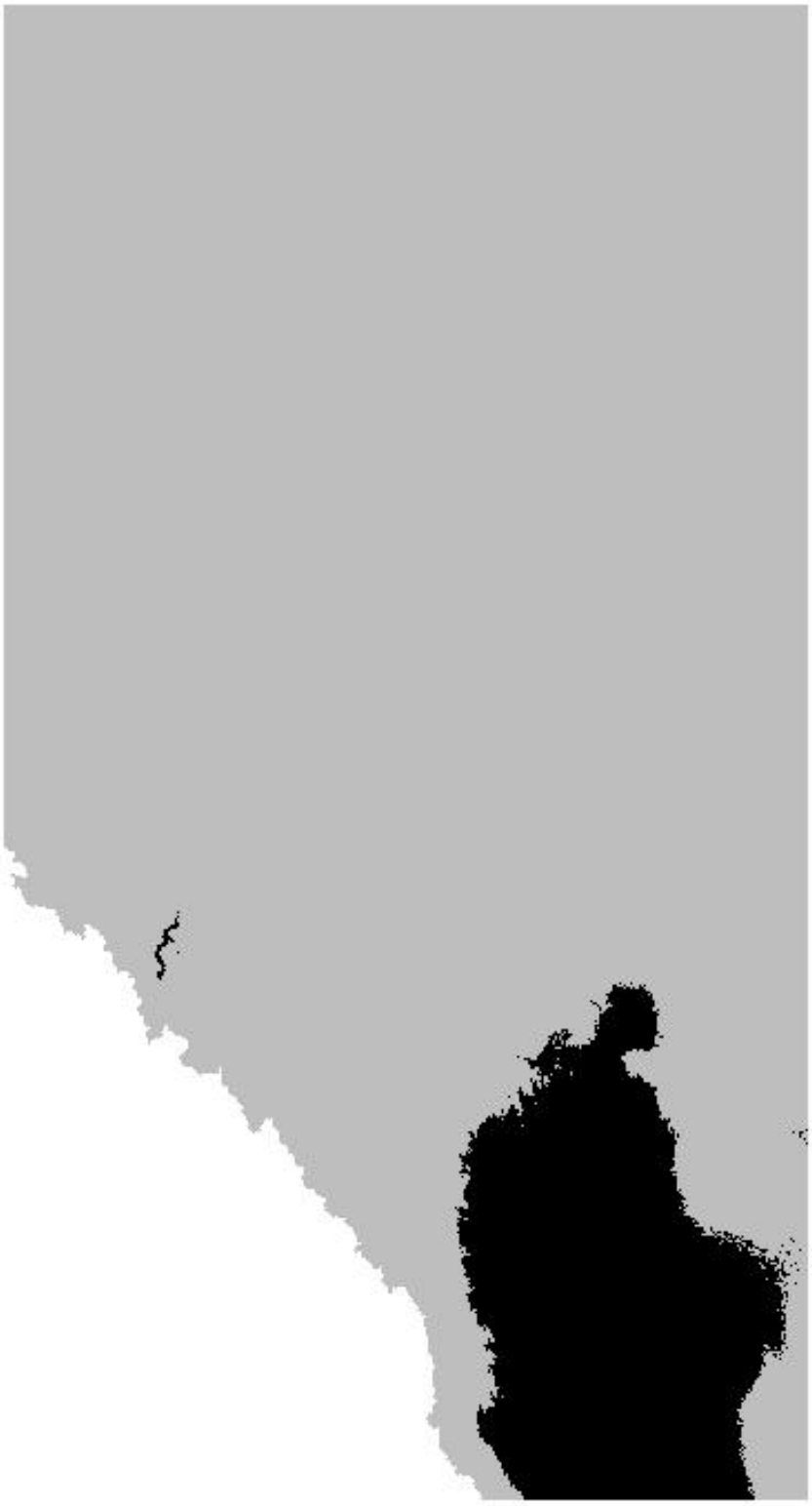

Figure 3.24 A binary map of the range of *Ixodes kingi* in Alberta. The distribution of the species was determined from 82 specimen records and

bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded using Google Earth (www.google.com/earth/index.html) to obtain latitude and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1) per grid cell from ten runs of Maxent was calculated. A threshold value of 0.5223 was calculated for the average of ten models using the "Equal test sensitivity and specificity" setting of Maxent. The threshold value was used within the raster package for R version 2.10 (www.r-project.org/) to convert the logistic output of Maxent (which will be a value between 0 and 1) to a binary (1 or 0) present/absent prediction, with the threshold value being the point at which an absent prediction becomes a present prediction. All cells which are predicted to have the species present are coloured black and those without are coloured grey.

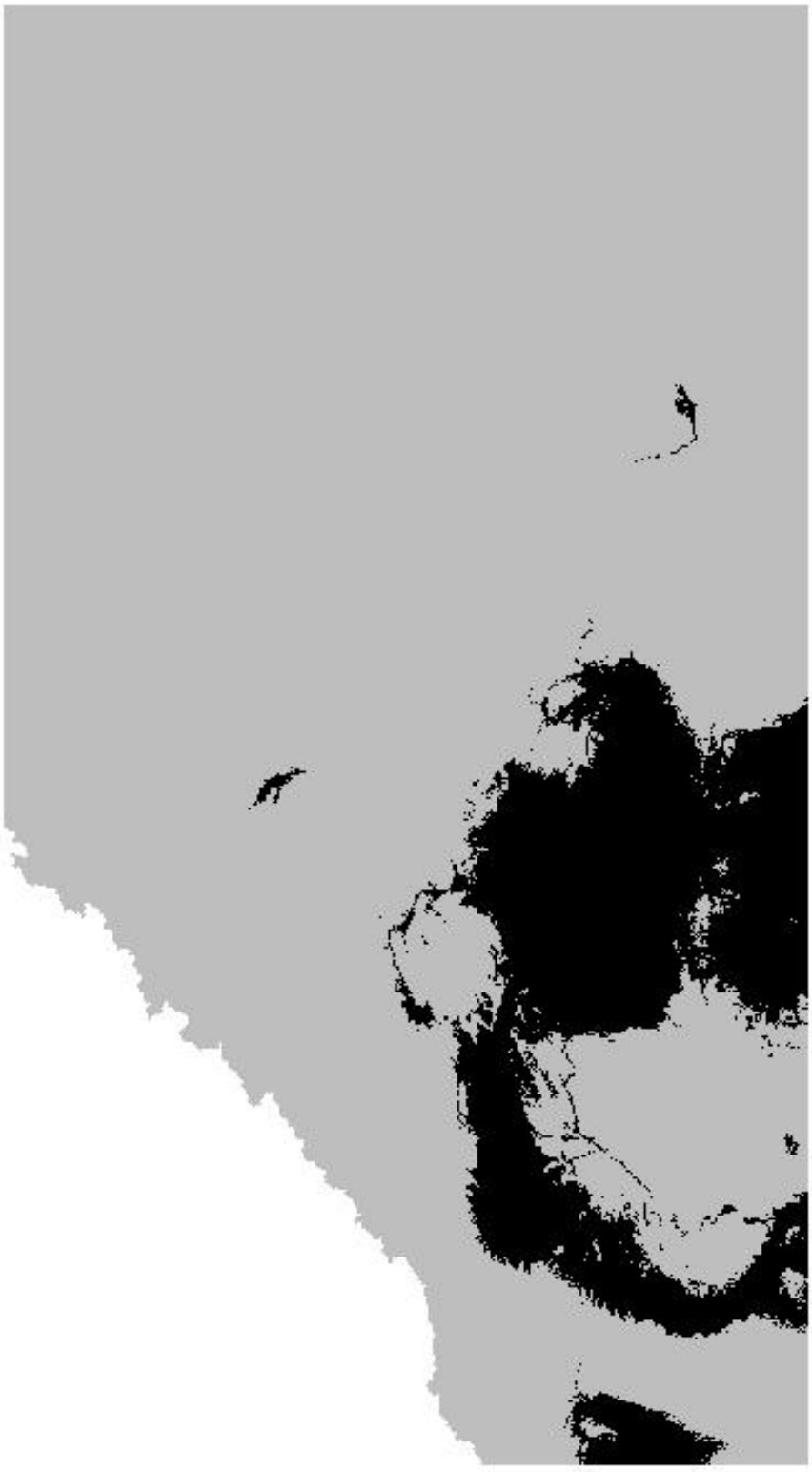

Figure 3.25 A binary map of the range of *Ixodes scapularis* in Alberta. The distribution of the species was determined from 41 specimen records and

bioclimatic data (www.worldclim.org) using the Maxent software (6). Specimens were collected between July 1, 2007 and December 31, 2010 and were geocoded using Google Earth (www.google.com/earth/index.html) to obtain latitude and longitude coordinates. All specimens had no associated out of province travel within the previous two months. The bioclimatic data used represented average values for the period from 1950 to 2000. The average output (0 -1) per grid cell from ten runs of Maxent was calculated. A threshold value of 0.5196 was calculated for the average of ten models using the "Equal test sensitivity and specificity" setting of Maxent. The threshold value was used within the raster package for R version 2.10 (www.r-project.org/) to convert the logistic output of Maxent (which will be a value between 0 and 1) to a binary (1 or 0) present/absent prediction, with the threshold value being the point at which an absent prediction becomes a present prediction. All cells which are predicted to have the species present are coloured black and those without are coloured grey.

Chapter 4. General Discussion and Conclusions

4.1. Overall research achievements

There are several outcomes from my research that are relevant for veterinary and public health practitioners, other researchers and the public. I have determined the most common tick species in Alberta, and where and when these species are active. I have shown that many ticks on companion animals would go unnoticed in the absence of veterinary examinations. I have identified environmental variables that most influence the distributions of several species. And overall, I have provided a detailed update to the understanding of the species composition and distribution patterns of Ixodoidea in Alberta, the first such survey since the 1950s.

I have shown that approximately one third of ticks were not noticed on dogs by the owners of those animals and that younger dogs had more ticks recovered than older dogs, mostly due to having to come in for vaccinations. But are younger dogs more likely to acquire ticks? Also, if we assume that older dogs interact with a veterinary clinic only when in poor health, do we assume that an older dog which is in poorer health is more likely to acquire a tick than a healthier older dog? My data cannot answer such questions as more data on the dog population of Alberta is needed. I have shown that dogs are useful sentinel animals for ticks, and have harbored a diverse set of species. Gathering data from dogs that did not interact with a veterinary clinic, healthy dogs, would increase the usefulness of dogs as sentinels for ticks and tick-borne pathogens.

The research presented here indicates that over 40% of dogs travelled out of Alberta and many brought ticks back with them. As mentioned in Chapter One for *A. marginale*, imported animals infected with a tick-borne pathogen can act as a vector and infect naive ticks established in Alberta. Concern exists for *A. marginale*-infected cattle being imported to Alberta, but imported dogs should also be a concern. There were a large number of rescue dogs brought in from different countries that had ticks recovered from them. My research should reinforce that any imported dog should be disease-free, as potential tick vectors may be established here now.

Knowledge of the tick species composition of Alberta can help to focus future tick-related activities. *Dermacentor variabilis* is clearly now an established species in Alberta. This tick has most likely spread through a range expansion from Saskatchewan. I have shown that *D. albipictus* and *I. kingi* comprised over 35% of the ticks found on hosts that have not left the province. However, the ability of these two species to act as vectors of pathogens needs to be examined more thoroughly. Their potential to vector pathogens such as bovine anaplasmosis (*Anaplasma marginale)*, Rocky Mountain spotted fever (*Rickettsia rickettsii*), Colorado tick fever virus (*Francisella tularensis* (tularaemia)), Powassan encephalitis virus, tick-borne encephalitis human anaplasmosis (*Anaplasma phagocytophilum)*, Lyme disease (*Borrelia burgdorferi)*, and other pathogens should be determined. *Ixodes kingi* and *D. albipictus* are among the most common ticks found in Alberta and their respective distributions encompass all major human population centres and most of the major livestock production areas. A lack of knowledge of what, if any, pathogens these ticks can harbour and transmit is a major gap in our knowledge. The lack of any outbreaks of tick-

borne diseases in these areas is not sufficient proof that these arthropods are a low risk to cause disease and morbidity in humans and livestock and companion animals. Specimens recovered in Alberta should be used in laboratory and field studies to better understand the risk posed by *I. kingi* and *D. albipictus*.

Ixodes scapularis was not reported as established in previous research on ticks in Alberta and has only been recovered infrequently (1-5). The previous reports of this tick in Alberta identify only a few sites where it was recovered in the province. I have identified 41 sites where this tick was acquired locally and an additional 18 sites where the tick might have been acquired out-of-province. Of the 814 ticks I identified, over seven percent were *I. scapularis*. The criteria put forth by the Consensus Conference on Lyme disease (12) requires recovering all three stages of a tick species in a locality for two consecutive years for a species to be deemed established in that locality. These criteria inform many Public Health policies on Lyme disease diagnosis, requiring a patient to have a travel history to an area meeting the criteria for *I. scapularis* establishment in order to consider a Lyme disease diagnosis. Using these criteria, the *I. scapularis* I have identified would be classified as adventitious. But if those criteria put forth (12) are too conservative, small populations of established *I. scapularis* could be deemed adventitious and the local risk of acquiring Lyme disease would be underestimated based on the requirement of travel to an area of established *I. scapularis*. The criteria used by Dennis et al. (6) for *I. scapularis* and also used by James et al. (7) for *D. andersoni*, for a species to be considered established is whether greater than six specimens or greater than one life cycle stage are recovered from a county (municipal district). Any area with fewer specimens than six

specimens are termed 'reported'. By these criteria, *I. scapularis* is established in Edmonton, Calgary, and the County of Strathcona. It is reported in the municipal districts of Rocky View, Parkland County, Sturgeon County, Leduc County, the municipal district of Wood Buffalo, and several others.

There are several implications to identifying local, endemic populations of *I. scapularis* in Alberta. The diagnosis of Lyme disease in a human is a clinical diagnosis and the travel history of a patient to an *I. scapularis* endemic or an area with a high incidence of Lyme disease does factor into that diagnosis. If there is a local population of *B. burgdorferi* infected *I. scapularis* in Alberta, then a lack of out of province travel is less important when forming a diagnosis. Also, having a local population of Lyme disease vector ticks may serve to raise awareness that Lyme disease could be acquired in Alberta which could lead to a more frequent consideration of a Lyme disease diagnosis. There is the possibility that cases of Lyme disease were misdiagnosed or had a delayed diagnosis due to there simply being no current evidence, until now, that ticks carrying Lyme disease were in Alberta. There are also implications for the citizens of Alberta. If there are local populations of *I. scapularis* in and around major centres such as Edmonton and Calgary, then people in those areas need to increase their vigilance for ticks on themselves and their pets. As a *B. burgdorferi*-infected *I. scapularis* requires between 24-72 hours of attachment to begin transmitting the bacterium to the host, checking for ticks frequently and removing any found is an effective way to reduce the risk of transmission. As 1/3 ticks on dogs were not noticed, people should also examine their pets more frequently and thoroughly for ticks.

Ogden (14) indicated that migratory birds have the potential to deposit millions of ticks over a wide area. They also state that estimating where migratory birds will introduce ticks is not straightforward because many birds have a large breeding range in Canada, sometimes extending east-west across the country. The American robin (*Turdus migratorius* Linnaeus 1766) can act as a reservoir for *B. burgdorferi* (15) and is a very common bird in Alberta, so it is of interest when discussing tick and *B. burgdorferi* dispersal. The distribution for *T. migratorius* in Alberta (16) covers basically the entire province. So if *T. migratorius* were depositing ticks during annual migrations a relatively even distribution across the province of *I. scapularis* and *B. burgdorferi* would be expected. The distribution of *I. scapularis* and *B. burgdorferi*-positive ticks determined by my research was found to be focused on the central area of the province, which is only one part of the Albertan range of *T. migratorius*. But *T. migratorius* is only one of many species of birds that migrate into Alberta and others could be responsible for depositing the ticks. A full examination of all migratory birds is too big a topic for this discussion. Instead, the genetic evidence from one Alberta tick from which *B. burgdorferi* was identified and sequenced (17) provides a useful suggestion as to where ticks with *B. burgdorferi* in Alberta came from. The sequence results (17) suggest a relation to the *B. burgdorferi* populations from the areas around the western side of the Great Lakes where there is a cluster of Lyme disease cases in humans. The number of reported cases of Lyme disease in humans in that part of the USA has been increasing over the past decade (18). So the number of infected ticks on migratory birds that have passed through that region would be expected to go up at the same time, presumably, resulting in the ticks deposited in

Alberta having a higher rate of *B. burgdorferi* infection than previously. I do not disagree with the hypothesis that migratory birds dispersed *I. scapularis* into Alberta. I think that it is a very plausible way for ticks to be introduced. What my research adds to this is a large number of locally acquired specimens of *B. burgdorferi*-positive ticks and a large number of *I. scapularis*. The question raised by such numbers is: why were there so few previous reports of *I. scapularis* in Alberta?

If *I. scapularis* was dispersed into Alberta via migratory birds, then one would expect reports of its occurrence in Alberta in past research on ticks (1,2,3). Gregson (1) found no reports of *I. scapularis* in Alberta while I found that *I. scapularis* comprised 10% of Alberta ticks. Bishopp and Trembley (3), Dennis et al. (6), Brownstein et al. (10) and Goodman et al. (11) all indicated that the distribution of *I. scapularis* in the United States has included the states on the Gulf of Mexico for several decades. The Mississippi and Central Flyways followed by migratory birds pass through parts of the Gulf States on the way to Alberta. So regardless of the current rising rate of Lyme disease along the western edge of the Great Lakes, migratory birds have been passing through *I. scapularis* habitat on an annual cycle for decades at least. While there is no data to confirm, it seems reasonable that some of those birds would have picked up *I. scapularis* on the way to Alberta and deposited some of those ticks in Alberta. Yet the first record of *I. scapularis* in Alberta was from 1998. Also, *Amblyomma americanum* is located around the gulf coast in the same area as *I. scapularis* (3) yet there were relatively few *A. americanum* recovered from dogs in Alberta. If migratory birds are the source of the *I. scapularis* recovered, it is reasonable

to expect to see *A. americanum* at the same level as *I. scapularis*. But the number of *A. americanum* recovered was much less than *I. scapularis.*
In the context of the potential for migratory birds to disperse ticks, the scarcity of previous reports of *I. scapularis* in Alberta and the lack of other tick species which could also have been transported by the same migrating birds, my findings provide a strong suggestion that the status of *I. scapularis* in Alberta needs to be further examined.

4.2. Directions for future research

The surveillance for ticks presently continues, and it should continue for some time. As the number of years of surveillance increase, the ability of this surveillance to monitor changes in the tick fauna of Alberta also increases. This will allow a faster, more directed response, and allow changes in composition and distribution to be analyzed for current and longer-term population trends.

The host range covered by the surveillance data in my analysis is not sufficient to obtain a complete estimation for all ticks in Alberta. For example, the Argasidae were essentially absent from the surveillance. Tick species previously recovered, such as *I. spinipalpis* among others (2), were not recovered. The inclusion of a more diverse range of hosts should increase the number of tick species recovered. Recovery of ticks should also be expanded to include activities such as flagging and trapping small animals, such as mice. A thorough examination of smaller hosts should result in the recovery of greater numbers of immature life cycle stages. These additional activities will most likely identify tick species not recovered by my surveillance on dogs and also indicate which, if any, species can no

longer be recovered in Alberta from among the previously reported species that I did not recover.

Current modelling techniques, such as Maxent, are powerful research tools and have the potential to generate very useful information on ticks. Knowledge of the environmental limits and requirements of a species, including microhabitat conditions, can then be used to refine the models. Such limits need to be determined for ticks recovered in Alberta. Drew and Samuel (8,9) determined some conditions required for *D. albipictus* reproduction in field and laboratory settings. Such work needs to be expanded and also carried out on the other tick species I identified. Ideally, a determination of the environmental minimum and maximum requirements of each tick species for a complete life cycle should be determined in the laboratory and the field. As much as possible, these experiments should be conducted on ticks recovered from known locations in Alberta. This will allow future modelling efforts to use the most relevant variables and allow modelling to be carried out within a known range of potential conditions. This could result in some areas without samples being excluded from use in the background, leading to a model less biased by pseudo-absence sites which did not fall within the potential range of suitable conditions. Note that this does not imply that all conditions within the minimum and maximum values are suitable, just that there are absolute conditions beyond which completion of the life cycle is not possible. The modeling I conducted using the Worldclim temperature and precipitation layers could be considered a general approach that is biased by the inclusion of sites in the background that were never suitable. I suggest that future research should determine a customized set of environmental conditions per tick species.

Another addition that future modelling efforts may include are data on host presence. Such data would allow a further refinement of the geographic range of a tick species. The tick species I identified have a wide host range, so multiple host species would have to be included in such a modelling effort. If we can identify which areas in the province lack the hosts for a tick to complete its life cycle, then those areas could be excluded from future modeling regardless of the environmental conditions at those sites. Projection of models onto future climate scenarios is another modelling effort that could be undertaken.

There are many pathogens that the ticks found in Alberta can potentially harbour and transmit to humans, pets and/or livestock. More information is needed to determine which of these pathogens occur in Alberta and where. My research has determined where certain vector species are currently found, and indicated that *B. burgdorferi* can be found in Alberta. But this is only a first step to determining the risk that tick-borne pathogens present to human health.

Direct testing of recovered ticks is needed for pathogens such as *R. rickettsii*, *A. marginale*, *Ehrlichia chaffeensis* (Ehrlichiosis), and many others. Many of these pathogens have the potential to expand their host ranges among ticks found in Alberta, and the presence of the other pathogens should also be investigated in tick and wildlife species. Humans, pets, livestock and wildlife should be tested for exposure. If a pathogen is found, the tick range predicted by the models can be used as a proxy for the geographic areas of risk for transmission. Effective monitoring of this scale will require efficient collaboration between researchers and veterinary and public health workers.

Another 40 years cannot pass before the province-wide tick fauna is re-examined. If the distribution of ticks is changing, we must monitor and constantly reassess the challenges posed by any shift in tick distributions, establishments of new tick species, or introduction of new pathogens.

4.3. References

(1) Gregson JD. The Ixodoidea of Canada. Sci Serv, Ent Div Canad Dept Agric Ottawa, Publ 1956;930:1-92.

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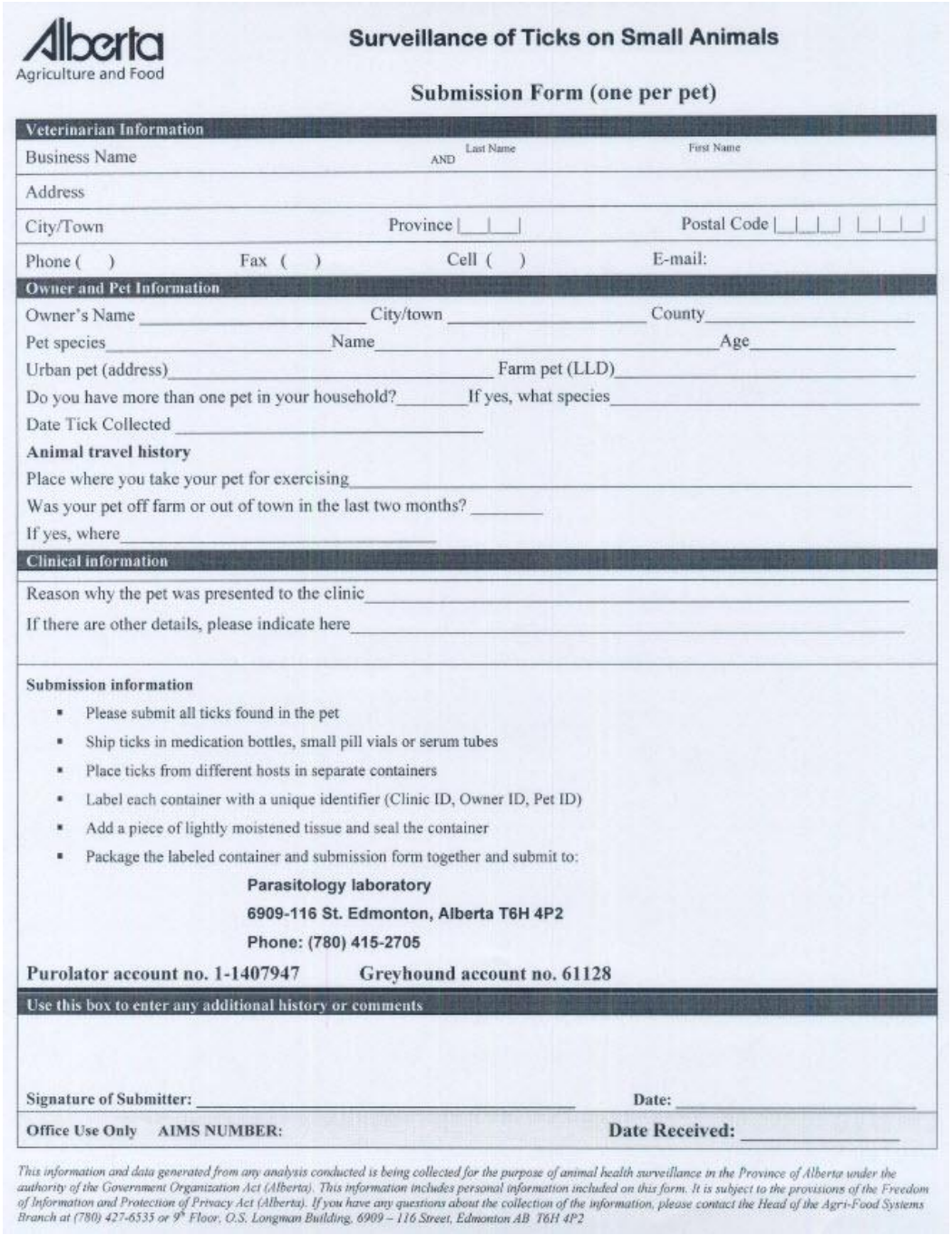
Chapter 5. Appendices

5.1. History sheet version 1



Alberta
Agriculture and Food

Surveillance of Ticks on Small Animals

Submission Form (one per pet)

Veterinarian Information

Business Name AND Last Name First Name

Address

City/Town Province |__|__| Postal Code |__|__|__| |__|__|__|

Phone ( ) Fax ( ) Cell ( ) E-mail:

Owner and Pet Information

Owner's Name _____ City/town _____ County _____

Pet species _____ Name _____ Age _____

Urban pet (address) _____ Farm pet (LLD) _____

Do you have more than one pet in your household? _____ If yes, what species _____

Date Tick Collected _____

Animal travel history

Place where you take your pet for exercising _____

Was your pet off farm or out of town in the last two months? _____

If yes, where _____

Clinical information

Reason why the pet was presented to the clinic _____

If there are other details, please indicate here _____

Submission information

- Please submit all ticks found in the pet
- Ship ticks in medication bottles, small pill vials or serum tubes
- Place ticks from different hosts in separate containers
- Label each container with a unique identifier (Clinic ID, Owner ID, Pet ID)
- Add a piece of lightly moistened tissue and seal the container
- Package the labeled container and submission form together and submit to:

Parasitology laboratory
6909-116 St. Edmonton, Alberta T6H 4P2
Phone: (780) 415-2705

Purolator account no. 1-1407947 **Greyhound account no. 61128**

Use this box to enter any additional history or comments

Signature of Submitter: _____ **Date:** _____

Office Use Only **AIMS NUMBER:** **Date Received:** _____

This information and data generated from any analysis conducted is being collected for the purpose of animal health surveillance in the Province of Alberta under the authority of the Government Organization Act (Alberta). This information includes personal information included on this form. It is subject to the provisions of the Freedom of Information and Protection of Privacy Act (Alberta). If you have any questions about the collection of the information, please contact the Head of the Agri-Food Systems Branch at (780) 427-6535 or 9th Floor, O.S. Longman Building, 6909 – 116 Street, Edmonton AB T6H 4P2