UNDERSTANDING ANIMAL MOVEMENTS TO INFORM BOVINE TUBERCULOSIS SURVEILLANCE

A FRAMEWORK FOR A TARGETED APPROACH

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JOÃO PAULO DO ROSÁRIO RIBEIRO LIMA

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SCOTT J. WELLS

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This dissertation is dedicated to my wife Maria. Thank you for your support, dedication and sacrifice so I could accomplish this professional goal.

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ABSTRACT

Bovine tuberculosis (bTB) is a zoonotic disease that affects domestic and wildlife species, with great impact in human, animal and wildlife health. In recent years in the US, a novel scenario for bTB has arisen, characterized by sporadic outbreaks in cattle populations across the country and by spillover of the infection to wildlife. The cattle production system in the US continues to change, with the number of cattle farms declining but the number of cattle per farm increasing. A consequence of cattle farms expanding in herd size, associated with better roads and transportation vehicles, is the potential for cattle to be moved great distances. Also the drought has encouraged a large number of cattle to move from severely affected southern states to northern states, such as Minnesota with available feed and water resources. This reality poses a threat to the biosecurity of the Minnesota cattle population and agricultural industry, due to the risk of introduction of bTB from which the state is now free.

The current bTB surveillance system is extremely costly and new science-based surveillance approaches are needed in order to respond to a novel disease scenario. The underlying objective of the studies presented here was to develop a new framework for bTB surveillance based on risk profiling of herds and regions. The fundamental concept from which the current work originates is that infectious disease transmission is not a random process but is modulated by risk factors that enhance its occurrence. The primarily concern in disease-free areas such as the state of Minnesota should be the

identification of those key players, in this case farms and/or regions, that are at higher

risk of disease introduction, and secondly to identify those farms and/or regions that

would cause greater impact in the cattle population and wildlife populations if infected.

The ultimate goal is to develop a targeted approach to bTB control and

surveillance in order to increase system accuracy and cost-effectiveness which, although

effective in the past, has failed recently to prevent continuing outbreaks in US cattle and

wildlife populations. One significant issue with the National bTB surveillance system is

the inherent challenge of detecting rare cases with the current diagnostic test. These tests

are laborious and untimely. In addition, the disease is easily spread among cattle and

wildlife, especially when animals congregate.

In Chapter 1, pathways for transmission and risk factors for bTB are reviewed,

emphasizing the epidemiologic triad: pathogen, host and environment and how those

relate to each other in impacting disease transmission. The agent that causes bTB,

Mycobacterium bovis (*M. bovis*), is a multi-host pathogen that survives well in the

environment. Under optimum conditions of temperature and humidity, *M. bovis* can

survive for long periods of time in different substrates, including feedstuffs. Since *M.

bovis* can infect several species, the transmission risks to cattle come not only from cattle,

but also from wildlife. In fact, in countries where disease spilled-over from cattle to

wildlife species disease control strategies have failed to eradicate bTB. This is because

wildlife has become the maintenance hosts for bTB. In the US (in both Michigan and

Minnesota), the wildlife species of concern is the white-tailed deer, although with

different resulting consequences. Humans are also susceptible to *M. bovis* and in some circumstances be a source of infection to cattle. Many studies have explored risk factors associated with bTB risk at the individual, farm and regional level. The main causes linked with outbreaks of bTB in cattle are the introduction of infected cattle through cattle movements and the sharing of ecosystems between cattle farms and wildlife. This is the current situation in the US; infected cattle were introduced from Mexico and transported across the US leading to outbreaks in several states. States with persistent and greater number of herds affected were those where wildlife was also affected. Understanding these transmission pathways and risk factors for bTB transmission to susceptible cattle populations is important. Several studies were planned in order to identify the risk factors of farms and regions for a targeted surveillance approach, specifically for the state of Minnesota.

In Chapter 2, using inter-state cattle movement data for the years 2009 and 2011, a risk model was developed by profiling cattle import movement to MN based on the type of cattle (beef or dairy), cattle production purpose (breeding or feeder), state of origin and size of the movement. Only about 5% of cattle premises in MN (total cattle premises = 27,406) had import cattle movements in either year with only 2% being classified in higher risk categories. In addition to identifying high risk farms, the model also identified high risk zones for bTB introduction in MN based on the density of farms in these higher risk categories.

In Chapter 3, by using complete movement data available from the MN Board of Animal Health (BAH) from the previously bTB infected area in northwestern MN, a risk score for disease introduction at farm level, founded on network analysis parameters and risk factors from the published literature, was constructed. The availability of complete movement data for cattle farms in a region allowed the development of a directed network of cattle movements for the first time in the US. The risk model targeted surveillance and also identified those farms that, if infected, would have greatest potential to facilitate ongoing transmission due to their connectivity with other farms. Furthermore the network analysis elucidated on the 2005 bTB outbreak and helped explain, due to the low connectivity of cattle farms in the affected region, why only 12 farms were affected after more than 5 years of introduction of an infected animal.

In Chapters 4 and 5, the specific objective was to evaluate risks of interaction between white-tailed deer in a previously infected area for bTB, defined as a transition zone between forest and agricultural land, where knowledge on deer ecology is limited. A total of 21 white-tailed deer were captured, in two different capture efforts, fitted with global positioning system (GPS) collars and followed up to a maximum of 15 months, dependent on collar functionality and mortality of the deer. The selected study area contained 25 cattle farms. During the study period, at 4 different times, the exact locations of cattle, cattle feeding sites and stored feed were geographically identified, and these locations were classified as farm locations. Study results showed that 25% of deer visited 20% of the cattle farms, with 70% of the locations within farms occurring in areas

where cattle were present. Deer visits occurred mostly during the night and from March to April. This study provided baseline information regarding cattle-deer interactions critical to transmission of bTB.

In Chapter 5, the objective was to evaluate a modeling approach on the risk for cattle-deer interactions based upon the landscape surrounding any given farm. Model results demonstrated that the proportion of agriculture land within a 500 meters radius was the variable with the greatest effect on resource selection, with a negative impact. On average there was a tendency for stronger selection for open areas outside forested areas in early Spring and Fall. Broad-scale patterns in the distribution of resource selection values show that the eastern portion of the study area has higher values, corresponding to higher densities of forest land. Also, five of the six cattle farms with deer visits, described in the previous chapter, had locations overlap with higher resource selection areas. In this study we show that the type of landscape surrounding a cattle farm can predict risk of greater deer-cattle interactions. Understanding factors that drive habitat use by deer can aid in the implementation of targeted approaches for infectious disease surveillance and control.

This combination of the studies provides a holistic perspective to bTB surveillance, especially at the wildlife and cattle interface. It develops a framework for a targeted surveillance system by developing approaches to identify at risk cattle premises and regions where disease is both more likely to be introduced and spread.

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CHAPTER 1 - PATHWAYS AND RISK FACTORS FOR BOVINE TUBERCULOSIS TRANSMISSION TO CATTLE: A FRAMEWORK FOR A RISK-BASED SURVEILLANCE SYSTEM

The objectives of this review paper were to identify the potential pathways of transmission of bovine tuberculosis (bTB) to cattle in the United States, and, based on published literature and recent occurrences of bTB outbreaks; identify the risk factors at the animal, herd and regional levels which maximize risk for bTB transmission and spread. The concept proposed aims to develop a framework for a risk based surveillance approach to bTB, in order to minimize cost and improve the ability of the system to detect early cases of disease by focusing on high risk herds and regions. This framework is essential to control a disease that continues to be a threat to the US cattle population even at very low levels of prevalence, not only for animal health-related consequences but also for the enormous economic burden resulting from outbreak occurrences. This is not only important for the government agencies and producers but also for the country as a whole due to potential trade restrictions. Bovine tuberculosis is a complex disease affecting a wide variety of hosts, domestic and wild species, and also constitutes a risk for humans due to its zoonotic potential. Under the current bTB situation in the US, a new approach to surveillance is needed in order to maximize detection and minimize overall control costs.

1.1. Introduction

The US National Bovine Tuberculosis Eradication Program started in 1917 and more than ninety years later, great advances have been made in reducing the disease to sporadic cases, but complete eradication continues to be a challenge. Recently, bTB cases have been reported in several states, including Minnesota, California, Michigan, New Mexico and Texas which at least temporarily lost their bTB accredited-free status as a result [1–3].

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* (*M. bovis*), is a chronic disease that affects livestock and wildlife but also constitutes a threat to humans due to its zoonotic potential. Spillover of bTB in humans is due to the lack of control measures for animal reservoirs and to associated co-morbidities such as HIV among susceptible individuals [4–12]

Control of bTB in cattle is complicated by the epidemiology of *M. bovis,* with differing patterns of transmission and host range [7]. The importance of the wildlife reservoir in the maintenance of this disease is evident from the fact that bTB has not been eradicated, even with aggressive control strategies applied, in countries like the United Kingdom, New Zealand and the United States [13–20].

Slaughter surveillance is the main tool of the bTB eradication program, and individual animal testing is often implemented only in response to detected cases or prior to cattle being moved across state boundaries [2, 21]. Slaughter surveillance is insensitive to detecting infected cattle in early stages of disease in part due to its reliance on detection of visual lesions at slaughter. Because bTB occurs infrequently in the US, there

is a risk for a lower level of awareness of inspectors to early stage lesions not easily

detectable [22]. This constitutes a primary problem of the existing national bTB

surveillance system, where, in the current low prevalence scenario; time from infection to

detection is extremely long (estimated median time of 5.75 years; [23]), with severe

consequences in terms of spread of disease to other cattle herds and wildlife.

While information is available from other locations in the world regarding bovine

tuberculosis, a thorough understanding of the disease in the United States is essential to

implement optimal national disease mitigation strategies, based on understanding of

transmission pathways and risk factors that maximize disease risk. Knowledge of these

risk factors could facilitate targeted surveillance of herds and regions at greater risk for

infection, thereby increasing the probability of early detection. This task is indispensable,

particularly with limitations of available resources for animal health surveillance and

control programs [24].

Therefore, the objective of this comprehensive narrative review was to

characterize bTB under the framework of the "epidemiologic triad" - pathogen, host and

ecosystem, including identify risk factors for occurrence in cattle, in order to frame new

risk-based approaches to bTB surveillance.

1.2. The pathogen: *Mycobacterium bovis*

1.2.1. Agent factors

Mycobacterium bovis is part of the *Mycobacterium tuberculosis complex*. Particle

sizes range from 0.5-7 μm, which is of sufficient size to be carried to the alveoli in

aerosol droplet nuclei [15] . The cell wall contains several complex lipids, which contribute to its relative resistance to acids, alkalis, and desiccation [25].

Mycobacterium bovis originated from a progenitor of the *Mycobacterium tuberculosis complex* and, likely through a mechanism of gene decay and deletion, has a smaller genome size than *Mycobacterium tuberculosis*, but is still >99.5 % identical to the agent that causes human TB [26].

1.2.2 Survivability

Several investigators have examined the ability of *M. bovis* to remain viable in the environment by performing studies under variable conditions. Morris et al., 1994, after summarizing findings of several studies, reported that: organism survival increased with reduced temperature and reduced UV exposure, with higher relative humidity and in the presence of organic matter. *M. bovis* in favorable conditions could be viable up to 2 years. Using a different approach, a New Zealand study simulated environmental contamination by inoculating cotton ribbons with *M. bovis* and placing them on pasture, forest floor, or possum dens. *M. bovis* was only isolated up to 4 days post-inoculation on pasture, but forest and possum den inoculates survived up to 14 days. No test strips had *M. bovis* isolated 28 days post-inoculation, which may have reflected a limitation of the method for isolation of an intracellular pathogen. Also, and consistent with previous studies, survival increased as mean daily temperature decreased [27]. In studies performed in the 1920's in England, fecal material collected from cattle shedding *M. bovis* in feces was spread on pastures in patches to approximate fecal contamination and cultured on monthly intervals. Survival in pasture varied from 1.5-7 months, with longer

survival times observed in the winter and autumn [28]. In a more recent study from Ireland, soils were sampled from badger sets and pastures of an affected Irish farm after depopulation of both cattle and badgers. One year later, after repopulation, the herd became infected again with bovine tuberculosis. Soil samples were then analyzed with PCR to detect *M. bovis* DNA and the presence of RNA, as an indicator of viable bacteria. Soil samples from badger sets and pasture were positive, indicating environmental persistence of *M. bovis.* This assumption was based on the absence of any cattle and also on the intense monitoring to avoid incoming badgers [29].

Another important aspect for potential transmission of *M. bovis* to cattle is survival in cattle feeds. Feedstuffs commonly provided as supplemental feed for deer (apples, corn, carrots, sugar beets, hay, and potatoes) were inoculated with *M. bovis* and stored at a variety of temperatures. Bacteria were successfully isolated from all samples after 7 days post-inoculation and survived as long as 112 days at freezing temperatures [30]. It should be noted that several of the above tested feeds are commonly fed to cattle, and this study demonstrated *M. bovis* survivability if inoculated in a variety of potential substrates. A Michigan study similarly evaluated survival of *M. bovis* in environmental substrates such as corn, hay, soil and water. The results showed that *M. bovis* can survive in favorable environmental conditions up to 88 days in soil, 58 days in water and hay and 43 days in corn [31].

No studies that evaluated survival of *M. bovis* in other types of feeds were found in the published literature, although it has been assumed that the low pH environment of silage may inhibit survival [15]. Overall, when considering feed contamination, the probability of *M. bovis* survival of and ability to infect cattle will be strongly influenced

by the weather conditions, time of contamination until it is fed and also by any processing of the feed.

Another potential source of infection is water. In one study, water samples were collected daily from troughs in holding pens with experimentally infected African buffalo. No water samples cultured positive for *M. bovis*, although samples were positive for other environmental mycobacteria [32]. Palmer et al (2001), in a study with experimentally infected white-tailed deer, was unsuccessful in isolating *M. bovis* from water. These results seem to indicate a low probability of *M. bovis* transmission through water sources.

Additionally, in a UK study, virulent *M. bovis* was discovered to survive amebic encystment, potentially allowing the organism to withstand environmental stresses such as desiccation or disinfectants [34]. While the role of protozoa in the epidemiology of *M. bovis* remains to be determined, the results of this study suggest that protozoa may be an important environmental reservoir as has been demonstrated for other bacteria.

In summary, *M. bovis* has the ability to survive under a variety of environmental conditions and in different substrates, which constitutes additional challenges when considering transmission to cattle populations.

1.2.3. Hosts

Mycobacterium bovis is found in a broad range of hosts, from domestic animals to wildlife to humans. Among domestic animals, cattle, goats, sheep, pigs, horses, farmed deer, as well as dogs and cats have been identified as susceptible to *M. bovis*, though with different levels of occurrence and pathogenicity [4, 7, 10, 35]. Related to wildlife, two

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recent review articles identified more than 40 species of animals with global distribution from which *M. bovis* was isolated [11, 35].

Related to the US bTB situation, it is important to specifically mention those species that have negatively impacted disease control and eradication efforts due to their confirmed role as maintenance hosts of the disease. Maintenance hosts are defined as being able to self-sustain infection. This is different than spillover dead end hosts that cannot sustain infection. In the UK the European badger (*Meles meles*), the brushtail possum (*Trichosurus vulpecula*) in New Zealand, elk (*Cervus Canadensis*) and wood bison (*Bison bison athabascae*) in Canada, African buffalo (*Syncerus caffer*) in South Africa, wild boar (*Sus scrofa*) in Spain and the white-tail deer (*odoicoileus virginianus*) in the United States, have been shown to undermine control efforts in cattle populations [9, 14, 15, 36–38]. The importance of these particular or similar species in the transmission of bTB to cattle in North America is further addressed in this paper**.**

1.3. Routes of Infection

Mycobacterium bovis can infect cattle through the nasopharyngeal, gastrointestinal, respiratory, reproductive, or cutaneous routes. The most common infection route, based on evidence from frequency and location of lesions from experimental studies, is the respiratory route [4, 8, 15, 39, 40]. However, reliance on distribution of lesions alone may not necessarily serve as adequate proof of the transmission route and should be interpreted with caution. The potential exists for ingested particles to aerosolize and cause the lesion pattern already described [41].

Therefore when considering the potential routes of infection to cattle, the two routes considered the most important in the spread of the disease to cattle are the oral and the respiratory routes. In an infection study where calves were fed corn inoculated with *M. bovis*, tracheobronchial and mediastinal lymph nodes were most commonly affected, while mesenteric lymph nodes were not lesioned. Because some kinds of feed may tend to form dust or fine particles, cattle exposed to *M. bovis* orally may develop lesions in the head or thorax [30].

In a study performed in Ireland, 10 groups of cattle composed of one un-infected in-contact steer from herds with no history of *M. bovis* infection and 2 reactor steers (i.e. positive to the skin test) from herds infected with *M. bovis* were independently housed together. After one year, transmission to the in-contact animal occurred in 4 out of the 10 groups, with *M. bovis* being isolated from mediastinal, bronchial and retropharyngeal lymph nodes. The respiratory route was associated with transmission in 3 of the 4 groups, based on isolation of *M. bovis* in a sample of a post-mortem tracheal-bronchial lavage in a reactor animal [42]. A more recent study demonstrated that infective doses as small as 1 CFU instilled into the tonsil were adequate to establish pulmonary lesions in cattle, adding strength to the theory that cattle are highly susceptible to infection by the respiratory route [43].

A series of experimental infection trials performed in England in the 1930's addressed oral route exposures by grazing animals in experimentally contaminated pastures. First, a trial was performed where pastures were inoculated with an emulsion of tubercular bovine lungs. Three naïve calves grazed on the contaminated pasture, and a separate group of 3 calves was given contaminated cut grass indoors, and another 3

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control calves were kept on the research dairy farm. After 5 months, all study cattle grazing on the contaminated pasture and 2 calves fed infected cut grass developed reactions to bTB skin test, and were found to have lesions at slaughter (pleural, hepatic, and mesenteric lymph node enlargement), while the nonexposed controls remained negative [44]. In a later study, the same researchers created a more approximate environment of natural infection by grazing the pasture with bTB positive calves that were fed highly contaminated milk and were shedding *M. bovis* in feces. After three weeks, the calves were removed and naïve calves were placed on the plots. Although exposed guinea pigs became infected in some cases, no cattle in this study were successfully infected from pasture exposure [45]. These results seem to suggest a risk albeit a low risk of infection from oral exposure to feces.

As indicated above, several studies showed long-term survival of *M. bovis* in contaminated feed, which points toward the potential for the agent to be transmitted through ingestion of contaminated feed [33, 46]. Also, oral routes of exposure to *M. bovis* are important when considering young calves fed tuberculous milk [39, 47, 48]. Experimentally, a dose of 9.9 x 10^2 CFU's given orally over a 5 day period, corresponding to 5 x 10^3 CFU's total, caused infection in naïve calves in one study [46].

In summary, bTB can be transmitted by different direct routes as well as indirectly as a consequence of the length of survival in the environment. These characteristics highlight the potential of *M. bovis* to affect different animal species as well as challenges in disease eradication. Furthermore, it indicates the high risk for emergence of disease reservoirs in ecosystems shared by domestic and wildlife species.

1.4. Pathogenesis

Whichever route the bacillus is presented, infection results when macrophages engulf the bacteria where it multiplies, and serves to facilitate spread to regional lymphatic tissues or to other parts of the body. *M. bovis* can ultimately destroy the macrophage, which leads to the infection of other macrophages and contributes to the proliferation of infection. The mechanism of bacterial survival within the macrophage is related to the inhibition of the phagosome with the lysosome, which would digest and destroy the bacteria [49]. Another cellular mechanism that has a role in the immune response to mycobacteria is autophagy, which causes the degradation of intracellular components, including pathogens [50]. The initial infection originates the formation of a primary focus, which, in conjunction with the compromised regional lymph node, forms the primary complex. In some individuals, this primary focus can disappear and limit the progression of the disease [47]. In experimental studies, gross lesions were observed as soon as 14 days after infection (7-11 days microscopically; [51]).

The presence of free live bacilli, after macrophage death, leads to systemic spread of the disease and priming of the immune system for a systemic response. This attracts more macrophages and other active immune cells that will destroy most of the bacilli. The bacteria that survive are usually confined in the granuloma (the typical tuberculous lesion), with infected macrophages at the center surrounded by connective tissue and lymphocytes. In case of rupture, bacilli are freed and disease transmission can occur [10, 47].

Previous research indicates that 9-19% of infected cattle shed *M. bovis* in nasal or tracheal secretions compared to 1 to 5 % of infected cattle that shed the organism in milk [39]. Another study found that animals with more extensive pathological change were more likely to shed *M. bovis* from nasal mucus and therefore posed a greater threat of infecting other animals [52]. However, experimental inoculation studies have also indicated that there is a latent period between inoculation and initiation of shedding. Once initiated, shedding is intermittent for up to 38 weeks [53]. The occurrence of a latent period associated with a longer incubation period, common in mycobacterial diseases, constitutes an extra obstacle in the diagnosis of bovine tuberculosis. bTB tests involving humoral immunity are generally of limited use in cattle because antibody titers are inconsistent and rise only in late stages of infection [54–56].

1.5. Sources of transmission

Figure i summarizes each potential transmission pathway for cattle prioritized through assignment of the relative weight (shown by thickness of lines in figure) of each path based on current knowledge.

1.5.1. Cattle to cattle

In cattle herds with natural infection, and when a bTB surveillance program is in place, the number of bovine TB-positive animals tends to be low. This occurrence has led to the belief that bovine tuberculosis may have low cattle to cattle transmissibility, low numbers of bacilli shed by infected animals, and/or a large infectious dose required to initiate new infection in naïve animals [57]. Phillips et al. 2003 instead associated this

low within-herd transmission to the long incubation period of the disease. Although some animals become infectious immediately after infection, the immune response will limit the progression of the disease, and even harbor the organism, with a corresponding low risk of shedding and infectiousness.

Two studies using mathematical modeling to estimate within-herd transmission of bovine tuberculosis in cattle populations reached similar conclusions and reported very low reproductive numbers (R_0), typical of chronic diseases [58, 59]. However, the recent outbreaks in the US, particularly in California and New Mexico, showed that introductions of infected cattle into a herd is a strong risk factor for an outbreak of bovine tuberculosis, due to within-herd transmission [2]. The study by Costello et al., 1998, described above, showed that transmission is likely to occur from infected to naive cattle when housed together in a relatively small confined area, which increases the contact rate and the likelihood of exposure to infected excretions.

Within-herd transmission might be low, but in favorable conditions with infectious individuals and high animal densities, typical of modern cattle operations, high rates of transmission could be expected.

1.5.2. Transmission of infection from other wildlife to cattle

1.5.2.1. Transmission of infection from white-tailed deer (*Odocoileus virginianus*) to cattle

Bovine tuberculosis transmission in the US initially occurs among cattle, with spillover to wildlife in certain situations. Although still possible to transmit disease, a

greater risk exists if the spillover host becomes a maintenance host, capable of perpetuating the infection in its own species and increasing the risk for inter-species transmission [60]. White-tailed deer, based on the most recent outbreaks of bovine tuberculosis, have been identified as the most important wildlife species for transmission of *M. bovis* to cattle in the US.

In 1994, in Northeastern Michigan, a male white tailed deer was found with suspect granulomatous lesions and subsequently diagnosed with *M. bovis*. Later investigations recognized that tuberculosis was endemic among white-tailed deer in this area and furthermore, genetic evaluations of strains found were indistinguishable between isolates from deer and cattle from northern Michigan [61, 62].

Similarly, shortly after the first affected cattle herd in the state was discovered in northwestern Minnesota in 2005, surveillance of hunter-harvested deer detected *M. bovis*-positive deer in the "Minnesota TB Management Zone". With increased surveillance of deer, 27 white-tailed deer with lesions were ultimately confirmed as *M. bovis* by bacterial culture from this management zone. The apparent prevalence of bTB in free-ranging white-tailed deer in northwestern Minnesota was estimated at 0.2% [63].

Several research papers have described the extent of pathologic tuberculous lesions in deer, with the retropharyngeal lymph nodes most commonly affected. Gastro-intestinal involvement was reported in rare cases [61, 62, 64]. Experimental infection studies have described the extent of lesions in infected deer, either through aerosol exposure or intratonsilar instillation with varying doses of *M. bovis*. After aerosol exposure, deer became infected and lesions were mostly pulmonary, while deer inoculated by intratonsilar instillation with high or low doses also developed extracranial

and extrapulmonary lesions [33, 65, 66]. In these deer, *M. bovis* was cultured from locations outside of the head and thorax including mesenteric lymph nodes, liver, spleen, kidney, supramammary, iliac, prefemoral and popliteal lymph nodes. In addition, *M. bovis* was also isolated from fecal and urine samples from study deer at postmortem examination [33].

This distribution of lesions shows the potential for transmission of *M. bovis* with emphasis on the respiratory route. To the authors' knowledge, there have been no published studies that have demonstrated transmission of *M. bovis* between cattle and deer by direct respiratory contact. However, experimental studies have shown that both species are susceptible to infection when *M. bovis* is aerosolized, and white-tailed deer do excrete the bacterium in respiratory secretions [66, 67]. Therefore, it can be inferred that deer and cattle in close contact could transmit *M. bovis* by the respiratory route.

Another potential route of transmission is through feed contaminated with nasal/oral secretions or feces. In one study demonstrating deer to deer transmission, experimentally infected deer were housed together with naïve deer. All in-contact deer became infected with *M. bovis* that was also isolated from pelleted feed and hay samples, suggesting feed as a possible means of efficient transmission [33]. In another study evaluating transmission between deer, feed exposed to but not consumed by experimentally infected deer (n=4), was given to 4 naïve deer on a daily basis in a separate pen to control for any direct transmission. All naïve deer developed tuberculous lesions [68]. Similar approaches were used to evaluate indirect transmission between deer and cattle, including pen exchange between experimentally infected deer and naïve calves and by feeding calves feed that was offered to but not consumed by experimentally

infected deer. In both cases, calves became infected and developed typical tuberculous lesions, demonstrating the risk of indirect transmission between species [46].

In Michigan, it has been proposed that deer were able to become maintenance hosts for *M. bovis* because of increased population size associated with long-term winter feeding. In this situation, increased deer densities allowed for efficient intra- (between deer) and inter- (between deer and cattle) species contact rates [61, 69]. Conversely, in Minnesota, the immediate and intense mitigation measures applied (removing many deer from the local landscape) with the goal of reducing deer densities appeared to have decreased the opportunity for the disease to be maintained and to spread further within the deer population, therefore deer did not evolve to become a fully established maintenance host [63].

The analysis of bovine tuberculosis cases in Minnesota deer and cattle also showed spatial clustering between affected cattle herds and infected deer. A summary of the outbreak investigations for the first 11 positive cattle herds in Minnesota found only two herds had no confirmed contacts with other affected cattle herds, and infected deer were harvested within 2 miles of the positive cattle premises. The spoligotype of *M. bovis* isolated from both deer and cattle in Minnesota was indistinguishable, implying that transmission between species had occurred, indicating that the risk for the emergence of maintenance host in the white-tailed deer population existed [40].

Based on the studies above, and with no evidence of intermediate hosts on farm, direct respiratory spread and indirect oral/nasal through contaminated fomites are the most important pathways to be considered for transmission between cattle and free-ranging white-tailed deer.

1.5.2.2. Transmission of infection from other wildlife species to cattle

Other wildlife species in different parts of the globe have a similar role as white-tailed deer in maintaining and spreading the disease to domestic cattle populations. It is important to classify these events in specific wildlife populations in terms of their ability to become spillover or maintenance hosts. Both can transmit the disease to cattle but the maintenance host can maintain infection in a population by intra-species transmission while infection in a spillover host can only persist if re-introduction of disease occurs [4, 17, 70]. A reservoir of disease emerges if one or more maintenance hosts, of different or the same species, maintain and transmit the pathogen within and between populations [71]. In some situations, it is possible that infection in wildlife populations requires continuous re-introduction from cattle. If infection in cattle were eliminated, infection in wildlife may not persist, meaning that wildlife are not a true reservoir of disease [71].

The ability of *M. bovis* to persist in a maintenance host is dependent on several factors, including individual susceptibility, population density, and the presence of factors that contribute to horizontal transmission. Therefore, a spillover host can potentially become a maintenance host, depending on favorable conditions for effective disease transmission, or remain as dead-end host, where disease will ultimately disappear if no spillover re-occurs [17].

Corner (2006), in addressing the role of wild animal populations in the epidemiology of tuberculosis, identified the following steps as necessary for assessing the risk of wildlife populations infecting cattle: 1) evaluate the evidence for infection and its prevalence in a particular species, 2) evaluate lesion pattern and potential routes of

excretion that could infect cattle, and 3) evaluate the spatio-temporal factors that influence direct or indirect interactions between infected wildlife and cattle. The concept of "*force of infection*", which characterizes the number of susceptible and infected individuals and infectivity of each population (related to the infective dose) is extremely important for efficient disease transmission [72].

The following section identifies wildlife species which can constitute a potential source of infection for cattle in the US.

Cervids: Elk (*cervus elaphus*) and red deer (*cervus cervus*) - Elk can become infected with *M. bovis* and potentially serve as a source of infection to other species. Several authors have described the extent of gross lesions in red deer and elk and found that the most common locations of gross lesions were the lung, medial retropharyngeal lymph nodes, and the mesenteric lymph nodes [73–79]. A review of bTB in captive deer in New Zealand similarly reached the same conclusion. These locations of infection could relate to farm management practices and the likely route of infection between individuals. Deer in intensively managed deer herds tend to have respiratory lesions more frequently than those in extensively managed herds where a higher proportion of nasal-oral lesions predominate [78].

A report from Michigan, using data from 2002-2004 from all hunter-harvested elk, showed that 23 (~7%) of 334 elk tested had lesions considered suspicious for *M. bovis* infection. Two animals were culture-positive for *M. bovis*, although without gross lesions [80]. Another report indicated that five of 2,164 elk (~0.2%) were culture-positive from 1996 to 2006, although three of the six counties where elk were harvested had *M. bovis*-infected deer [81]. These data indicate that transmission of bTB from deer to elk is

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apparently rare, perhaps based on the fact that deer and elk maintain geo-spatial separation, even when located in the same geographical area [82]. Elk in Michigan are not considered a maintenance host for bTB [9, 80].

In North America (United States and Canada), cases of bovine tuberculosis have occurred in spatially adjacent cattle and elk. The direction of transmission, however, has not been clear [83–85]. In Riding Mountain National Park in Canada, indirect transmission was presumed to be the most likely route of transmission between cattle and elk, as a consequence of both species sharing the same food resources [84]. To date, no experimental infection trials involving elk have been published to demonstrate that infected elk can efficiently infect cattle, but empirical data provides strength to this hypothesis. If elk were a known risk, mitigation measures should be similar to those applied to prevent deer to cattle transmission.

Carnivores: Coyotes (*Canis latrans*), wolf (*Canis lupus*), fox (*Vulpes vulpes*), and bobcat (*Felis rufus*) - Carnivores can become exposed to bTB by consuming the tissues of infected animals. Long-term wildlife surveillance has found *M. bovis*-infected coyotes in the bTB-endemic region of Michigan [86, 87] . Gross lesions observed included nodules in the lung and enlarged mesenteric lymph nodes. In a cross-sectional study, 10 coyotes were trapped from each township within the bTB-endemic 4-county area of Michigan. The apparent prevalence of *M. bovis*-infected coyotes was 33%, with most lesions located in the gastrointestinal tract [88]. In contrast, no infected coyotes were found in the Riding Mountain National Park region of Manitoba, although two wolves were confirmed to be infected with the same strain found in elk and cattle of the area [89, 90] .

In a preliminary experimental infection trial in Michigan, coyotes were infected with 10^5 CFU of *M. bovis* orally, and no organism was cultured from the animals' tissues or feces, which appears to indicate a low risk of coyotes as a source for cattle infection [91]. Furthermore, there are few known direct or indirect interactions between cattle and coyotes. Both red foxes and bobcats have also been found to be infected with *M. bovis* in Michigan [9].

In summary, several carnivore species have been found with *M. bovis* infections in areas where they have access to infected prey or carcasses. Lesions tended to be localized to mesenteric lymph nodes and lung. No infection studies have been performed to explore the infectiousness of wild carnivores to other species, although the lack of disseminated disease suggests these animals are likely spillover dead end hosts [9].

Medium-sized mammals: Raccoon (*Procyon lotor*) and Opossums (*Didelphis virginiana*) - Both raccoons and opossums are opportunistic scavengers, and are often found around farm buildings, potentially eating cattle feed, particularly corn. Surveillance of raccoons in the bTB-endemic region of Michigan has found a small number with *M. bovis* infections [9, 87]. Experimental infection trials have demonstrated that raccoons were able to reliably develop infection when given high doses for 5 consecutive days, although no *M. bovis* was isolated from fecal or urine samples [92]. Surveillance of opossums in the bTB-endemic region of Michigan has similarly found a small number of animals with *M. bovis* infection [9, 93]. Experimental infection trials have included inoculating opossums by intramuscular injection, oral dose in food, and aerosol exposure [94, 95]. While all inoculation routes produced culture-positive opossums, no animals became infected with disseminated disease, and fecal shedding was rare.

Although opossums and raccoons are susceptible to *M. bovis* infection and infected individuals have been discovered in the wild, the lack of disseminated disease and the low incidence of fecal shedding observed suggest such species are spillover dead-end hosts.

It is important to distinguish the brush tail possum (*Trichosorus Vulpecula*) that has an important role as a maintenance host for bovine tuberculosis in New Zealand with the North American opossum. Although in the same order, *Marsupialia*, they belong to different families [96]. While sharing similarities (being nocturnal and omnivorous), their potential for disease transmission appears to be quite different.

Small rodents - mouse (*Mus musculus*), vole (*Microtus pennsylvanicus*), and rat (*Rattus norvegicus*) - Small rodents can be ubiquitous on farms, particularly if large stores of grain are present. In an experimental infection study, voles, mice and rats were inoculated with *M. bovis* [97]. Intranasal and oral inoculation with a dose of 10^5 CFU was sufficient to produce detectable *M. bovis* infection in more than half the voles and mice, although only one rat developed infection. Several voles also had *M. bovis* isolated from their fecal material. Voles and mice have demonstrated susceptibility to *M. bovis* under experimental conditions, and infected voles can shed *M. bovis* in their feces. Rodent sampling in the bTB-endemic El Paso area of Texas and in East Sussex, England however did not yield any positive cases of *M. bovis* infection [98, 99]. Extensive sampling has not been performed in bTB-endemic regions to determine the extent of infected individuals, so it is unknown if natural conditions promotes disease transmission to and within these populations.

Rabbits (family *Leporidae*) - Rabbits have long been used in experimental conditions as a model for tuberculosis pathogenesis [4]. However, surveillance has not detected infected rabbits in natural settings. In one example, a survey of mammals living in a bTB-endemic region of England included several hundred rabbits. No lesions or culture-positive tissues were found [98]. Such surveillance has not been reported in the United States, so it is unknown if rabbits may be infected, or have the potential to serve as a maintenance host.

Birds - **Pigeons *(Columba livia)*, starlings *(Sturnus vulgaris)*, crows *(Corvus brachyrhynchos)*, and mallard ducks (*Anas platyrhynchos)*** are all frequently seen on farm operations. Experimental infection trials of these species did show that pigeons were susceptible to intratracheal and oral exposure. *M. bovis* was detected in the feces of one bird 60 days post-intratracheal inoculation and one day post oral inoculation [100]. Crows and starlings only developed lesions after receiving intraperitoneal inoculation; oral exposure did not induce lesions nor yield any culture-positive tissues or feces [101]. Mallard ducks were similarly not susceptible to infection after oral or intratracheal inoculation [102]. Pigeons were among the species sampled in the El Paso region for infection with *M. bovis*, but none were found to be infected [99]. Bird surveillance for *M. bovis* has not been reported in Michigan, and birds naturally infected with *M. bovis* have not been reported in the literature to the authors' knowledge.

Domestic dogs (*Canis lupus familiaris*) and cats (*Felis catus*) - Historically, infection with *M. bovis* has been reported in dogs and cats on bTB-positive cattle operations [4]. Similar to wild carnivores, domestic carnivores can exhibit scavenging

behavior of potentially infected species. In cattle herds with high levels of infection, exposure to unpasteurized milk carrying *M. bovis* is also a potential source.

A recent case report from the United Kingdom describes a dog that potentially became infected with *M. bovis* from its owner, through the respiratory route [103]. A semiferal cat in Michigan was diagnosed with *M. bovis* infection, with likely exposure to infected deer carcasses in the bTB-endemic region of the state [104]. A cross-sectional field study of dogs and cats residing on infected cattle farms however failed to find immunological and bacteriological evidence of infection with *M. bovis* [105]. A more recent report from England describes three different cases of *M. bovis* infection in dogs after being bitten by wildlife: a badger, a squirrel and a fox [106].

Similar to wild carnivores, domestic dogs and cats can potentially become infected with *M. bovis*, although it is not known whether they could shed the organism or constitute a source of infection. They are more likely to be defined as a spillover dead end host.

1.5.3. Transmission from humans to cattle

The routes by which humans can become infected with *M. bovis* are gastrointestinal, respiratory and direct contact through broken skin [7, 12, 107]. Historically, humans were infected mainly through ingestion of unpasteurized milk, and the resulting lesions were typically extrapulmonary. With the widespread adoption of milk pasteurization in developed countries, predominant syndromes of disease have switched from involving gastrointestinal or cervical lymph nodes to greater prominence of the pulmonary form followed by the genitourinary. In developing countries, the non-

pulmonary form continues to be the most common [108, 109]. This may reflect differences in routes of infection, with ingestion and handling of unpasteurized dairy products not constituting a major contributor to infection in developed countries with low disease prevalence in humans.

Human tuberculosis caused either by *M. tuberculosis* or *M. bovis* is often clinically and pathologically indistinguishable. Infected individuals may have a latent form of infection that evolves to active shedding form of disease without apparent symptoms [7, 109].

Human-to-human transmission of *M. bovis* is believed to be of low epidemiologic importance although a few case reports are published [107, 108]. In the United Kingdom, a cluster of six human bovine tuberculosis cases were reported and only one person in the cluster was confirmed to have consumed unpasteurized dairy products and occupational exposure to cattle, while the remaining cases had social contacts with one another [110]. This report supports the potential for human-to-human transmission of bovine tuberculosis via respiratory route, particularly in immunocompromised individuals. In this case study, four of the six people involved had predisposing conditions such as HIV infection, drug abuse, and diabetes. This risk of human-to-human transmission may be higher in developing countries, where the burden of HIV is enormous [5, 111].

Although infections with *M. bovis* in the United States are believed to comprise 1-2% of the total number of human TB complex cases, endemic tuberculosis in cattle populations is present in Mexico and other developing countries [112, 113]. Additionally, consumption of raw milk is widely practiced in Mexico, placing the population at risk of infection. As a probable consequence; Mexico has one of the highest rate of isolation of

M. bovis from symptomatic human tuberculous patients [114, 115]. Recently, a report from Mexico indicated that dairy farm workers exposed to *M. bovis* affected cattle had a higher prevalence of latent and pulmonary TB compared to non-exposed controls. In this study, two individuals had pulmonary TB caused by *M. bovis* and, in one of the cases, the strain spoligotype was identical to that from cattle on the same farm [116]. In San Diego County, California, epidemiologists reported 45% of children with tuberculosis were infected with *M. bovis*, and 6% of adult cases and nearly every case of *M. bovis* occurred in persons of Hispanic ethnicity [117]. The close contact between the Hispanic communities in San Diego and bordering Tijuana, Mexico was hypothesized to be an important factor in the occurrence of bovine tuberculosis in this region. A review of pediatric TB in the same area found extrapulmonary disease was more frequent in cases caused by *M. bovis* infection than those caused by *M. tuberculosis*, with both gastrointestinal and cervical lymph node forms observed, which indicates a likely mode of transmission to be consumption of contaminated dairy products [118].

As demonstrated above, humans infected with *M. bovis* may harbor granulomas in the respiratory, gastrointestinal, and genitourinary tracts. Viable bacteria have been demonstrated from the sputum and urine of tuberculous patients, though it is unknown if fecal shedding can occur. In a recent report, a veterinary nurse from the United Kingdom with a history of testing affected cattle herds developed a pulmonary infection of *M. bovis*. Her household dog also developed pulmonary disease and was culture-positive for *M. bovis* on postmortem [103]. This case, in addition to the human-to-human cluster mentioned above, demonstrates the potential for humans infected with *M. bovis* to transmit infection by the respiratory route.

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A few case reports link human infection to a cattle herd breakdown with bovine tuberculosis. A 72-year old man in Switzerland with a previous history of *M. bovis* infection as a child died with pulmonary and renal tuberculosis. The patient worked occasionally on a bTB-positive cattle herd that had no apparent link to affected cattle herds. Strain typing of the isolates from the human case and the cattle were similar, indicating that the man may have infected the herd [119]. In a 1965 paper, incidence of bTB in Danish herds detected by routine herd testing was compared with the occurrence of human cases of bovine tuberculosis, using epidemiologic links between the cattle herds and human cases to propose humans as the source of the herd breakdowns [109]. Also, a report from Germany described several cases in which human workers allegedly infected cattle through urination on cattle feeds [120]. More recently, in a case-control study performed in Ethiopia, a significant statistical association was reported between owners with active tuberculosis and increased tuberculin reactivity in their cattle, in comparison with herds where the owners did not have active tuberculosis. Also, both *M. bovis* and *M. tuberculosis* were isolated from cattle and human patients, clearly indicating the inter-species transmission of tuberculosis [121].

In two US studies, human contacts on dairies positive for bTB were investigated, though human infection with *M. bovis* was not demonstrated as a source of cattle infection. In the El Paso region, 124 people who had contact with animals or unpasteurized milk from bTB-affected and non-affected dairies were interviewed, skin-tested, and had a urine culture performed. Thirty-nine percent of study participants had a positive skin test (i.e. Mantoux), but did not have *M. bovis* isolated from urine or during follow-up testing [99]. In California, a questionnaire and tuberculin skin test was given to

78 people who either worked on the dairy and drank unpasteurized milk from the farm, or performed slaughter of infected cows during herd depopulation. Forty-two percent tested positive, with Mexican-born participants at 15.8 times more risk of a positive skin test when compared to US born individuals. Of the test-positive individuals, 61% received a chest radiograph but none had pulmonary lesions. It was concluded that no test-positive individuals had evidence of active (clinical) TB infection [122].

In summary, humans infected with *M. bovis* can develop infections in the respiratory, genitourinary, and gastrointestinal tracts. The best evidence for transmission of infection is by the respiratory route, although urinary shedding of bacteria also appears likely. Although transmission of tuberculosis from humans to cattle is possible, it is unknown how frequently persons infected with *M. bovis* would have direct or indirect contact with cattle in a manner that would be sufficient for TB transmission to cattle. Therefore the risk of human to cattle transmission of bTB is currently undefined.

1.6. Risk factors for BTb introduction and spread in a susceptible population

Identification of the risk factors for disease transmission is essential to prioritize resources in the context of a surveillance program [123]. In this section, we aim to distinguish between risk factors for disease and those that affect test results. The latter are not causal mechanisms for disease introduction into a susceptible population.

In only one U.S. study were herd-level risk factors identified. This was a case-control study of 17 positive herds and 51 location and size-matched controls located in Michigan. A statistically significant association between farm management factors and increased risk of positive bovine tuberculosis status was found, but only in univariate

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analysis. Factors that significantly increased risk of herd TB infection included increasing herd size, fence-line contact with other livestock, presence of ponds or creeks, loose hay fed to cattle, and hay stored outdoors. Factors that significantly decreased risk of herd TB infection were variables associated with limiting the access of deer to cattle areas, including use of barbed or electrified wire fencing, and cattle housing [124].

In studies from other countries, several authors have evaluated risk factors for test-positivity with consistent results observed. At the individual animal level, age is consistently a strong risk factor, with older cattle more likely to test positive, explainable by a longer exposure to *M. bovis* and also as a consequence of being tested more times than young cattle [125, 126]. Breed has also been identified as a significant factor, with cattle of *Bos Indicus* breeds less likely to test positive to the skin test than those of *Bos Taurus* breeds [7, 127]. A possible explanation, in addition to genetic host resistance, is that genetically improved cattle are placed under more stress due to production demands, which can lead to immunosuppression and increased susceptibility of infection. In addition, greater animal densities increase transmission rates, as demonstrated from a study performed in Tanzania in which a higher proportion of lactating animals, more likely to be confined, tested positive [6]. A study from England displayed similar results, where herds with dairy cows were more likely to test positive for bTB [128], likely related to the type of management and number of incoming movements that increase the risk for introduction of infected cattle to the herd. Sex was also identified as an important risk factor for a positive bTB test but also is directly related with management practices, such as retaining males longer than females in the herd, or vice-versa [6, 129, 130].

When aiming to identify risk factors for herd positivity, herd size in several papers has been identified as an important risk factor, with larger herds more likely to test positive [124, 128, 130–133]. This factor should be interpreted with caution, because larger farms are more likely to test positive as a consequence of testing multiple cattle with a test with non-perfect sensitivity [55, 134]. On the other hand, some studies have shown that the effect of larger herds is related to factors that could play a role in disease transmission, such as increased animal density and higher herd turnover leading to more purchased cattle [128]. The latter is a highly important risk factor, associated in several studies with increased probability for disease introduction at the farm level [125, 131, 132, 134–139]. These studies indicate that the importance of mechanistic management factors for disease transmission (i.e. cattle introduction and animal densities) versus risk factors that are only associated with a positive test result (i.e. herd size and type) [140].

Studies performed in countries struggling with the problem of a wildlife reservoir of *M. bovis* have reported that cattle herds closer to wildlife habitat are more likely to be infected compared to herds not exposed to this habitat [19, 124, 126, 134]. In the case-control study performed in Michigan described above, a significant difference was detected between the percentage of surrounding land classified as "open, non-agricultural" between case and control farms [124]. The proportion of forest cover surrounding cattle areas has been associated with more deer locations within those areas [141]. In Minnesota, a cross-sectional study evaluated the approximate percentage of surrounding land, and found that an increasing percentage of land classified as woods, swamp, or other wildlife habitat was significantly associated with increasing risk of deer accessing stored feeds on the farm [142].

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In order for disease transmission to occur between white-tailed deer and cattle, both species must be present in the same location in enough numbers to facilitate interactions between species [143]. A risk assessment carried out by USDA-APHIS-Veterinary Services evaluated the spatial association between positive deer and positive cattle herds in northwestern Minnesota, and found that positive deer were significantly clustered with one another, and all positive and suspect deer were within 6.8 miles of positive cattle herds [144].

A recent USDA-APHIS VS report summarized the current US situation for bTB by describing the different outbreaks that have occurred. The primary risk factors identified for California and New Mexico herds, which can be extended to states with similar management practices, were the import and mixing of Mexican-origin steers, management practices used by raisers of dairy replacement heifers, and the large numbers of purchased cattle. On the other hand, for a state like Michigan where only one *M. bovis* strain among cattle and deer was identified, the risk for transmission and spread of disease between both species appeared due to close contacts as well as sharing of a common source of contaminated feed or water [2]. This highlights the magnifying role that wildlife species such as the white tailed-deer can have in maintaining and spread the disease in domestic cattle [63, 145].

The summary of risk factors for bTB infection reported here has the advantage of framing this section in conjunction with different pathways of transmission. The statistical associations shown in the risk factor studies discussed are then understood under a causal path of transmission.

1.7. Summary of herd level and slaughter cases of bTB in the United States from 2001-2011

A description of bTB outbreaks in the US, including potential causes, characteristics of herds affected and areas affected, is extremely important to provide a basis from which to identify mechanistic risk factors critical for both the cattle population nationally and at the state level.

We obtained data on reported bTB cases in US cattle herds and at slaughter in cattle from 2001 to 2011 from the United States Department of Agriculture - Animal and Plant and Inspections Service - Veterinary Services (USDA-APHIS-VS). The outbreak data identified the state and year of the outbreak, the type (beef or dairy) and size of the positive herd, the number of reactors to the skin test by positive herd, and the reason for detection. The reason for detection for each herd was categorized as followed: Routine testing (live-animal testing that included regulatory testing required for movement, sale/market, herd accreditation, and diagnostic testing), Slaughter (herd identified because at least one infected animal from the herd was identified through slaughter surveillance), Trace (herd identified as a result of a trace investigation from an infected herd) and Wildlife (herds tested because infected wildlife was detected in region). Wildlife was involved only in the states of Michigan and Minnesota. Most of the affected herds from these states were attributed to wildlife since those herds were in an area where white-tailed deer were also infected with the same bTB strain and herds were subjected to a more intense testing protocol (area testing and annual testing). However, this did not indicate that the source of infection was always or only white-tailed deer. As described

earlier, in MN only two herds (out of twelve) were not connected to affected herds through fence line contacts or through cattle movements [40]. Similarly, in the state of MI, from a total of 49 affected herds from 1975 to 2010, 47 herds had a history of purchasing cattle before becoming positive, though white-tailed deer were suspected to be the cause of infection in 37 cattle farms [20].

From 2001-2011, 15 states were affected by bTB (Table i). When evaluating herd type (beef or dairy) and herd size, we propose that these variables are not causal risk factors but instead are related to a common type of production system (in terms of cattle type and herd size) in each of the states affected. Overall, 70% of affected US herds from 2001 to 2011 were predominantly beef cattle, 28% dairy and 2% mixed. Affected farms with ≤100 cattle (48% of total) were mostly beef cattle herds located in MI and MN, while the majority of farms with ≥ 1000 cattle (20% of total) were dairy cattle and were located in states bordering Mexico.

States where bTB spilled over into wildlife had a higher number of bTB affected herds than states without spillover to wildlife (Figure ii). Categorizing affected into the geographical regions of Border with Mexico, Great Plains, Midwest and Southeast allowed inference of the most likely pathways of transmission at a regional level. Based on the number of farms detected by tracebacks, states located in the Mexican border and Great Plains groups had most of the cases that likely originated introduction of an infected animal in susceptible cattle populations (cattle movements). In the Midwest, due to the spillover of infection into the wildlife population in MN and MI, direct and indirect transmission among deer and cattle was a likely source of infection, even though the origin was often imported cattle, at least in Minnesota.

The summary of US slaughter-detected cases highlights that most US slaughter cases originated in Mexico (Figure iii) during this time period and most were detected in the state of TX (Figure iv). USDA APHIS has reported that the large majority of cattle coming from Mexico and entering the United States enter to Arizona, California, New Mexico, Texas and Colorado [2]. However, an unknown number of cattle continue to move across the country reaching as far as Washington State [146].

The higher number of positive bTB herds found through traceback investigations and the higher number of bTB infected cattle found at slaughter were located in states bordering Mexico and Great Plains states (particularly southern states in this region), which generally correlates with the higher number of cattle imported to these states from Mexico. This indicates that these states continue to serve as ports of entry for infected bTB cattle in the US which is and should remain a focus for enhanced surveillance.

1.8. Conclusion

The summary of bTB outbreaks in the US and current knowledge about bTB transmission pathways highlights the need for further research on factors that increase the risk for bovine tuberculosis infections and between-herd spread in the US cattle population, with specific focus on cattle movements and cattle-wildlife interactions. While the primary source of bTB introduction to the US appears to be the introduction of infected cattle to herds, most likely originating from imported cattle from Mexico, the impact of bTB outbreaks may be amplified if that production system allows for interaction of cattle with a potential wildlife reservoir (i.e., the white-tailed deer in the U.S.).

The US currently lacks a comprehensive cattle identification system that would allow cattle to be fully traced which would facilitate control of disease outbreaks. Future research need to identify risk factors that increase the risk for disease introduction at the herd and regional level based on a profile of cattle movements. This can be achieved using data available on cattle movements from state and federal animal health agencies. With the goal of developing a new risk-based surveillance, it is essential to identify which herds and regions have higher risk for a disease outbreak so resources can be focused on these areas to improve early detection of the disease and reduce the overall cost of the surveillance program.

In regions where susceptible wildlife are known to be present and in contact with cattle, a better understanding of its ecology and characteristics of the ecosystem surrounding farms is essential, in order to understand which factors might increase the risk for cattle-deer interaction. This could be extremely useful, particularly when responding to an outbreak, since this data could inform which targeted areas for disease control should be of focus: wildlife population reductions, measures to mitigate cattle-wildlife interactions and also the delineation of buffer zones to contain an outbreak based on knowledge of animal movements.

In addition, is also necessary to test the efficacy of new diagnostic tests. Alone or in conjunction with other diagnostics, testing can increase the overall sensitivity of the surveillance system, and consequentially decreasing the time from infection to detection.

This review identifies pathways for bTB introduction and spread in susceptible cattle populations and recognizes wildlife as a central player in undermining control efforts, and more importantly frames the bTB transmission process in a holistic way. It is

the understanding of the epidemiologic triad (pathogen, host(s) and environment and the mechanistic risk factors), at the herd and regional levels, which modulate the relations between these components that will inform effective disease control programs. This will ultimately improve the likelihood of success in control of a multi-host zoonotic disease.

1.9. Figures
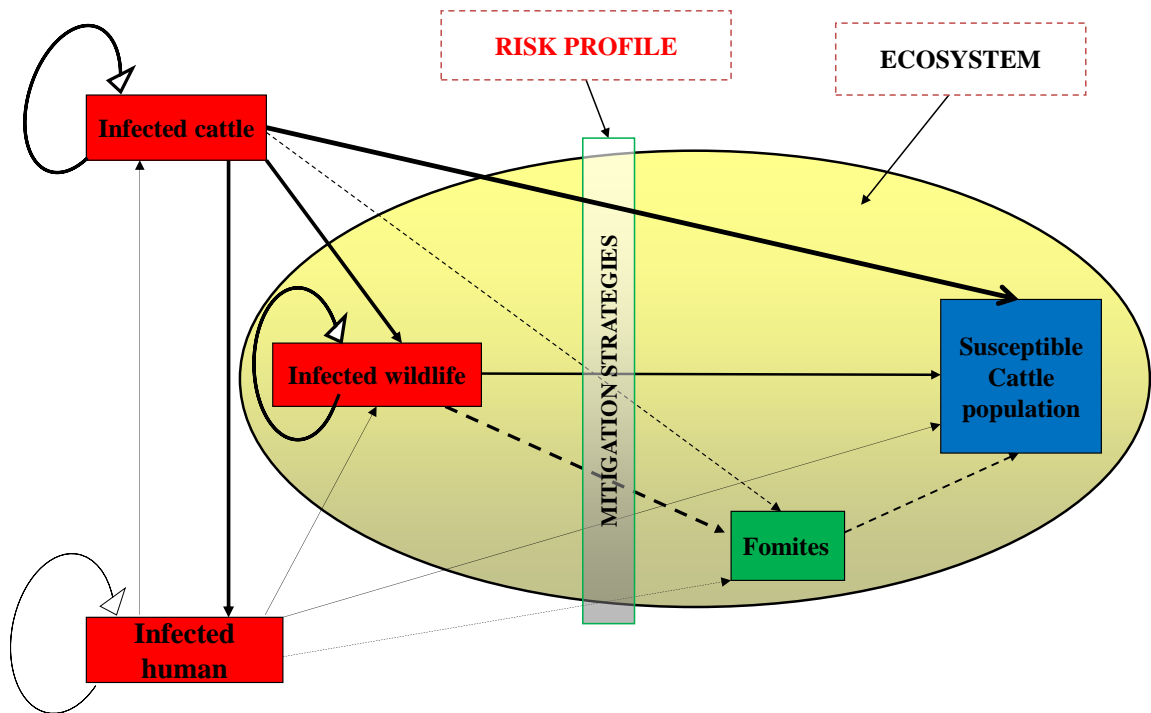
Figure i. Potential pathways of transmission of bovine tuberculosis to cattle

Line weights represent different strength of evidence for each pathway of transmission of

bTB to cattle. Dashed lines indicate indirect transmission and solid line direct

transmission.
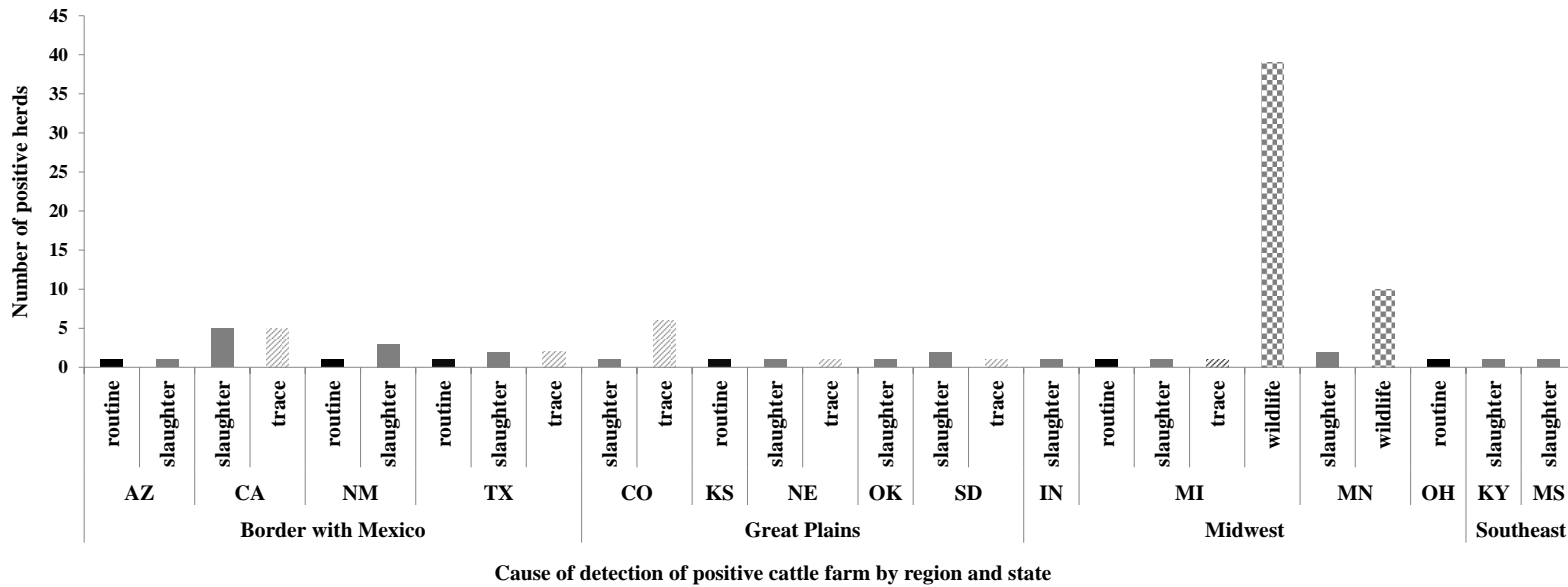
Figure ii. Summary of bovine tuberculosis herd level cases from 2001 -2011 in the United States by cause of detection of positive bTB cattle farm by region and state.
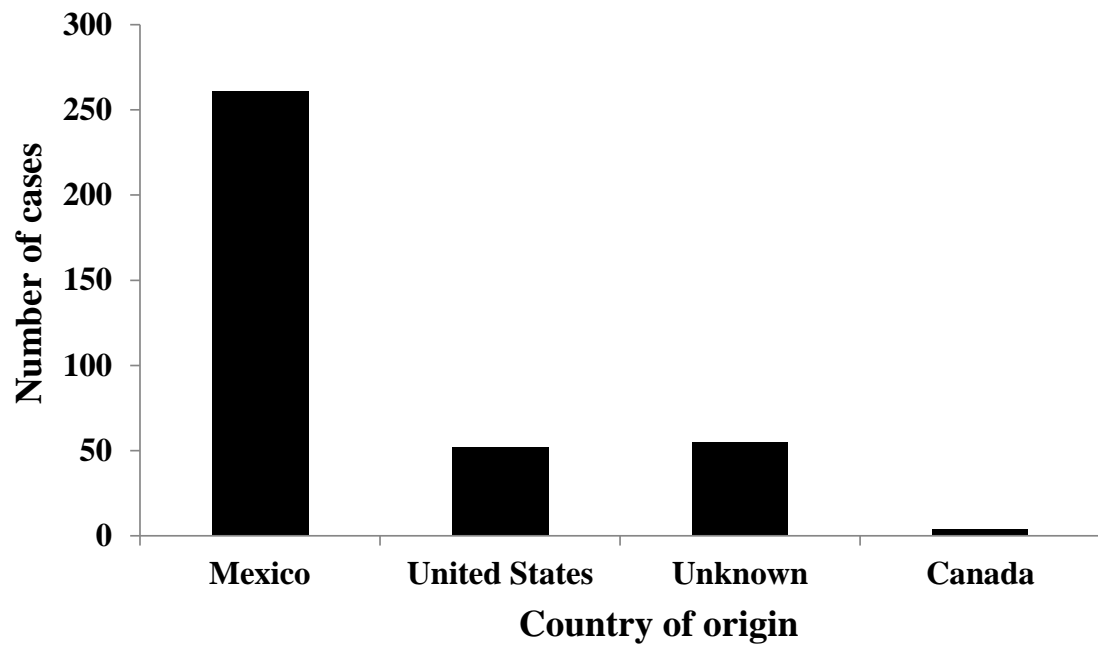
Figure iii. Summary of bovine tuberculosis slaughter cases from 2001 -2011 in the

United States by country of origin of cattle.
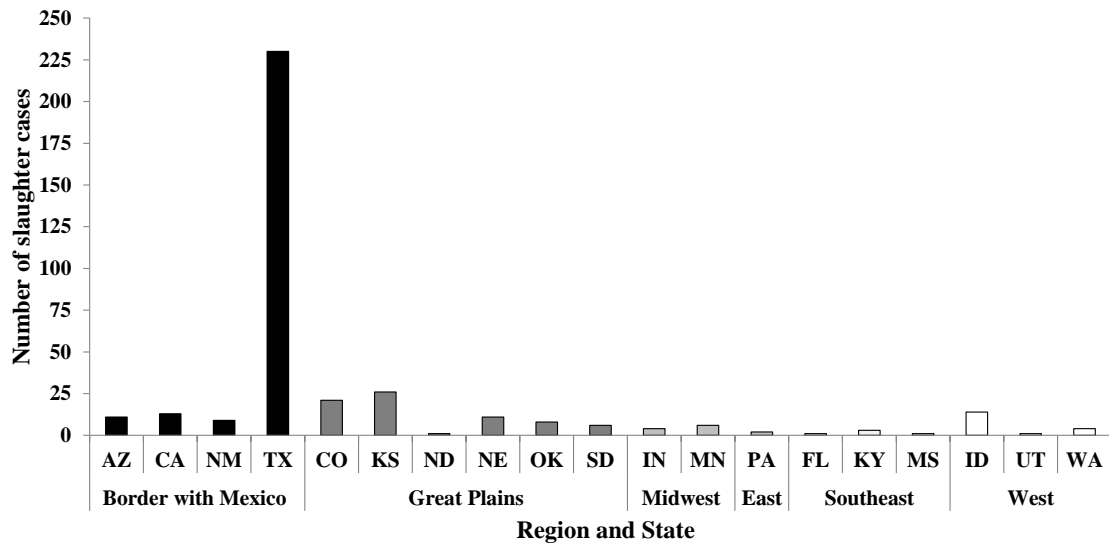
Figure iv. Summary of bovine tuberculosis slaughter cases from 2001 -2011 in the

United States by region and state of occurrence.

1.10. Tables

Table i. Summary of bTB herd level cases in the US by region, state, number of positive

herds and total number of reactor cattle to the skin test.

Region	State	bTB positive dairy herds (n)	bTB positive beef herds (n)	Reactors (n)
Border	AZ	1	1	3
	CA	10	0	213
	NM	3	0	180
	TX	2	2	128
Great Plains	CO	1	6	111
	KS	0	1	3
	OK	0	1	3
	NE	0	2	3
	SD	0	3	7
Midwest	IN	0	1	23
	OH	1	0	1
	MI	8	34	116
	MN	0	12	35
Southeast	MS	0	1	2
	KY	0	1	1

CHAPTER 2 - TEMPORAL AND SPATIAL PATTERNS OF CATTLE IMPORTS TO MINNESOTA FOR THE YEARS 2009 AND 2011: IDENTIFYING CATTLE FARMS AND AREAS FOR RISK-BASED SURVEILLANCE

In this study, a framework for a risk-based surveillance system for cattle herds was developed. This was achieved by identifying high risk herds and also high risk areas for bTB introduction into the state, which can direct resource allocation for a state animal health agency. Data on import interstate cattle movements was obtained from the Minnesota Board of Animal Health (MNBAH) for the years 2009 and 2011. Descriptive analysis was performed for both years 2009 and 2011. Movement data was summarized at the premises and county level and, for both levels, the distribution of cattle moved and number of movements was evaluated. In order to develop a strategy for a targeted surveillance system at the herd level for bTB in MN, each import movement was risk profiled based on known risk factors. The data was analyzed by fitting a linear regression model at the county level, to determine which variables associated with higher number of movements at the regional level. Results showed that, in each year, about 1500 MN herds had imported cattle. Most of the cattle imported were beef and feeder cattle and originated in northern US states. The peak of incoming cattle and import movements was the Fall season for both years. The risk model identified four risk groups. About 500 Minnesota cattle premises (~2% of total cattle farms in MN) were categorized in the Very High and High risk groups for either year. Premises were ranked, within category, by

number of incoming movements. The southeast and southwest regions of the state had the

highest density of cattle premises with import movements and also of cattle premises in

the higher risk groups. This study identifies an approach for risk-based surveillance for

bTB using available data and outlines a method that can be applied in other locations and

in other disease scenarios.

2.1. Introduction

The cattle production system in the United States (US) is constantly changing,

with number of cattle farms dropping but the number of cattle per farm increasing

through time [147, 148]. A consequence of cattle farms being larger and also more

specialized, along with improved roads and vehicles, is that cattle are moved long

distances for production and trade purposes [149]. More recently, an additional factor that

has promoted a large number of cattle moving from south to north in the US was a

drought [150]. This severely affects the southwest part of the country and makes

Minnesota (MN) and other northern states very attractive for agriculture and raising cattle

due to its water resources. This situation poses a biosecurity threat to the MN cattle

population, due to the risk of introduction of disease for which the state is now free.

In recent years, several US states have reported bovine tuberculosis (bTB) cases

in cattle herds which led to the loss of bTB accredited-free status. When herds test

positive, livestock producers have been strongly encouraged to depopulate their herds,

with indemnification costing millions of dollars annually to producers and government [151]. In addition, livestock trade is severely restricted in affected regions. These dramatic regulatory responses occur even when prevalence of the disease is low; a single detected animal with bTB can ruin producers' livelihoods and a few cases restrict trade across entire regions. The US bTB eradication program is primarily funded by appropriated federal funds, which until recently remained at about $15 million per year since 2003 and is usually augmented by additional emergency funds, obtained by the United States Department of Agriculture, Animal Plant Health and Inspection Service, Veterinary Services (USDA-APHIS-VS), estimated at $207 million per year since 2001 [24].

Slaughter surveillance is the main tool of the bTB surveillance and eradication program, and individual animal testing is only implemented either in response to an outbreak or prior to cattle being moved across state boundaries [2, 21]. Slaughter surveillance is insensitive to detecting infected cattle in early stages of disease and because bTB is a rare disease there is the risk for a lower level of awareness of inspectors to early stage lesions [22]. This constitutes one of the main problems of the existing national bTB surveillance system, where in the current low prevalence scenario; time from infection to detection is extremely long (estimated median time of 5.75 years; [23]), with severe consequences in terms of the spread of disease to other cattle herds and wildlife. In addition, slaughter surveillance has a differential coverage of herd sizes and

cattle types. A lower percentage of cows from beef cattle herds are culled each year to slaughter (less than 10% on average) compared to dairy herds (25-30%). Similarly, fewer cattle are shipped to slaughter from smaller herds compared to larger herds. A recent report estimated for a cattle herd with <50 cows, over a 5 year surveillance period, a 15% likelihood of detection for an infected beef cattle herd versus 20% for an infected dairy herd, and for a herd with ≥500 cattle the likelihood of detection was of 35% for a beef cattle herd versus 99% for a dairy herd [152].

In an assessment conducted by USDA APHIS the importation and comingling of Mexican-origin steers, the management and biosecurity practices used by calf raisers for dairy replacement heifers, and the influx of purchased additions were identified as risk factors associated with bTB infection in US areas without a wildlife reservoir [2]. The role of cattle movements has been identified of primary importance, particularly in low prevalence or disease-free areas, where disease can be introduced through the importation of cattle from infected areas [137, 153, 154].

The USDA APHIS in 2009 proposed a new approach to manage bTB in order to respond to a novel scenario of this disease in the US, characterized by low prevalence of bTB in cattle herds, most slaughter identified infected bTB cattle from Mexican origin, fewer and larger cattle herds with cattle being moved across large distances and the risk of spillover to wildlife. The surveillance system, according to the same document, needs to focus in reducing the risk of introduction of bTB into the U.S. from imported animals

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and wildlife; improve surveillance for bTB and move from a State classification system in terms of bTB status to a science-based zoning approach to address disease risk [24].

The major obstacle in implementing any of these measures in the US is the lack of a comprehensive national cattle identification and traceability system. This challenges the states' abilities to trace infected cattle (reported to be successful in only 70% of bTB occurrences; [21]), identify infected herds and also implement compartmentalization or zoning approaches when responding to a disease outbreak. The World Organization for Animal Health states, in its Terrestrial Animal Health Code, that there is a strong correlation between the existence of animal identification systems and the success of traceability of animals and products of animal origin [155]. In the US, most cattle movements across state borders need to be identified through a Certificate of Veterinary Inspection and reported to state animal health agencies [2]. This constitutes the only official document that controls inter-state cattle movements in the US, although its application to inform disease surveillance has been limited [156].

In the current climate of budget constraints, there is a need to develop more efficient surveillance strategies that respond to the new challenges of disease control. This can be achieved by focusing on the strata of the population more likely to have disease as a consequence of their risk of profile [123, 157]. In this study, we propose to develop a framework for a risk-based surveillance system for cattle herds by creating a risk profile of each import cattle movement to the state of MN. This approach will

identify high risk herds for bTB introduction into the state of MN and identify areas at

higher risk for disease introduction, which can direct resource allocation for the state

animal health agency.

2.2. Material and Methods

2.2.1. Data collection

2.2.1.1. Cattle movement data for Minnesota imports

Data on import interstate cattle movements was obtained from the Minnesota

Board of Animal Health (MNBAH) for the years 2009 and 2011. Cattle imported into the

state of MN must be accompanied by a Certificate of Veterinary Inspection (CVI) issued

by an accredited veterinarian, with a few exceptions. In addition to a CVI, breeding

cattle, rodeo cattle, and all cattle for exhibition entering the state of MN must be

officially identified. Breeding cattle was defined by the MNBAH as all cattle except the

following (classified as feeder cattle): heifers of beef breed less than 18 months of age

maintained for feeding purposes; bulls under ten months of age maintained for feeding

purposes; and steers and spayed heifers.

CVI's for 2009 and 2011 existed only in a hardcopy format, thus from June to

August 2012, the data was entered into an Excel® spreadsheet, creating a row for each

CVI corresponding to a cattle movement, with respective available information (Table ii).

Data was collected on the origin of the cattle to the city level, and for each receiver

within MN address level data was collected. Each movement was characterized by movement date, number of cattle, cattle type: dairy or beef, and cattle production purpose: feeder or breeding.

In order to simplify data collection and entry, the following decisions were made: CVIs with both feeder and breeding cattle on the same CVI were separated and entered into the database in two different rows; rodeo and exhibition cattle were classified as breeding cattle, since were considered high value animals; CVIs received for cows with calves at side were entered as breeding and the calves were included in the total cattle count for the movement; cattle breeds that can be used for either dairy or beef purposes were entered as beef with the exception if the CVI consignee was referenced as a dairy herd; cattle marked as "mixed" or "crossbred" were classified as beef with the exception of cattle referenced as a mix or crossbred of a specific breed, and were classified as either beef or dairy cattle by using the breed referenced (i.e. Holstein cross was entered as dairy); and breeds of cattle identified as dual beef/dairy were classified as beef cattle. Cattle movements to slaughter plants were not included in the data collection.

2.2.1.2. MN herd level data

Data regarding inventory of MN cattle operations were collected for purposes of the data analysis (described below) and was available from different state agencies:
A - MN Board of Animal Health

1. Database of all registered cattle farms in MN, with incomplete information about herd type and herd size.

2. Database of state and federal inspected livestock markets

B - MN Department of Agriculture

1. Database of all dairy cattle farms with a permit to ship milk in the state of MN that is updated on a yearly basis.

C - MN Pollution Control Agency

1. Database of all feeding operations in the state of MN with more than or at least 50 head of cattle.

2.2.4. Data analysis

A descriptive analysis was performed on the cattle movement data for both years of data collection. The data was summarized at the premises and county level and for both levels the distribution of cattle moved by covariates and number of movements was evaluated.

In order to develop a strategy for a targeted surveillance system at the herd level for bTB in MN, each import movement was risk profiled based on the following variables: cattle type, cattle production purpose, number of cattle and state of origin. The latter two were categorized in three categories each, respectively: large size movements - >100 head, medium size movements – 11-100 head, and small size movements - ≤10

head; level 1 states (Arizona, California, Colorado, Michigan, New Mexico and Texas) -

states with the most imports from Mexico based on USDA reporting or states with lower

than free state bTB status in cattle for the year of movement [2], level 2 states (Iowa,

Kansas, Missouri, Nebraska, Oklahoma, Washington) - states other than the ones

classified as high risk states where cattle originated from Mexico are likely to be moved

after entering the US [146], and level 3 states - all other states from where cattle were

moved to MN . The structure of the 2009 movement data was then explored using PROC

LCA, a SAS® (Version 9.3 SAS Institute Inc., Cary, NC, USA) procedure for latent class

analysis (LCA). This analysis identifies a group of mutually exclusive latent classes

based on responses to a set of variables [158]. The data structure was explored by fitting

several models with 2, 3, 4 and 5 latent classes. Model fit was evaluated based on Akaike

Information Criterion (AIC) and entropy. The latter reflects accuracy of assignment of

each movement to a latent class based on the categorical variables (aka latent class

indicators). The entropy was calculated using the posterior probabilities of assignment of

each movement to a particular class, which are calculated by applying Bayes theorem.

The model chosen had 4 latent variables and a maximum entropy level, meaning that

each movement was assigned with a very high probability (>90%) to a specific class.

Since the latent class model was data driven and not based on previous knowledge of

risk, the results of the model were then confronted with expert opinion regarding the

profile of each latent class. Class membership assigned by the LCA was then adjusted

based on the previous knowledge of those risk factors. Although a simple empirical approach could have been used, the LCA was needed to explore the data structure and to assign number of classes based on model fit parameters. Otherwise, could constitute an extenuating process due to the multitude of possible classes and some could have a very small number of movements due to the uncommon combination of the variables collected (ex: large movements of breeding cattle from level 1 states).

The final classes after adjusting for expert opinion were the following:

Class 1 - Very High Risk (VHR) - All size movements of dairy and beef breeding cattle from level 1 and level 2 states.

Class 2 - High Risk (HR) - All size movements of dairy and beef feeder cattle from level 1 and level 2 states.

Class 3 - Medium risk (MR) - All size movements of dairy and beef breeding cattle from level 3 states.

Class 4 - Low risk (LR) - All size movements of dairy and beef feeder cattle from level 3 states.

From the initial latent class assignment only 106 movements of breeding cattle from high risk states were moved from class 3 to class 1 and 1 movement of beef breeding cattle was moved from class 2 to class 1 (Table iv).

For the 2011 movement data, the same class structure was implemented, without performing the latent class analysis (Table v).

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The movement data, for each year separately, was summarized at the premises level (includes all types of premises with the exception of slaughter plants) in Excel® and, for each premises, four variables were created (Very High, High, Medium and Low risk) that reflected the number of movements from each risk category. Cattle farms were classified in the higher risk category for which at least one movement was present. A cattle farm could have a majority of LR movements but if it had one VHR movement, this farm was considered VHR in the respective year of analysis.

The data was summarized at the county level (n=87) and analyzed by fitting a linear regression model, using the SAS® PROC GLM procedure, to determine which variables were associated with higher number of movements at the county level. The variables included in the model were the following: total number of cattle farms, total number of farms with movements, presence of a state or federal inspected market (binary), presence of feedlots with ≥500 head capacity (binary) and presence of dairy farms with ≥ 500 head (binary). Since the correlation for the same dependent variables between 2009 and 2011 was extremely high at the county level (>90%), the mean value between both years was used as the outcome variable. A similar mean value was used for the total number of farms with movements. The dependent variable was transformed to the natural log scale to not violate the normality assumption. Statistical significance was determined at $\alpha \leq 0.05$.

Finally, to the movement data summarized at the farm and county level, a smoothing method was implemented by applying a kernel function to map overall cattle farm density and VHR and HR farm densities using ArcGIS®.

2.3. Results

2.3.1. Descriptive Analysis

By the end of 2012, the state of MN had 27,406 active cattle farms registered in the MNBAH database. A higher density of cattle farms formed a diagonal line across the state from southeast to northwest. However, the two areas with greater density of farms were in the southeast corner of the state and the center of the state (Figure v).

The import movement data shows that the temporal trends were very similar between the years 2009 and 2011, with a peak in the number of incoming cattle and incoming movements in the Fall months, with the latter also having an increase at the end of the Winter and early Spring season (Figure vi). For the years 2009 and 2011, there were a total of 7,185 (Table iv) and 8,107 (Table v) import cattle movements to MN, respectively.

Most of the cattle imported in both years were classified as beef and feeder cattle. This can also explain the peak in the Fall months, corresponding to the weaning season in cow-calf operations in this region and the time when calves are sold to feeding operations to be fed for slaughter. Most of the breeding cattle were in the dairy category (Table iii).

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The states that sent more cattle and had more movements to MN were located in the northern US where beef cow-calf cattle farms predominate [159]. Some of the southern states, potentialy at greater risk for bTB due to increased cattle introductions from Mexico, also sent high numbers of cattle (Figure vii) to MN but in very few movements (Figure viii).

For each year, 2009 and 2011, there were about 1,500 cattle premises that received cattle from other states. However, a much smaller proportion of those, for both incoming cattle and movements, concentrated most of the trade activity, approximately following the 80-20 rule (aka Pareto principle) (Table vi). Only 624 (~2% of total cattle premises in MN) importing cattle premises had incoming movements in both 2009 and 2011 and from these, 21% were in the VHR and HR groups for both years.

2.3.2. Spatial distribution of cattle premises by risk category

The risk model results showed similar results for both years, with greater density of cattle premises with incoming movements in the southwest and southeast corner of states, also corresponding to greater density of VHR and HR premises (Figure ix). The further north in the state, the fewer VHR and HR premises were observed (Figure x). For both years, there were a higher number of cattle premises in the MR and LR versus the VHR and HR categories (2009 - 39 vs. 61%, 2011 - 35 vs. 65%; Table vii).

Although farms were classified in the same risk category, the level of risk

changed depending on the number of incoming movements, which was used to rank

premises within risk category and overall. Figure x shows a map with combination of

premises in the VHR and HR categories ranked by number of incoming movements,

where nodes' size corresponds to more incoming movements and thus a higher rank.

2.3.3. Spatial distribution of high risk areas

The regional level results showed that there was no strict overlap between density

of cattle premises in MN (Figure v) and the density of VHR and HR cattle premises

based on our model (Figure xi). The latter, as previously stated, tended to be located in

the southwest and southeast corners of the state and more sporadically across the rest of

the state. The map with the kernel density of VHR and VH in Figure xi departs from the

individual analysis of each premises and allows a regional perspective of the areas where

there were higher densities of higher risk farms.

2.3.4. Factors affecting higher regional density of movements

The total number of herds with movements and presence of large feeding

operations, as defined above, were associated with higher number of incoming

movements at the county level ($P<0.05$). The total cattle farms in the county was

borderline not statistically significant (*P*=0.06), but when the presence of large dairies and markets were removed from the model, it became statistically significant (*P*=0.02).

2.4. Discussion

The method described in this manuscript develops a framework for a targeted surveillance approach to bTB, using data available from a state animal health agency and that is available in all US states. The application has limitations, since risk is never null and there is always a probability, although small, for a cattle farm in a lower risk category to be the source of disease into the state. However, a targeted surveillance approach for bTB will not eliminate the current baseline tool of the surveillance system, slaughter surveillance, but instead could enhance testing of cattle on premises considered to be at higher risk for disease introduction into a disease free area or a non-endemic area. The latter point is extremely important since the state of MN has returned to bTB free status and the state goal is to avoid bTB re-introduction into the state.

As stated before, the fundamental problems with bTB slaughter surveillance are the long period of time between when an animal is infected and when it is detected by an inspector at slaughter and also the unequal coverage of different types of farms by herd size and cattle type [23, 152]. The approach described here overcomes both problems by focusing on mechanistic factors that increase risk of disease being introduced into a cattle farm and not on unrelated characteristics of the farm itself. Furthermore, by increasing

bTB testing intensity in the herds more likely to be the index herds of an outbreak, based on risk profile of cattle movements, time from infection to detection will consequentially be reduced. This mitigates the impact of disease by limiting its spread in the MN cattle population. Also, by limiting the impact of disease, the control cost will be reduced either in scenarios of depopulation or test and removal.

The concept of targeted surveillance and identification of individuals more susceptible to infection has been presented by other authors as the fundamental factor that could enhance a targeted surveillance effort [123, 157, 160].

When representing the total cattle premises population in MN with the most recent number of 27,403 cattle farms, it is easy to conclude that it is non-feasible to include all herds in a target population for random sampling when responding to an outbreak. Random sampling approaches are the paradox of infectious disease surveillance, knowing that disease does not behave randomly but it has underlying risk factors that enhance or limit its spread [161, 162]. For bTB, this is not different, and several authors have shown the link between number of cattle introductions and origin of trace-in cattle as risk factors for bTB occurrence [135, 137, 139]. Furthermore, the USDA indicated in one report that 90% of purchased cattle the index herd in the 2005 MN bTB outbreak originated in other states, adding more evidence to this reality [2].

More recently, when applying network analysis methods to infer vulnerability to infection in a different system, in-degree, the number of incoming contacts to a specific

node or individual in the network, a similar concept to the number of import movements in the current analysis, was shown to be the most important parameter linked with risk of infection [163, 164].

The current analysis has the advantage of being performed on CVI data available from all US states. Although with limitations, it constitutes a resource that could be used more frequently to inform disease surveillance. To the authors' knowledge, this has not yet been done, with the exception of a recent manuscript that developed novel methodology to model cattle movements in the US, as part of the national cattle movement modeling effort [165].

The assignment of cattle premises into risk categories based on the risk profile of the cattle movements going in each premises constitutes a novel approach. Many risk factor studies evaluate associations between farm characteristics and disease status. These studies fail to identify the causal risk factors for disease introduction but instead report statistical associations that could be misleading. In disease transmission, it is of great importance to consider both the number of contacts and also the quality of those contacts for efficient transmission, since both are not randomly distributed [166]. In the current analysis, the same rationale applies, where it is not only how many incoming movements but also the type of movement premises received.

The OIE defines compartment as the "an animal subpopulation contained in one or more establishments under a common biosecurity management system with a distinct

health status with respect to a specific disease or specific diseases for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade" [155]. As an extension, we propose to identify areas at higher risk for disease introduction, grounded on known risk factors and thus allow allocation of resources to those "high risk compartments". This pertains not only to increased animal testing but also the collection of detailed data regarding cattle movements from high risk premises in those areas, in order to increase efficiency of traceability in response to a potential outbreak.

Since most of the cattle moved were in the categories of beef and feeder cattle, it was not surprising that the presence of large feeding operations (as previously defined) was an important explanatory variable for more import movements into a region. However, the high level of incoming movements in the southwest and southeast region of MN was also associated with a higher density of cattle premises with VHR and HR movements. As a result, both of these regions are hubs of cattle trading into MN, with impact for the rest of state. The higher density of farms in those areas indicates a higher risk for disease spread to cattle and wildlife, if introduced, when compared with other regions in MN with lower density of cattle farms and wildlife, particularly white-tailed deer. However, the regional approach alone presents limitations by neglecting some VHR and HR farms that are located in other regions of MN.

For bTB, a disease with a long incubation and latent period, we suggest combining at least the 5 previous years of CVI data in order to inform the targeted surveillance system, based on what is known about the time between infection and detection at slaughter surveillance. In the current analysis, only two years of data were evaluated and a total of 2,390 cattle premises had movements in either year, which would constitute the underlying baseline population where targeted surveillance would be performed. For other diseases, the same approach could be used, but adjusting the time period for data collection in accordance with the disease transmission parameters.

The fundamental goal of risk-based surveillance is to improve early detection of disease in order to mitigate its impact and as a consequence reduce the overall economic burden of an outbreak [123]. By focusing on the strata of the population at high risk to introduce disease into a disease-free or non-endemic area, the probability of detection is increased and the cost-benefit of a surveillance and eradication program improved [157].

The current study was conducted with the goal of developing a risk-based surveillance strategy for bTB to enhance the probability of early detection and mitigate disease impact in the cattle and wildlife populations, while reducing the financial burden of a potential outbreak. This task was achieved in a comprehensive way by using available data from the MNBAH, which can be applied in any other states across the US. The application of such method will reduce the impact of a bTB outbreak and the concept could also be applied in other disease surveillance systems.

2.5. Figures
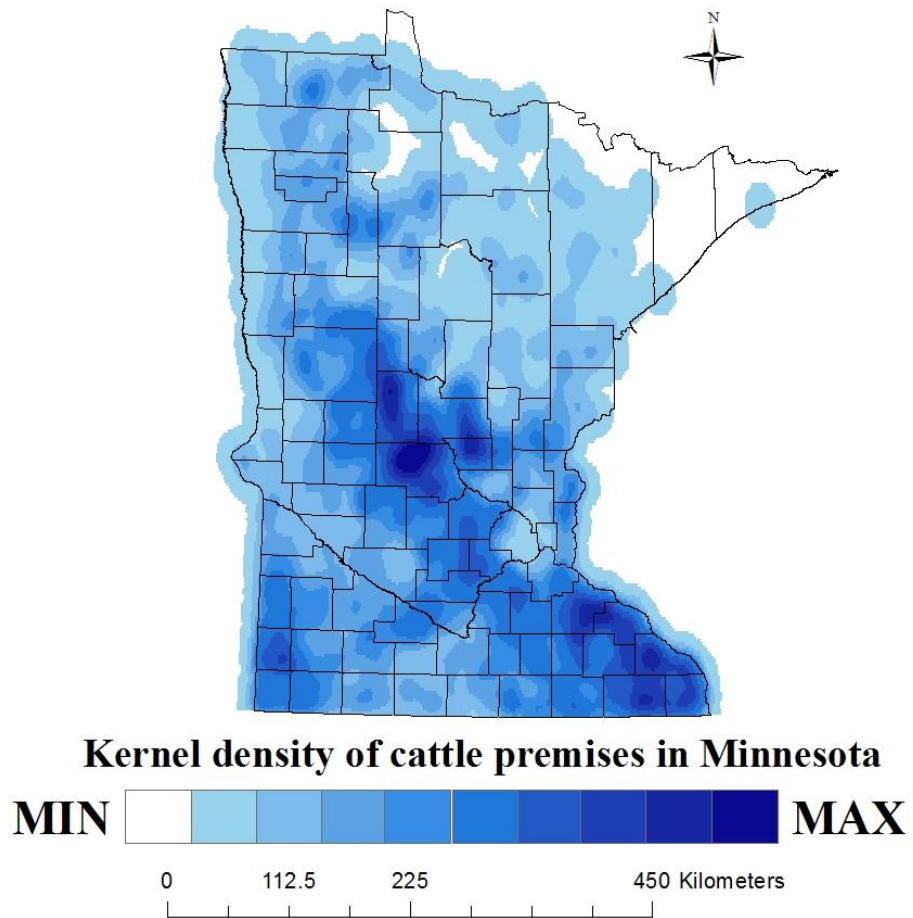
Figure v. Kernel density for cattle premises in the state of MN (n=27,406).
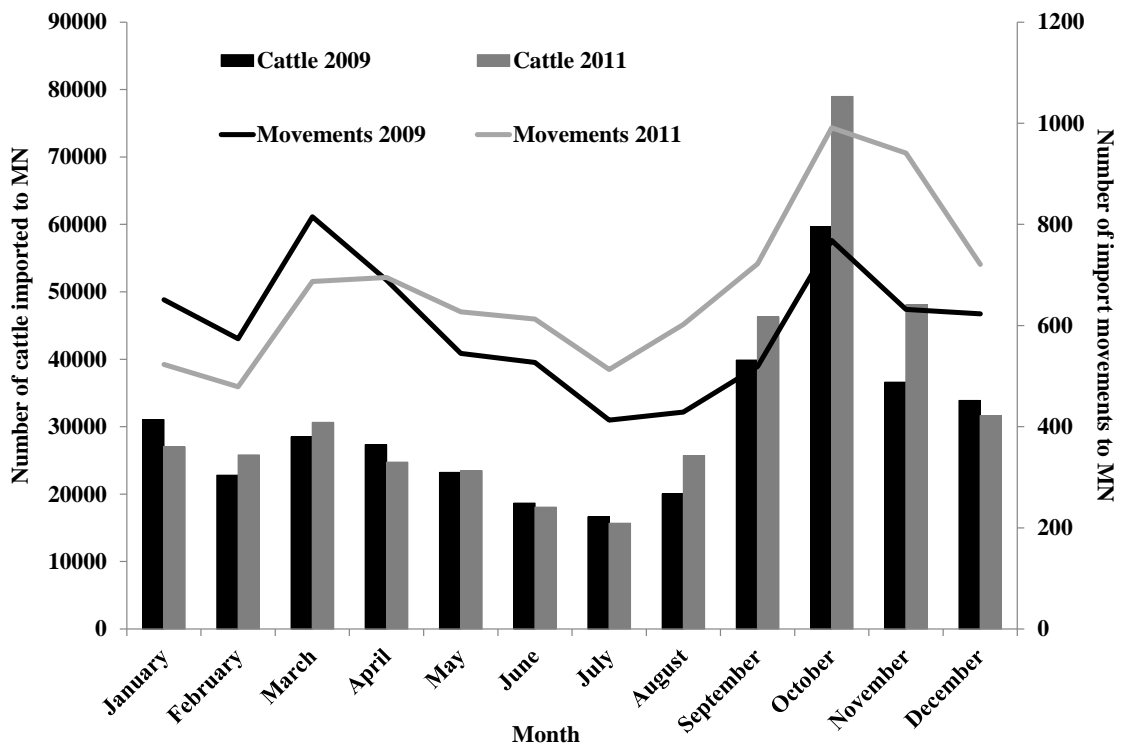
Figure vi. Histogram showing number of import movements and cattle moved to MN by month for the years 2009 and 2011.
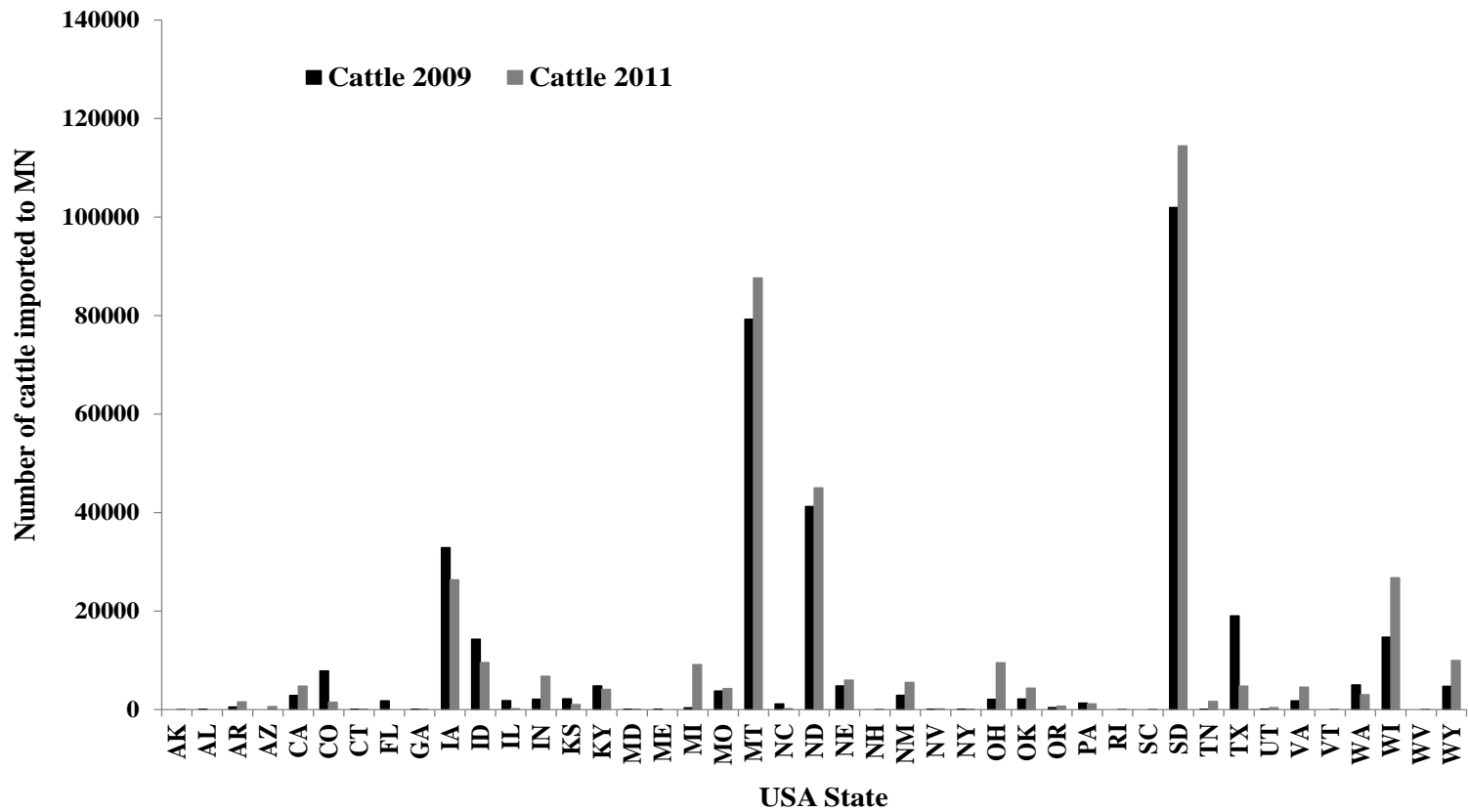
Figure vii. Histogram showing number of cattle imported to MN by state of origin for the years 2009 and 2011.
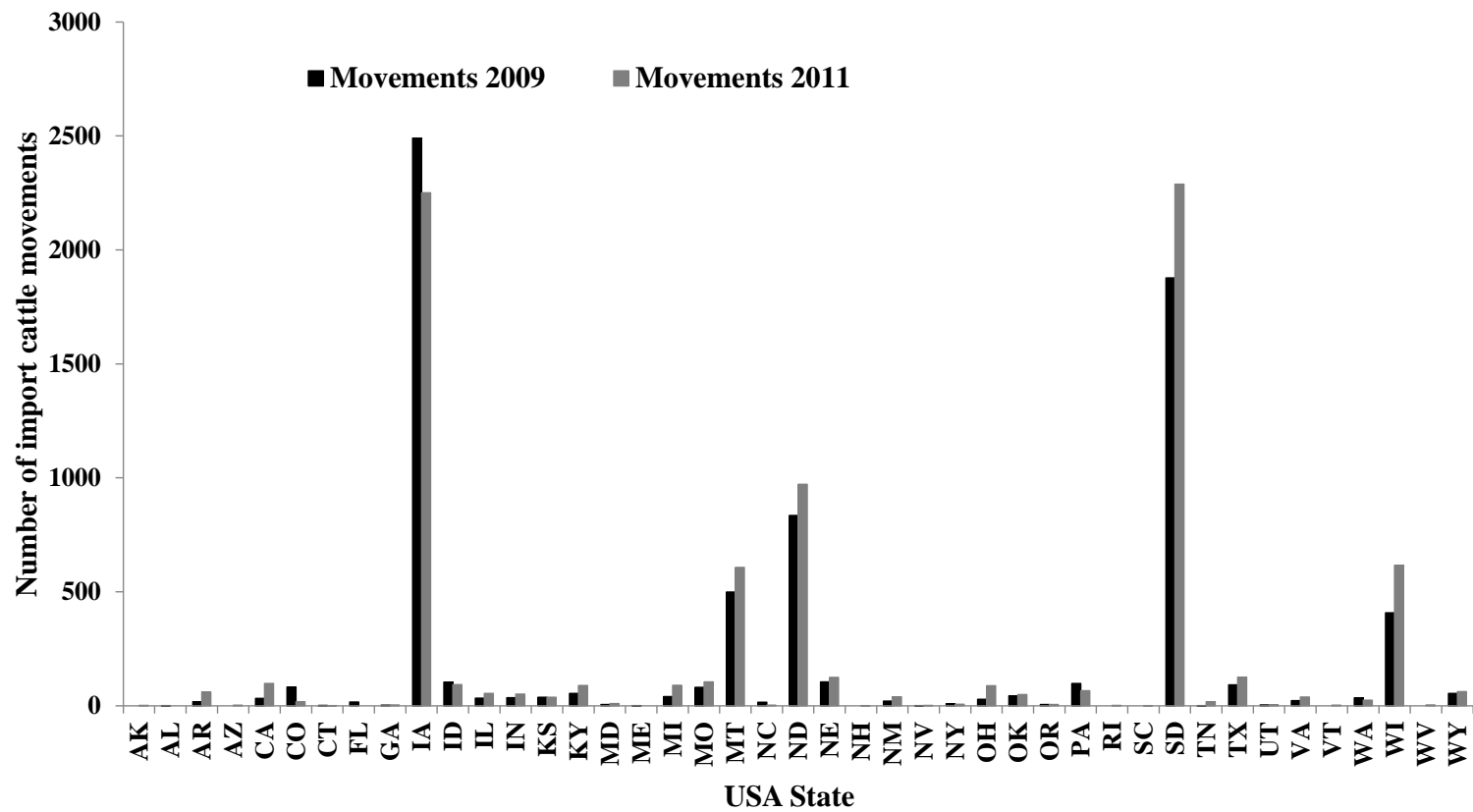
Figure viii. Histogram showing number of import movements to MN by state of origin for the years 2009 and 2011.
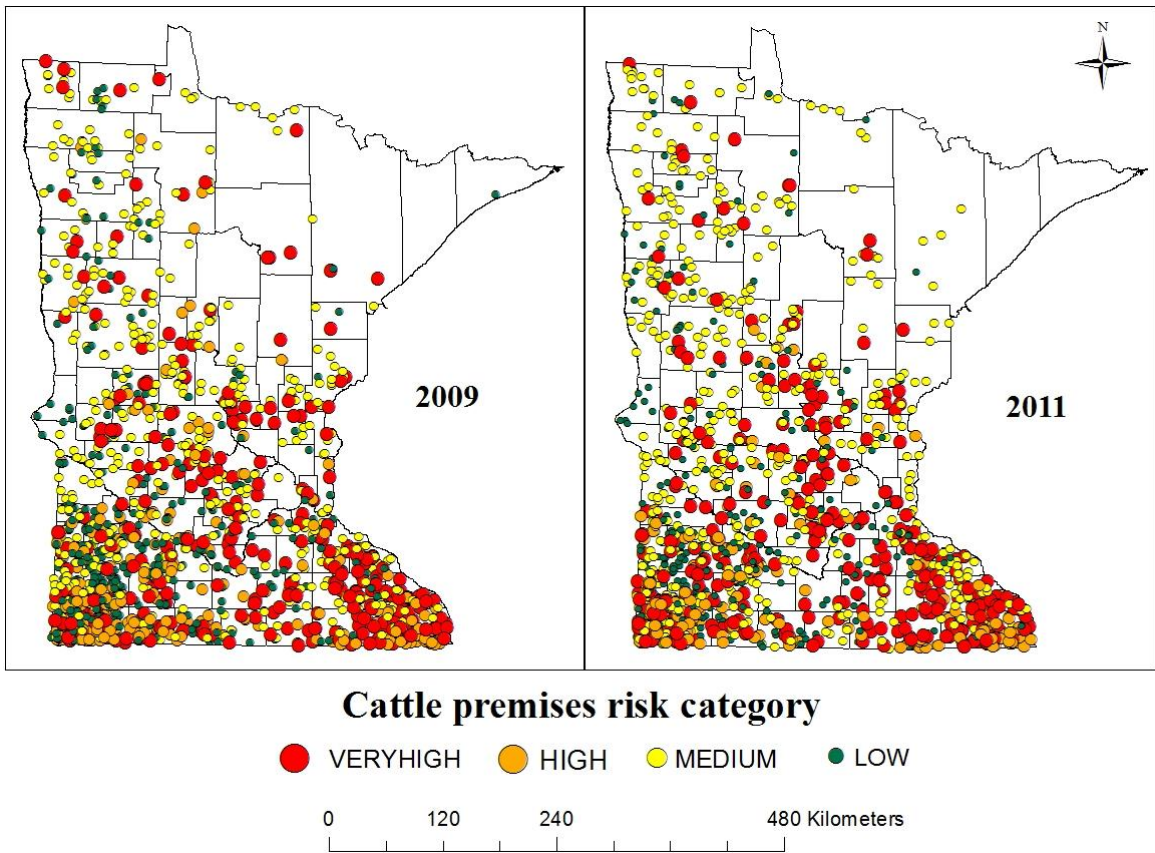
Figure ix. Map with distribution of cattle farms in MN by risk group for BTb for the years 2009 and 2011.

Ranking of cattle premises in the Very High and High risk category by number of incoming movements

MIN · · · • • • • • • • • • • • • • • • MAX

0   120   240   480 Kilometers
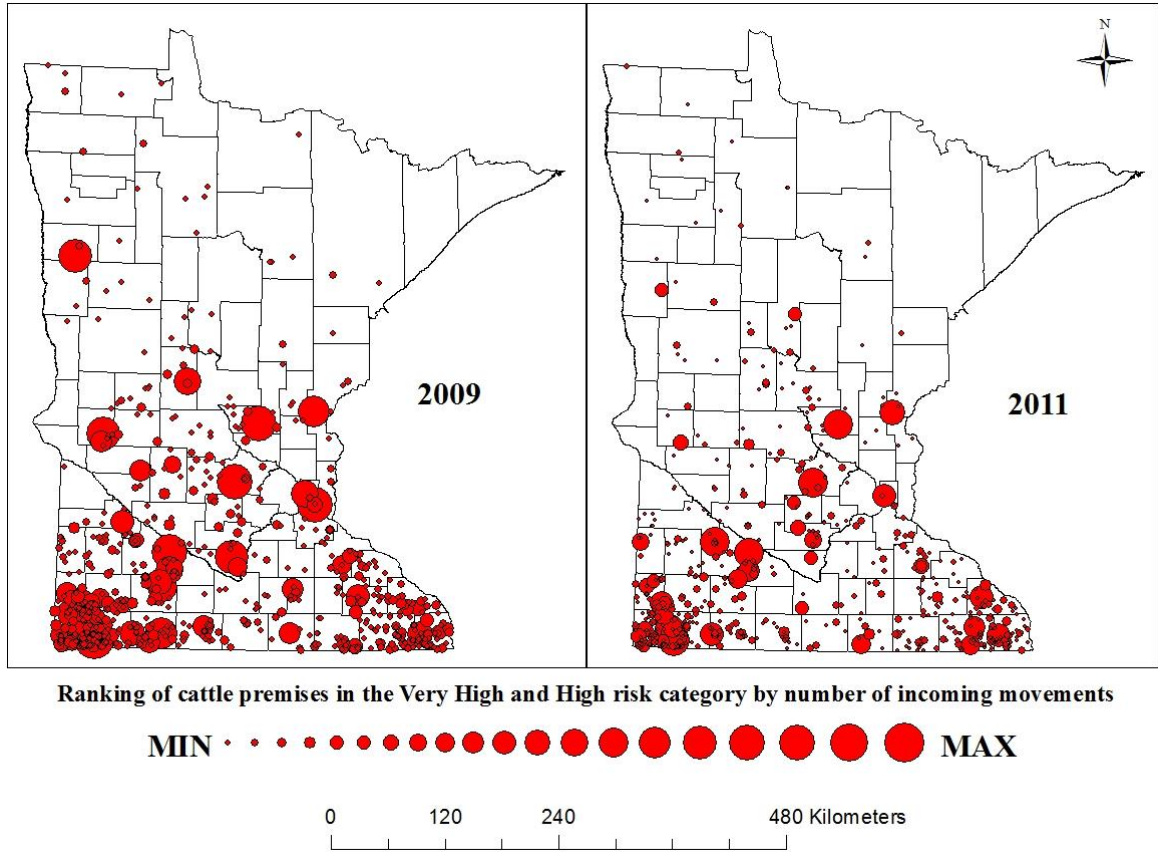
Figure x. Map with cattle premises in the Very High and High risk category, with nodes sized by the sum of the number of incoming movements in both categories for the years 2009 and 2011.
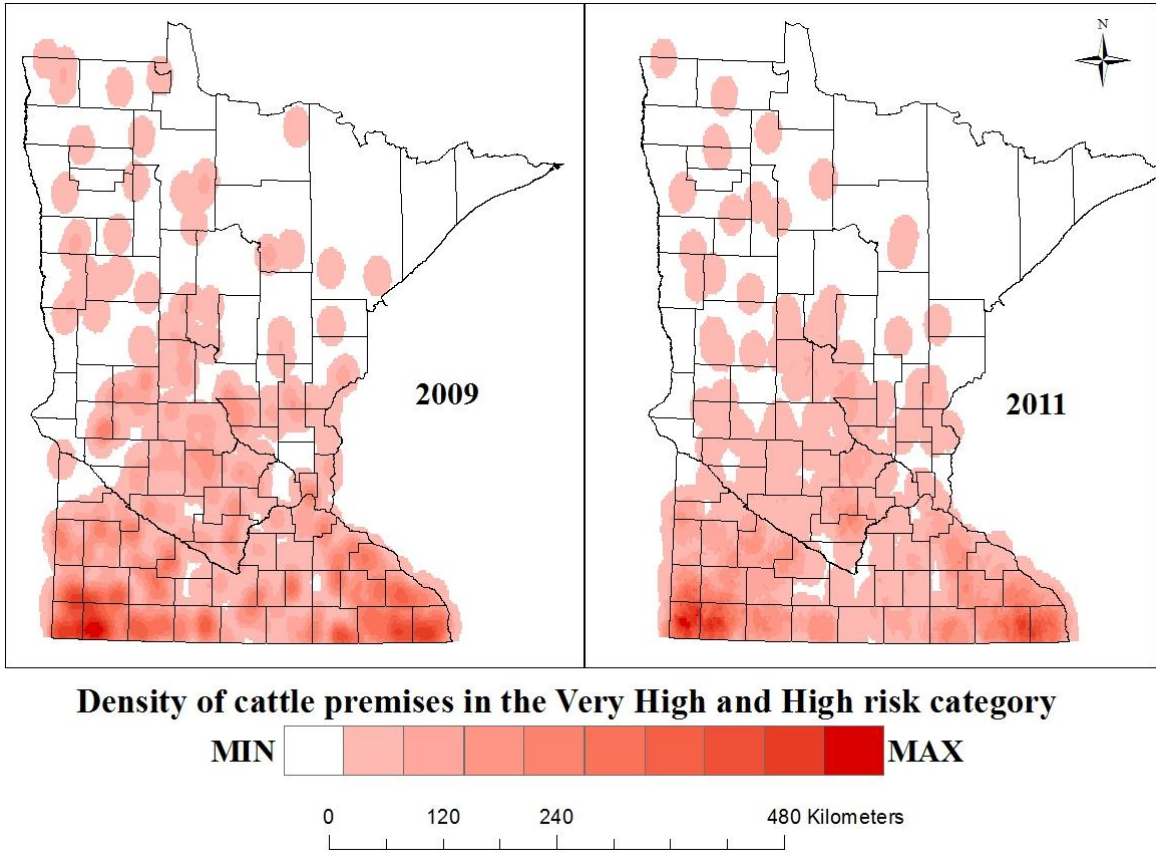
Figure xi. Kernel density for location of Very High and High risk premises in the state of MN for the years 2009 and 2011.

2.6. Tables

Table ii. Data collected from each certificate of veterinary inspection for import cattle movements into MN for the years 2009 and 2011.

CVI number	Number of identification for the certificate of veterinary inspection
Number of head	Number of cattle in each CVI, corresponding to an import movement
Cattle type	Dairy or Beef cattle
Cattle production purpose[1]	Feeder or Breeding cattle
Movement Date	Date of movement
Sender City	City of Origin
Sender State	State of Origin
Receiver premises ID	Unique official number for premises within MN - with associated name, address, city, county, zip code and latitude and longitude information

[1]Breeding cattle is defined by the MNBAH as all cattle except the following (classified as feeder cattle): heifers of beef breed less than 18 months of age maintained for feeding purposes; bulls under ten months of age maintained for feeding purposes; and steers and spayed heifers.

Table iii. Summary of number of cattle imported to MN in each class for the years 2009 and 2011.

Year	Class	Breeding[1] (n) (%)	Feeder (n) (%)	Total by class (n) (%)	Total by year (n) (%)
2009	Beef (n)	7,054	258,329	265,383	358,134
	(%)	(2%)	(72%)	(74%)	(48%)
	Dairy (n)	29,000	63,751	92,751	
	(%)	(8%)	(18%)	(26%)	
2011	Beef (n)	10,394	262,808	273,202	394,410
	(%)	(3%)	(67%)	(69%)	(52%)
	Dairy (n)	54,462	66,746	121,208	
	(%)	(14%)	(17%)	(31%)	
Total by production purpose (n)		100,910	651,634	752,544	
(%)		(13%)	(87%)		

[1]Breeding cattle is defined by the MNBAH as all cattle except the following (classified as feeder cattle): heifers of beef breed less than 18 months of age maintained for feeding purposes; bulls under ten months of age maintained for feeding purposes; and steers and spayed heifers.

Table iv. Summary of number of movements by risk category for the year 2009.

Variables		Risk category				Total	Total movements[5]
		Very High[1]	High[2]	Medium[3]	Low[4]		
Number of head	>100	6	207	18	821	1052	
	11-100	499	435	598	1484	3016	
	≤10	1537	371	952	257	3117	
Cattle type	Beef	390	611	786	2334	4121	
	Dairy	1652	402	782	228	3064	7185
Cattle production purpose[6]	Breeding	2042	0	1568	0	3610	
	Feeder	0	1013	0	2562	3575	
State of origin[7]	High Risk	106	160	0	0	266	
	Medium Risk	1936	853	0	0	2789	
	Low Risk	0	0	1568	2562	4130	

[1]Very High Risk (VHR) - All size movements of dairy and beef breeding cattle from high and medium risk states.

[2]High Risk (HR) - All size movements of dairy and beef feeder cattle from high and medium risk states.

[3]Medium risk (MR) - All size movements of dairy and beef breeding cattle from low risk states.

[4]Low risk (LR) - All size movements of dairy and beef feeder cattle from low risk states.

[5]Number of total incoming movements to MN in 2009.

[6]Breeding cattle is defined by the MNBAH as all cattle except the following (classified as feeder cattle): heifers of beef breed less than 18 months of age maintained for feeding purposes; bulls under ten months of age maintained for feeding purposes; and steers and spayed heifers.

[7]Level 1 states (Arizona, California, Colorado, Michigan, New Mexico and Texas) - states with the most imports from Mexico based on USDA data or states with lower than free state BTb status in cattle for the year of movement [2], Level 2 states (Iowa, Kansas, Missouri, Nebraska, Oklahoma, Washington) - states other than the ones classified as high risk states where cattle originated from Mexico is likely to be moved after entering the US [146] and Level 3 states - all other states from where cattle was moved to MN .

Table v. Summary of number of movements by risk category for the year 2011.

Variable		Risk category				Total	Total movements[5]
		Very High[1]	High[2]	Medium[3]	Low[4]		
Number of head	>100	31	180	48	898	1157	
	11-100	577	297	1080	1442	3396	
	≤10	1549	324	1309	372	3554	
Cattle type	Beef	435	471	888	2404	4121	
	Dairy	1722	330	1549	308	3064	
Cattle production purpose[6]	Breeding	2157	0	2437	0	4594	8107
	Feeder	0	801	0	2712	3513	
State of origin[7]	High Risk	227	145	0	0	372	
	Medium Risk	1930	656	0	0	2586	
	Low Risk	0	0	2437	2712	5149	

[1]Very High Risk (VHR) - All size movements of dairy and beef breeding cattle from high and medium risk states.

[2]High Risk (HR) - All size movements of dairy and beef feeder cattle from high and medium risk states.

[3]Medium risk (MR) - All size movements of dairy and beef breeding cattle from low risk states.

[4]Low risk (LR) - All size movements of dairy and beef feeder cattle from low risk states.

[5]Number of total incoming movements to MN in 2011.

[6]Breeding cattle is defined by the MNBAH as all cattle except the following (classified as feeder cattle): heifers of beef breed less than 18 months of age maintained for feeding purposes; bulls under ten months of age maintained for feeding purposes; and steers and spayed heifers.

[7]Level 1 states (Arizona, California, Colorado, Michigan, New Mexico and Texas) - states with the most imports from Mexico based on USDA data or states with lower than free state BTb status in cattle for the year of movement [2], Level 2 states (Iowa, Kansas, Missouri, Nebraska, Oklahoma, Washington) - states other than the ones classified as high risk states where cattle originated from Mexico is likely to be moved after entering the US [146] and Level 3 states - all other states from where cattle was moved to MN .

Table vi. Cumulative proportion of import movements and cattle moved to MN for the

upper 20th percentile of cattle premises with more incoming movements and incoming

cattle for the years 2009 and 2011.

Year	2009		2011	
	Total	Upper 20th percentile of farms (%)[1]	Total	Upper 20th percentile of farms (%)[1]
Number of cattle	358,126	322,008 (90%)	395,933	364,211 (92%)
Number of movements	7,185	5,446 (76%)	8,114	6,419 (79%)
Number of farms	1537	307	1478	296

[1] Cattle farms were ranked from highest to lowest for either number of incoming cattle or

number of incoming movements.

Table vii. Number of cattle premises by risk category for each year of analysis, 2009 and

2011.

	Risk category[1]				
Year	VERY HIGH[2] n (%)	HIGH[3] n (%)	MEDIUM[4] n (%)	LOW[5] n (%)	Total n (%)
2009	327 (1.2%)	267 (1.0%)	548 (2.0%)	395 (1.4%)	1537 (6%)
2011	327 (1.2%)	193 (0.7%)	608 (2.2%)	344 (1.3%)	1472 (5%)

[1]Percentages in each category are calculated with of total number of licensed cattle farms

in MN.

[2]Premises with at least one movement in the Very High Risk (VHR) category.

[3]Premises with at least one movement in the High Risk (HR) category.

[4]Premises with at least one movement in the Medium Risk (MR) category.

[5]Premises with at least one movement in the Low Risk (LR) category.

CHAPTER 3 - NETWORK ANALYSIS OF CATTLE MOVEMENTS IN A PREVIOUSLY INFECTED AREA WITH BOVINE TUBERCULOSIS IN MINNESOTA, US – A FRAMEWORK FOR A RISK-BASED SURVEILLANCE

Bovine tuberculosis (bTB) was first detected in 2005 in cattle in northwestern Minnesota (MN) through slaughter surveillance**.** By the end of 2008, 12 cattle herds were infected with bTB, and the main cause for infection was determined to be the movement of infected animals between herds. USDA granted *split-state* status to MN in 2008, upgrading most of the state to modified-accredited advanced (MAA) and only a smaller area of 6,915 km^2 in northwestern Minnesota as modified accredited (MA). The state was again declared bTB free in 2011. From January 2008 to 2011, all cattle movements within the bTB MA were recorded electronically. The objective of this study was to characterize cattle movements in an area previously infected with bTB in MN and to create a risk score based on network parameters and known risk factors from the published literature that would identify those herds with a higher risk of becoming infected and/or infecting other herds. During the period that data was collected, 57,460 cattle were moved in 3,762 movements corresponding to permits issued to 682 premises, mostly representing private farms, sale yards, slaughter facilities and county or state fairs. Although sale yards represented less than 2% of the premises (nodes), 60% of the movements were to or from a sale yard. The network showed an overall *density* of 0.4%, a *clustering coefficient* of 14.6% and a *betweenness centralization index* of 12.67%.

These reflect the low connectivity of this cattle network, which explains the low number of cattle herds affected in the 2005 bTB outbreak. The degree distribution showed that 20% of nodes performed 90% of the movements. This analysis provides a baseline description about the contact structure of cattle movements in an area previously infected with bTB and develops a framework for a targeted surveillance approach for bTB to support future surveillance decisions.

3.1. Introduction

The State-Federal program for eradication of Bovine tuberculosis (bTB) in cattle populations in the US was initially developed in 1917 and has been extremely efficient in reducing disease prevalence, nearly to the point of eradication [3]. The backbone of the surveillance system for detecting infected animals is slaughter surveillance, which has an estimated median time-to-detection of 5.75 years [23] and an average herd-level sensitivity of 39% [2]. Individual animal ante-mortem tests for bTB are available; however, testing is not routinely performed. Currently, individual ante-mortem animal testing is only performed prior to cattle movements or during disease eradication efforts in infected areas.

The main problems with the current surveillance system include: (i) the long time-to-detection (often identifying disease years after its introduction into an initially disease-free population); and (ii) the unequal probability of inspection of cattle farms of different

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types and sizes, with large dairy herds being tested more frequently than small beef herds (for example, due to the former sending more cattle to slaughter) [2]. The delay in detection allows bTB to spread to other animals and other herds. Once detected, this results in high eradication costs due to the widespread herd tracing and animal depopulation that is required. In order to detect bTB cases earlier in disease free areas, and as a consequence minimize the spread of disease, factors associated with a greater risk of disease introduction or transmission must be incorporated into the surveillance system. In the field of animal health, risk is defined in the Animal Health Code (OIE) as "the likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health" [155]. This is the concept behind *risk-based surveillance* programs, which seek to focus funding and resources toward subsets of the population with a higher risk of the health event of interest, improving surveillance system sensitivity and cost-effectiveness [123].

A recent assessment conducted by USDA APHIS identified a variety of risk factors associated with bTB infection in US areas without a wildlife reservoir, including the importation and comingling of Mexican-origin steers, the management and biosecurity practices used by calf raisers for dairy replacement heifers, and the influx of purchased cattle [2]. The role of cattle movement has also been identified to be of primary importance, particularly in low prevalence or disease-free areas, where disease can be introduced through the importation of cattle from infected areas [137, 153, 154].

Characterizing patterns of cattle movements before a disease outbreak occurs is thus critical to identifying which herds have the highest risk of infection and which would be most likely to transmit an infection to others. Because cattle movements are directed, since cattle moves from one farm to another and not in both directions, these risks (risk of becoming infected and likelihood of transmitting infection to other farms) may be different depending on a herd management factors, including whether the herd engages primarily in the exportation or importation of cattle, and the origin of the imported cattle. Furthermore, these risks do not exclude the role of wildlife for disease spread after it is present, but confirm the importance of cattle movements as the primary source for disease introduction in a disease free area [167, 168].

Network analysis, a methodology arising from the social sciences with recent applications to the spread of human and animal infectious diseases, has proven to be an useful tool in understanding the structure of contacts within and between animal populations as well as the role of high risk herds in the transmission of infectious diseases in livestock [161, 169, 170]. In a network analysis framework, a population is described in terms of a set of nodes and the edges that describe the interactions between them. In the case of describing the spread of bTB, nodes represent cattle herds, where an edge between two herds represents the movement of animals from one herd to another and a potential pathway for disease introduction. A variety of measures have been developed to characterize network features, including measures of *connectedness*, *clustering*, and

distance, as well as the specific role of individual nodes in the network, including measures of importance or *centrality* [171]. Certain network measures have been linked with the risk of infection, such as *in-degree*, the number of incoming connections to a node; while others have been associated with both the risk of infection and also of transmitting disease to other nodes in the network, such as *betweenness centrality* [163, 172].

Given the association of livestock movements with outbreaks of bTB [153], the objectives of this study were (1) to characterize cattle movements in the region of a past bTB outbreak and (2) to identify which herds have a higher risk of becoming infected and/or of infecting other herds based on known risk factors and network analysis parameters. We made use of cattle movement data collected following a recent bTB outbreak in Minnesota. Bovine tuberculosis was first detected in 2005 in cattle in northwestern Minnesota through slaughter surveillance**.** By 2009, 12 cattle herds were found to be infected with bTB. Almost all herds in the outbreak could be linked to an infected herd through cattle movements [40]. The US Department of Agriculture granted *split-state* status to Minnesota in 2008, upgrading most of the state to modified-accredited advanced (MAA) and only a small area of 6,915 km^2 in northwestern Minnesota as modified accredited (MA). In 2011, the state was declared bTB free. From January 2008 to 2011, cattle movements within the MA region were recorded electronically as part of an eradication program.

The USDA recently issued a document outlining a new approach to bTB surveillance. This includes the development of targeted approaches, with the objective of responding to the new challenges posed by this disease which currently include: most cases detected at slaughter are imported animals; the danger for a wildlife reservoir to emerge as in the state of Michigan; and the greater cost of disease control and eradication due to increased herd size and long distance cattle movements across the country [24].

The objective of this analysis is supported by the hypothesis that the assumption of randomness does not hold in infectious disease transmission due to the heterogeneity of the populations and their contacts. In our study, the focus is on the heterogeneity of cattle movements between herds, where each herd contributes differently to the overall risk of disease transmission [162, 172]. Furthermore, the longitudinal component of the current data set allows the estimation of network parameters across several years and evaluation of the influence of the most prominent nodes of the network of cattle movements. The ultimate goal is to develop a risk-based surveillance framework for bTB, in order to identify in which herds to focus surveillance resources to minimize disease burden and reduce the cost of disease control.

3.2. Material and Methods

3.2.1. Data collection

Data on cattle movements for the bTB MA zone from 2008 through 2011 were obtained from the Minnesota Board of Animal Health (MNBAH). Due to Minnesota's split-state status over this time period, all cattle movements to and from farms in the MA zone were recorded through animal movement certificates and radio frequency identification (RFID) tagging of individual animals. The MNBAH has made these data available as a part of the electronic *Generic Database* (software made available to states by USDA-APHIS). The RFID tag data reflects all individual animal movements, including within-herd movements (i.e. within the same owner but between different locations), between-herd movements, to and from sale yards, to slaughter facilities, and to and from county or state fairs.

3.2.2. Study population

The bTB area was located in the interface between four counties in northwestern Minnesota: Beltrami, Lake of the Woods, Marshall and Roseau. Cattle operations in this area are predominantly beef farms (versus dairy farms) (Table viii).

3.2.3. Inclusion criteria

We included all cattle movements entering, exiting, and within the MA zone that had a complete movement record in our analysis. For cattle movements within the MA zone or between the MA zone and the rest of Minnesota, we excluded cattle movements

for which either the origin or destination farm could not be identified from our data. For movements entering or exiting the MA zone with origins or destinations outside the state of Minnesota, we did not exclude any movement, as long as the state of origin or receipt of the movement was available. From 2008 through 2011, 3,467 movements satisfied the inclusion criteria, representing a total of 46,717 cattle moved between 559 premises, including private farms, sale yards, slaughter facilities and county or state fairs (Table ix). One farmer may own or manage multiple premises; since cattle might move seasonally to different locations depending on availability of feed, we were only interested in movements between distinct operations and we combined movements to and from premises operated by the same farmer.

3.2.4. Analysis and definitions

From these data, we constructed a network reflecting the structure of cattle movement between premises. In the network, each cattle operation (private farm, sale yard or other) is represented by a node and edges (connections) represent the movement of cattle from one node to another. We constructed a directed network to account for the fact that cattle movements are not necessarily reciprocal; some cattle operations may serve primarily as suppliers, with many out-going connections, while other operations may predominantly import cattle, with many in-coming connections. We constructed separate networks for each of the four years in the study period, reflecting the specific

cattle movements of any given year. We also constructed an aggregate cattle movement network, which reflects the cattle movements over the entire four-year period. The network of cattle movements was analyzed as both a valued, to account for the strength of relationship between nodes (i.e. multiple movements between the same nodes were considered) and also as dichotomous network [171].

We analyzed each cattle movement network using UCINET®, a software package for network analysis [173]. We characterized each network in terms of standard network features, including the network's overall density, mean degree, and betweenness centrality index (for definitions, see Table x) [171, 174–176]. We also constructed a *degree distribution* of the overall degree as well as of *in-degree* and *out-degree* separately to evaluate proportion of nodes involved in the majority of movements and cattle moved [162]. Spearman correlations were calculated to evaluate the association between centrality parameters for incoming and outgoing movements.

A complete summary of relevant network features is summarized in Table x. We considered "influential" nodes in the network to be those nodes with high degree [177–180]. For each of these important nodes, we constructed *ego-centric networks*, which represent the network of direct links, incoming or outgoing, for one node of interest. The objective was to identify in detail which nodes were receiving cattle from these more central nodes.

After characterizing the network features of cattle movements, we proposed a risk-score for private farms designed to reflect the risk of bTB infection based on a farm's role and position in the network. Such a score could be used in developing targeted surveillance strategies to improve the efficiency and timeliness of bTB detection in the region. We based the risk-score on the number of cattle movements and movement characteristics that affect risk, which was informed by published literature (summarized in Table xi). Thus, the risk score incorporates not only the heterogeneity in the number of contacts, but also in the origin of the cattle moved, which is known to affect risk of disease introduction.

The risk-score was calculated at the movement level and then summarized at the farm level on an annual basis to account for year-specific differences. The final risk-score was the Total Risk Score (TRS) over the four-year period. To account for parameter uncertainty, we randomly sampled parameter values from appropriate probability distributions and calculated the resulting mean risk score and the associated 25th and 75th percentiles over 10,000 samples (Table xii). The model was built in Microsoft Excel$^{®}$ using ModelRisk$^{®}$, add-in software for risk analysis. The parameter values for the Pert distributions for risk associated with import movements (i.e. movements from out-of-state) and movements from sale yards was based on published literature (Table xi) and on expert opinion. This distribution is used to model expert opinion when information

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regarding the parameters is difficult to infer from published literature, and only requires estimates of the minimum, most likely and maximum values for the parameter [181].

The equations below explain the risk score at ith movement, the jth farm in the kth year:

1) $RS_i = (1 + SY + IM) \times W$

2) $RS_{jk} = \sum_{i=0}^{n} RS_{ij}$

3) $TRS_j = \sum_{k=1}^{4} RS_{jk}$

Equation 1 refers to the risk score for each incoming movement that includes the following variables: 1) to account for each movement, which when summed corresponds to the *in-degree* for each farm; SY) movement from a sale yard; IM) import movement with origin outside of MN and W) weight accounting for number of cattle in the movement (1 = movement with ≤10 cattle, 2 = movement with 11 to 100 cattle and 3 = Movement with > 100 cattle). Equation 2 sums the individual incoming movement risk scores from equation 1 at farm level for each year of the study. Equation 3 sums the risk scores from each year in order to obtain the TRS for each farm in the full period of the study (Table xii).

3.3. Results

3.3.1. Descriptive analysis with trends by year and month

The number of movements of cattle entering, exiting, and within the MA zone peaked in January and December each year, although a significant number of cattle movements occurred at other times of year (Figure xii). The number of cattle moved was maximal in the same months (January and December), but exhibited a stronger seasonal trend, hitting a minimum in the summer months (Figure xiii).

We also saw the temporal effects of the change in status in the bTB MA area on the number of recorded cattle movements. Since movements were self-reported by cattle owners, we observed a slow increase in the number of recorded movements at the beginning of 2008 as the MNBAH began enforcing the mandatory reporting of movements. Similarly, we also noted a drop in the number of recorded movements at the end of 2011 corresponding to the change in status of the bTB MA area to bTB-free in October 2011. Data collected in 2009 and 2010, when no changes in status occurred, are therefore the most complete.

3.3.2. Network analysis of the complete network and by year

The networks representing the structure of cattle movements in the bTB MA zone, both on an annual basis and over the entire four-year period, were characterized by being highly disconnected, meaning that there were far fewer edges (i.e. movements) present in the network compared to the total number of possible edges, as reflected in a low *density* and a high *fragmentation* (Table xiii). Furthermore, the nodes connected to a

given node were unlikely to be connected themselves (low *clustering coefficient*). Most nodes were not along the shortest path between other nodes (low *betweeness index*) and since the network had low *density*, most of nodes were unable to reach other nodes in the network (low *closeness index*). These two parameters indicated that the network "*power*" was concentrated in a very small number of nodes. Appropriately, we noted the presence of two main hubs in the network, which corresponded to two sale yards located in northern Minnesota just outside the bTB MA area with a huge number of incoming connections (Figure xiva). Aside from these two hubs, the majority of nodes in the network had more outgoing movements than incoming movements (Figure xivb). The longitudinal component of the data showed very little variation between years for the network parameters (Table xiii).

3.3.3. Correlation between centrality parameters

In the current network, the correlation among parameters related to incoming and outgoing movements was extremely high (Table xiv). This high correlation between degree and Bonacich Power, for both incoming and outgoing movements can be explained by the strong influence of two sale yards, with the majority of movements being performed either to or from these facilities. In other words, those nodes that have more movements are also more likely to have movements to or from those highly influential nodes, thus resulting in very little difference between both parameters [182].

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3.3.4. Degree distribution

Further analysis of the *degree distribution* showed that 20% of the nodes with highest degree account for 90% of the movements and 86% of the cattle being moved. Again the two main sale yards were the dominant influence on these results (Figure xv).

When evaluating only the private farms within the bTB area, most farms had low *degree*, with the most connected farm having a degree value of 26 (Figure xvi). Considering *in-degree* and *out-degree* separately, it is clear that most farms had more outgoing (Figure xiv-b) than incoming (Figure xiv-a) movements, reflecting the management practices of the study area, where cow-calf beef operations prevail.

3.3.5. Sale yards

The network had 12 sale yards and 7 were located within Minnesota. From the latter, 2 had a central role in the network of cattle movements to and from the bTB area. Overall, 61% of the total number of cattle moved and 64% of the total number of movements went through these 2 sale yards (Table xv). The ego-networks, incorporating nodes that send and receive directly to and from both sale yards, included 45% and 47% of total number of nodes for sale yard 1 and 2, respectively (Table xv). Both sale yards had a greater number of incoming cattle than outgoing cattle, in part as cattle sold to farms outside of the bTB MA area are not accounted for. This difference was much greater in the case of sale yard 2, reflecting differences in sale yard operations: sale yard

1 dealt more with young stock, while sale yard 2 managed more cull cattle, which were less likely to be sold to other farms in the bTB MA area. Because beef cow-calf herds were the predominant cattle operation type in the bTB MA area, many more farms sent cattle to sale yards than imported from them. This regional feature highlights the ability of the sale yards in dispersing cattle with diverse origins, and potentially disease as well, across a broad area.

3.3.6. Risk score for private farms within the bTB area

The TRS associated with risk for disease introduction showed that the variability was higher with high risk farms, and decreased to a null value in low risk herds with no incoming movements across the 4 years (Figure xvii). Farms were ranked based on the TRS and divided into high, medium, and low risk groups based on the score and its variability. However, the cutoff values could be adjusted depending on the intensity of the surveillance implemented by the animal health agency. From our cut-off values, the higher risk group included 14% (n=50) of the farms and comprised 80% of the cumulative risk for the farms in the bTB area, the medium risk group included 46% (n=169) of farms accounting for the remaining 20% of the cumulative risk, and the low risk group consisted of farms with a null risk score, corresponding to the absence of incoming movements and were 40% (n=149) of the total number of farms. The average

correlation between the mean risk score for each year, 2008 through 2011, was 0.41 ±
0.18.

3.3.6.1. Evaluation of the impact of farms in the high risk group

Once infected, not all herds at high risk for infection are equally important to
disease transmission. Figure xviii differentiates among farms with a high probability of
infection, based on the risk score, and those that may have a higher or lower impact on
disease transmission based on out-degree or flow betweenness centrality. With *out-degree*, there was more spread of the results, since more farms had out-going movements,
which impacted the network locally, i.e. to their immediate connections, after excluding
movements to slaughter plants. Concerning *flow betweenness*, since it is a parameter that
measures connectivity to the whole network, fewer farms had the potential for
widespread impact in the overall network. Furthermore, the farms with the highest risk of
infection within the high risk group did not have the higher values for *out-degree* and
flow betweenness (Figure xviii).

3.4. Discussion

In this analysis, we developed a potential approach to targeted surveillance by
incorporating network analysis parameter (in-degree) and known risk factors for bTB
associated with cattle movements. This analysis was only possible since complete cattle

movement data was available, which to the authors knowledge constitutes a unique situation. The ability to score farms and to identify those at high risk for disease introduction could greatly improve the effectiveness of disease surveillance by detecting disease more quickly while reducing cost. The method developed here has limitations, particularly the absence of estimates for the parameters used in the risk model in disease free scenarios, such as the Minnesota situation. However, using current established knowledge on bTB risk factors for disease introduction into a disease-free area adds confidence to our assumptions.

The application of network analysis to understand the heterogeneous structure of contacts in a population has been extremely important in identifying the high risk players, or "*superspreaders*", that play a key role in the spread of infection [162, 172]. For example, we know from disease modeling in scale-free networks, where the *degree distribution* follows a power-law (i.e. few nodes have more connections versus a random distribution of connections), that *nodes* with the highest *degree* are more likely to become infected and, once infected, to rapidly spread disease throughout the network [160, 163, 164]. However, the vast majority of these conclusions have been based on analyses of undirected networks, where nodes may both infect and be infected by their direct contacts. In contrast, cattle movements are inherently and importantly directed, making the distinction between risk of infection and risk of transmission essential. Each of these types of risk can only be assessed using a directed network approach. We used *in-degree*

as the primary parameter indicator of risk for disease introduction, as centrality measures

relating to incoming movements were all highly correlated. Outgoing movements were

more variable, so we assessed the risk of disease transmission through an analysis of both

out-degree and more complex parameters such as *flow betweenness centrality* [175, 183].

Both the *in-degree* and *out-degree* are local measures in the network, calculated based on

immediate edges to each node. On the other hand, centrality measures such as *flow*

betweenness centrality, evaluate the role of each node in the overall network. Unlike the

more commonly used measure, *betweenness centrality*, which only considers geodesic

paths between nodes, *flow betweenness centrality* considers all possible paths between

two nodes and can account for different strengths of connections (e.g., differences in the

number of cattle moving from one premise to another). Nodes with a higher *flow-*

betweenness have a higher influence in the network's overall connectivity, which

extrapolated for risk of disease transmission could be interpreted as an increased risk of

transmission if infected.

Analyzing the directed movements of cattle between premises has allowed us to

observe important differences in import and export behaviors. The distribution of *in-*

degree of premises in the MA bTB region exhibits the characteristics of a power law

distribution, where a few actors incorporate most of the activity of the study population

[162]. However, we did not see this trend to the same extent in *out-degree* connections,

which is likely related to the types of cattle production system in the MA bTB area. This

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area has a predominance of beef cow-calf operations, with most herds shipping calves after weaning and few behaving as buyers, and many of the private farms having no incoming movements at all throughout the 4 years.

One of the primary findings in the movement data for the MA bTB area was the highly influential role of two sale yards that acted as hubs in the network and connected many different farms together through cattle movements. Sale yards or animal markets and their role in disease have been studied previously in other circumstances and have been found to be extremely important factors in disease transmission [184, 185]. In an analysis of foot-and-mouth disease (FMD) outbreaks in the UK from 1992 to 2003, authors concluded that the primary factor contributing to a large outbreak size was an FMD-infected animal going through a market [178]. A descriptive investigation of the initial spread of the 2001 UK FMD outbreak also highlighted the pivotal role of a sale yard in disease spread [177]. Anecdotally, after the 2005 Minnesota bTB outbreak began, official veterinarians identified cattle from over 20 states in sale yard 1 while performing animal-level testing. This reinforces the importance sale yards play in facilitating mixing between cattle from many different areas, with a high potential for disease transmission.

The centrality of sale yards in the current network analysis highlights the importance of keeping thorough records of animals moving through these holdings. Such records would be extremely informative in the event of an outbreak disease investigation. Tracking movements to and from sale yards constitutes one potential targeted disease

surveillance measure that could save great amount of resources in labor and consequentially reduce the financial toll when facing a disease emergency. This is particular important when considering the fact that sale yards tend to include movements that cover longer distances, working as bridges between otherwise geographically isolated areas, increasing the risk for a more widespread outbreak [186]. Changing the organization of sale yards could also mitigate the risk of disease spread, such as segregating cattle originating from areas with different levels of risk for disease from each other.

Among private farms, low values of overall *flow betweenness*, as with low *density*, confirm the low level of connection of this network of cattle movements and the minimum impact caused by any of the farms, if infected, in the overall network. Very likely, disease would spread slowly and would affect a small number of farms. The network analysis presented here sheds light in understanding what potentially happened in the bTB outbreak in MN, where only 12 farms were infected during a 5 year period after disease was introduced. The great risk for spread will increase if an additional host emerges in the wildlife population, creating additional routes for disease propagation [143].

Studies investigating the risk of disease attributable to cattle movements are limited and in general rely on data collected in bTB endemic areas. However, risk factors such as cattle movements, and more specifically movements from sale yards or areas

known to be infected, have been shown in the published literature to be highly influential. Strengthening these conclusions is the fact that the index herd of the 2005 bTB outbreak in Minnesota had 90% of its imported cattle originating from out-of-state. This is in contrast to the other bTB case herds, in which only 3% of imported cattle originated from outside Minnesota [2].

The proposed model for targeting surveillance based on the risk score for bTB does not exclude the standard slaughter surveillance applied to every animal. The goal is to implement higher levels of surveillance expressed by more field testing or increased submission of tissues from slaughter, for farms identified as high risk. This targeting will increase the surveillance system sensitivity. Furthermore, the identification of such farms and the higher level of surveillance implemented would allow a faster response when performing contact tracing [172]. The main objective is to perform targeted surveillance on farms that are more likely to become infected with bTB and consequentially mitigate bTB spread into the overall cattle population and potentially to wildlife populations. The number of farms to test will depend on availability of resources, but instead of based on a random selection process it could be based on a risk score. Targeting education to high risk farms before a disease incursion in order to improve response to an outbreak has also been proposed [186].

The current dataset had the advantage of incorporating 4 years of data, allowing for a longitudinal analysis of network features and farm risk scores. This is particularly

important when dealing with a disease with a long latent period such as bTB, where risk behaviors in one year can still influence the population four years later. However, the completeness of the data was not consistent over the entire observational period, with fewer movements recorded in early 2008 and late 2011 than during other times. Since cattle movements were self-reported by farm owners, adherence may have been low at the beginning of the mandatory reporting period and enforcement of this mandatory reporting likely declined once the status of the MA bTB area changed to bTB free in 2011. These observations highlight some of the problems of relying on self-reported cattle movements, as these may not reflect what in fact occurs in the field. This can be improved by education and by clear policy measures that reward those who adhere to the rules and penalize violators.

Though we considered individual movements between premises, we ignored the specific timing of these movements. The order in which movements occur over time is important, as any exports occurring from an uninfected herd prior to the importation of an infected animal have no potential for disease transmission. The ordering of movements is particularly important when performing trace back investigations after an outbreak, where the specific chain of infection must be identified. However, for this analysis, which aimed to identify the high risk farms for disease introduction into a disease-free area, this variable was less critical, since we addressed the first movement of a potential chain of movements to follow.

This evaluation of the impact of high risk farms in the network of cattle movements shows the potential of using network parameters to quantify risk and target surveillance. However, an analysis based on cattle movements alone does not necessarily capture all transmission pathways. For example, animals in neighboring farms may come into contact with each other at fence lines, with potential for bTB transmission. While the risk score does not incorporate proximity to high risk farms, in an outbreak situation, farms with fence line contact to high risk farms would be immediately included in a high surveillance zone. The presence of bTB-susceptible wildlife, such as white-tail deer, may also facilitate unmeasured contact between farms [142]. Wildlife may move freely through farmland and come into contact with animals from different herds, facilitating disease spread. As control and eradication are even more difficult in wildlife populations, once infected these populations may become maintenance hosts [71]. Thus, when assessing the potential impact of high risk farms in the network, incorporating their potential for interacting with white-tail deer is necessary. Empirical studies of the behavior of deer and other relevant wildlife in the farm landscape are needed in order to properly account for wildlife mediated contact between farms in the network and risk models.

Network analysis is a growing tool in veterinary epidemiology, however very few papers have used it to inform disease surveillance in a practical way. The current model, although limited in its application in a specific area in Minnesota, can serve as a basis to

be applied in similar circumstances to other regions. In addition, the model is simple in its application and in the data required, and could therefore be feasible for use by animal health agencies. The objectives of animal health surveillance can be greatly enhanced by using targeted approaches, particularly in areas that are disease-free in order to act more effectively and minimize the high costs associated with investigating and controlling disease outbreaks.
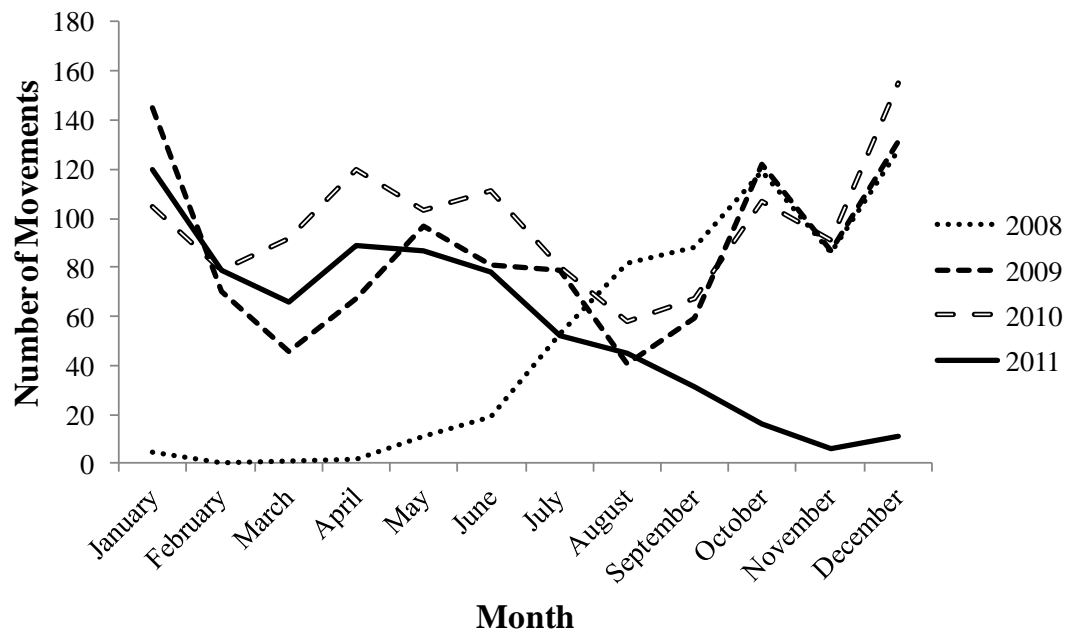
Figure xii. Number of cattle movements in the MN bovine tuberculosis Modified

Accredited area by month, with each series representing a year.
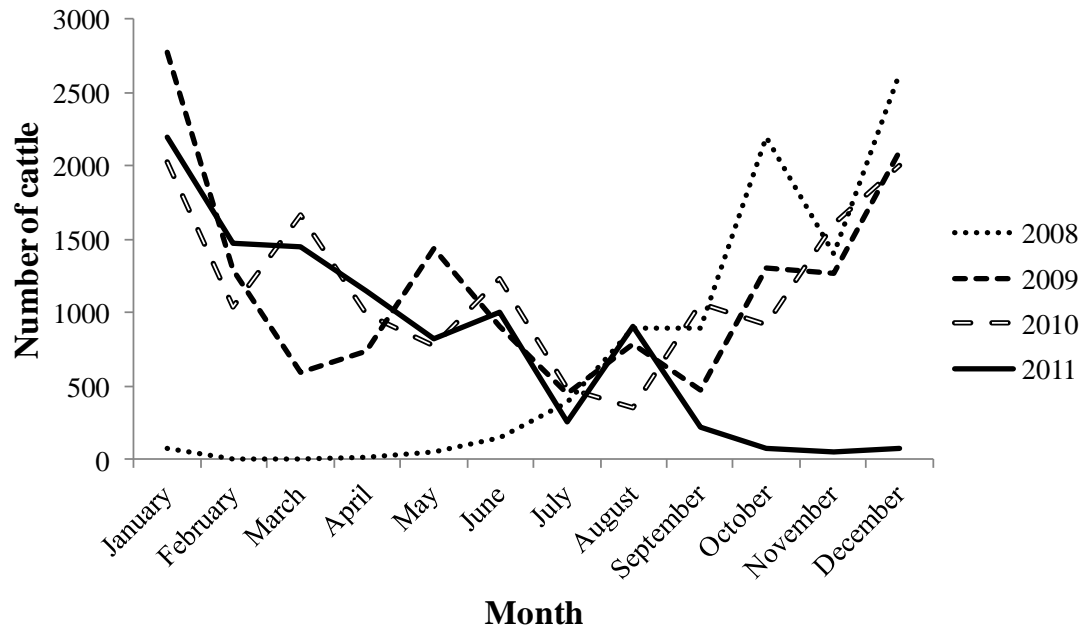
Figure xiii. Number of cattle moved in the MN bovine tuberculosis Modified Accredited by month, with each series representing a year.
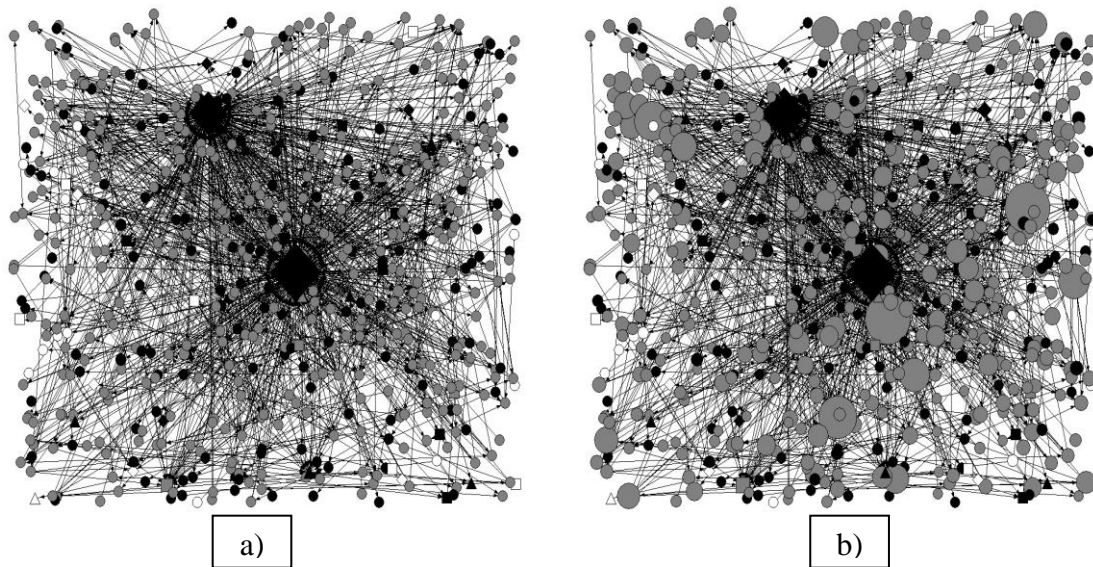
Figure xiv. Network graphs for all the cattle movements between 2008 and 2011 in the MN bovine tuberculosis Modified Accredited area.

Shapes represent type of node: ● - private farms, ◊ – sale yards, ■ – other and ▲ – slaughter facilities. Colors represent location: white – outside MN (other state or Canada), black – MN outside bTB area and grey – bTB area. Nodes are sized by a) in-degree for a valued network and b) by out-degree for a valued network. Location of nodes in the graph is random and not related with geographic location.
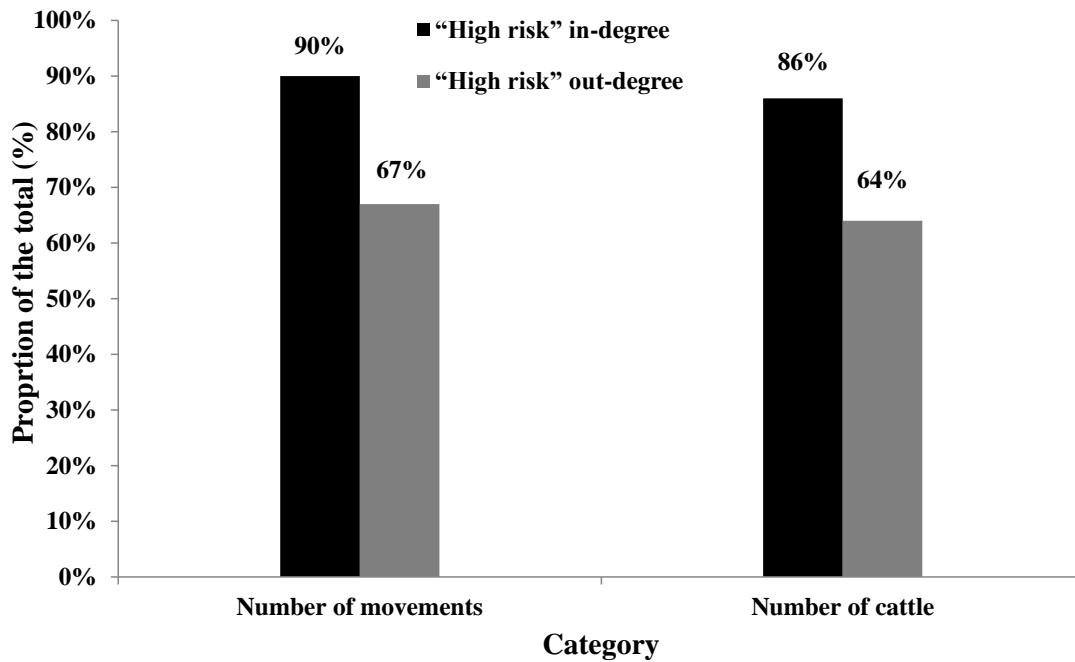
Figure xv. Distribution of the proportion of movements and proportion of cattle moved in relation to the total for the upper 20% of nodes with higher in and out-degree in the MN bovine tuberculosis Modified Accredited area.
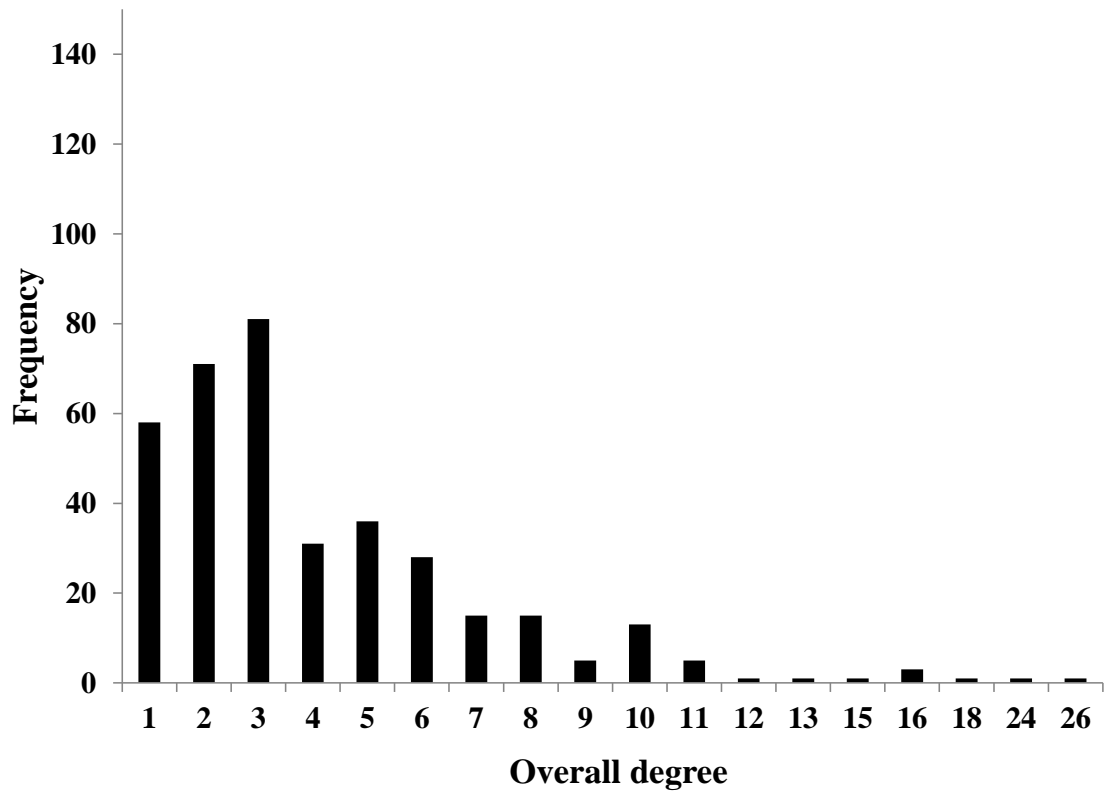
Figure xvi. Histogram with the distribution of overall degree (in and out) for the binary network for private farms (n=367) within the MN bovine tuberculosis Modified Accredited area.
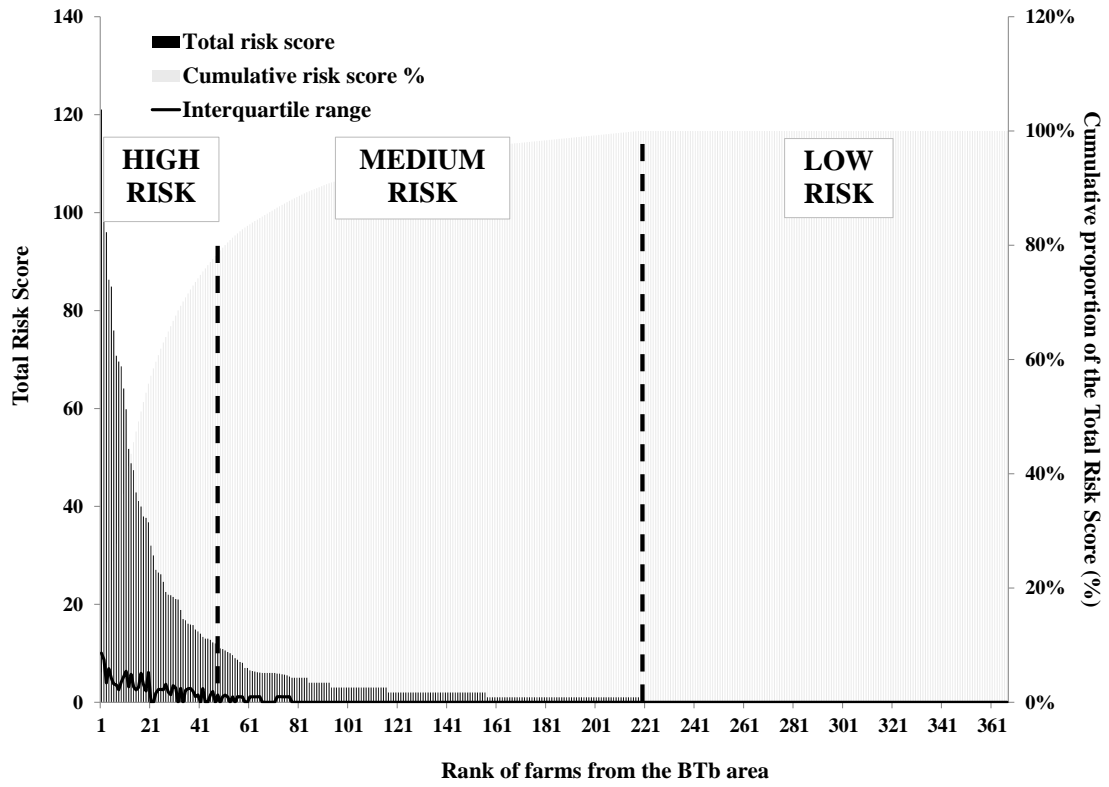
Figure xvii. Histogram with total risk score and interquartile range (25th to 75th[th]

percentile) of the Total Risk Score after 10000 iterations for private farms within the MN

bovine tuberculosis Modified Accredited area.

Dashed lines indicate thresholds for targeted surveillance based on combination of the

cumulative proportion of the Total Risk Score and variability of the interquartile range.
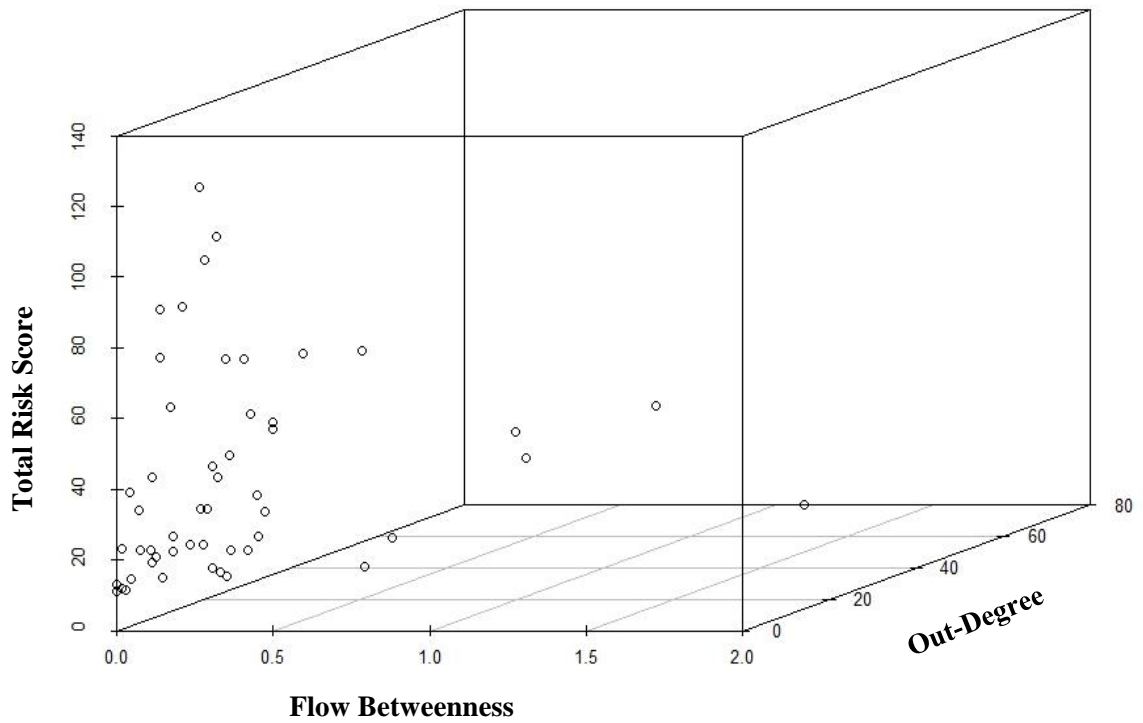
Figure xviii. Scatter plot with 3-dimensions with Total Risk Score (after 10000 iterations) by flow betweenness and out-degree for a valued network for herds in the bovine tuberculosis Modified Accredited area, including only herds in the high risk category from the risk score model.

3.6. Tables

Table viii. Farm characteristics in the MN counties included in the BTb area[1].

County	Average size of farm (km^2)	Total cattle/total farms (n/n)	Beef cattle/ total farms (n/n)	Dairy cattle/ total farms (n/n)	Rank of total cattle[2]
Beltrami	1.27	19,706/261	9,975/228	975/15	43
Lake of the Woods	1.74	4000/49	1800/46	200/-	-
Marshall	2.62	11,668/167	5,160/143	1,823/7	61
Roseau	2.02	17,948/249	8,009/211	1,422/22	49
Overall	1.91	53,332/726	24,944/628	4,420/44	

[1] Data obtained for the 2007 Agriculture Census form the USDA-NASS.

[2] Position in the ranking of total cattle for counties in MN.

Table ix. Summary of cattle movement data included in the network analysis.

Items	Years				Overall
	2008	**2009**	**2010**	**2011**	
Number of premises	302	386	375	280	559
Number of movements	594	1025	1168	680	3,467
Number of cattle	8702	14140	14181	9694	46,717

Table x. Network parameters calculated and its description and equation.

Parameter	Description	Formula
Density (D)[1]	Contacts that occur between pairs of nodes as a fraction of the total number of contacts that could occur	$D = \dfrac{L}{g(g-1)}$
Clustering coefficient (CC)[2]	Probability that two nodes connected to another same node are connected between them	$CC = \dfrac{1}{N}\displaystyle\sum_{i=1}^{N}\dfrac{e_{jz}}{k_i(k_i-1)}$
Fragmentation (F)[3]	Proportion of pairs of nodes that are not connected as a fraction of the total number of contacts that could occur	$F = 1 - \dfrac{2\sum_i \sum_{j<i} r_{ij}}{g(g-1)}$
Degree (d)[4]	total number of contacts (i.e. cattle movements) for each node	$d(n_i) = \displaystyle\sum \ell_i$
Mean Degree[5]	Mean degree of the network	$d = \dfrac{\sum_{i=1}^{g} d(n_i)}{g}$
Betweenness centrality (C_B)[6]	Number of times a node is on the shortest path (geodesic path) that connects two other nodes.	$C_B(n_i) = \displaystyle\sum_{j<k}\dfrac{s_{jk}(n_i)}{s_{jk}}$
Flow betweenness Centrality (C_F)[7]	Number of times a node is between all paths that connect two other nodes.	$C_F(n_i) = \dfrac{\sum_{j<k}^{n}\sum p_{jk}(n_i)}{\sum_{j<k}^{n}\sum p_{jk}}$
Betweenness centralization index (NBc)[8]	Overall level of betweenness centrality for the network	$NB_c = \dfrac{\sum_{i=1}^{g}[C_B(n^*) - C_B(n_i)]}{(g-1)}$
Closeness centrality (C_C)[9]	How close a node is connected to all the other nodes in the network	$C_c(n_i) = \dfrac{1}{\sum_{j=1}^{N} d(n_i, n_j)}$

Closeness centralization index (NCc)[10]	Overall level of closeness centrality for the network	$NB_c = \dfrac{\sum_{i=1}^{g}[C_C(n^*) - C_C(n_i)]}{[(g-2)(g-1)]/(2g-3)}$
Bonacich Power (c$_i$)[11]	Centrality is defined not only by degree but also by how central are the nodes with which the node is connected.	$c_i(\alpha, \beta) = \sum_{j} R_{ij}(\alpha + \beta c_j)$

[1] L - the number of contacts in the network; g $(g-1)$ - the total number of possible contacts on a g x g matrix.

[2] N- nodes in the network; e_{jz} – number of connections between nodes j and z that are neighbors of node i; k_i – degree of node i.

[3] $r_{ij} = 0$ if nodes i and j are not connected or $r_{ij} = 1$ if nodes i and j are connected.

[4] Degree was also calculated for only incoming movements = in degree (d_i) and only outgoing movements = out-degree (d_o); ℓ_i – total number of movements for node i.

[5] Mean degree was also calculated for both in degree (d_i) and out degree (d_o).

[6] $s_{jk}(n_i)$ – number of shortest paths through node i; s_{jk} – total number of shortest paths.

[7] This parameter was normalized, i.e. for each node i the flow that passes through i divided by the total flow that does not include node i.

[8] $C_B(n^*)$ – highest value of betweenness centrality in the network.

[9] Closeness was calculated also for both out-contacts (out closeness) and in contacts (in-closeness); $d(n_i, n_j)$ – length of shortest path between nodes i and j (if does not exist maximum distance possible is assumed).

[10] $C_C(n^*)$ – highest value of closeness centrality in the network.

[11] R_{ij} = adjacency matrix ; α = normalizes the parameter ; β = increases centrality if positive or decreases it if negative. Needs to be smaller than the reciprocal of the largest eigenvalue of the adjacency matrix - $\beta < 1/\lambda$.

Table xi. References used to estimate parameters for risk score model.

Variable	Author	Parameter value/description
Cattle bought from markets	Ramírez-Villaescusa et al., 2010	OR=1.95; 95% CI (1.05, 3.63)
	Johnston et al., 2005	OR = 3.26, 95% CI (1.71, 6.21)
Cattle bought from farm sales	Johnston et al., 2005	OR = 1.93, 95% CI (1.03, 3.60)
Bought-in cows	Reilly and Courtenay, 2007	OR = 22.5; 95% CI (4.0, 124.9)
Purchased cattle	Goodchild and Clifton-Hadley, 2001	Majority of BTb incidents were associated with purchased cattle
	Marangon et al., 1998	OR = 5.79; (1.80, 18.61)
In-coming movements	Gopal et al., 2006	30 out of 31 herd level outbreaks were associated to cattle movements
Range of source operations	Gopal et al., 2006	Wide range of source operations was associated with BTb infection
Cattle bought from infected herds	Munroe et al., 1999	OR = 48.8; 95% CI (9.7, 245.2)
	Gilbert et al., 2005a	Main predictor for BTb infection
Out of state movements	USDA, 2009	Index herd in MN had 90% of purchased animals from out-of-state

Table xii. Summary of risk model variables and parameters at the movement level to characterize herds within the BTb MA area by risk of introduction.

Variables	Parameters	Definition	Model Inputs
In-degree[1]	One incoming movement	Incoming movement for each farm	1
Import Movement (IM)[2]	Not a movement from out-of-state	Increased risk for farms with movements from out-of state versus farms with no movements from out-of state	0
	A movement from out-of-state		Pert(5, 10,15)
Sale Yards (SY)[2]	Not a movement from a sale yard	Increased risk for farms with movements from sale yards versus farms with no movements from sale yards	0
	A movement from a sale yard		Pert(3, 5, 7)
Number of cattle in each movement (W)[3]	≤10 cattle	Increased risk for farms with movements with more cattle	1
	11-100 cattle		2
	>100 cattle		3
Risk score[4]	1) $RS_i = (1 + SY + IM) \times W$ 2) $RS_{jk} = \sum_{i=1}^{n} RS_i$ 3) $TRS_j = \sum_{k=1}^{4} RS_{jk}$		

[1] The sum of the value 1 for each farm in all the years will originate the total in-degree $\sum d_i$.

[2] Import movement (IM) and movements from Sale Yard (SY), were modeled using PERT distributions.

[3] Number of cattle in each movement (W) was applied as a weight to the risk score and was divided in three categories with respective weights.

[4] Equation 1 refers to the risk score for each incoming movement (RS_i) that includes the following variables: 1) to account for each movement, which when summed corresponds

to the in-degree for each farm; SY) movement from a sale yard; IM) import movement with origin outside of MN and W) weight accounting for number of cattle in the movement (1 = movement with ≤10 cattle, 2 = movement with 11 to 100 cattle and 3 = Movement with > 100 cattle). Equation 2 sums the individual incoming movement risk scores from equation 1 at farm level for each year of the study (RS_{jk}). Equation 3 sums the risk scores from each year in order to obtain the total risk score (*TRS*) for each farm in the full period of the study.

Table xiii. Summary of network parameters by year of movements and overall after dichotomizing the matrix[1].

Parameters	Years				Overall
	2008	2009	2010	2011	
Density (%)	0. 5	0. 4	0. 5	0. 6	0. 4
Fragmentation (%)	99.3	89.5	85.6	86.4	77.0
Cluster coefficient (%)	8.0	8.5	10.4	10.8	14.6
Average in-degree (range)	1.427 (0 - 133)	1.672 (0 - 182)	1.709 (0 - 156)	1.543 (0 - 120)	2.257 (0 - 261)
Average out-degree (range)	1.427 (0 - 8)	1.672 (0 - 19)	1.709 (0 - 18)	1.543 (0 - 17)	2.257 (0 - 32)
Betweenness network centralization Index (%)	0.10	6.28	8.90	9.50	12.67
Closeness network in-centralization Index (%)	0.71	1.44	1.32	1.29	0.64

[1]The parameters were calculated for a binary network, considering only one movement between a pair of nodes even if multiple movements occurred.

Table xiv. Spearman rank correlation between different centrality measures calculated for the complete network dichotomized[1] to compare incoming and outgoing parameters.

	In-degree	In-Bonacich Power[2]	Out-degree	Out-Bonacich Power[2]	Betweenness	Flow Betweenness
In-degree	1	0.91	0.05	0.04	0.67	0.58
In-Bonacich Power[2]	0.91	1	0.05	0.04	0.70	0.61
Out-degree	0.05	0.05	1	0.92	0.62	0.56
Out-Bonacich Power[2]	0.04	0.04	0.92	1	0.56	0.49
Betweenness	0.67	0.70	0.62	0.56	1	0.80
Flow Betweenness	0.58	0.61	0.56	0.49	0.80	1

[1]All the network parameters were calculated based on a binary network, with the exception of flow betweenness that was calculated for a valued network.

[2] Centrality is defined not only by degree but also by how central are the nodes with which the node is connected (i.e. when comparing two nodes with the value 1 for in-degree, a node with low degree connected with a node with high degree will have a higher Bonacich Power compared with a node with high degree connected with a node with low degree).

Table xv. Summary of number of movements and cattle moved for the two primary sale yards included in the network of cattle movements (valued network) for the MN bovine tuberculosis Modified Accredited area.

	In-degree	Out-degree	Incoming cattle	Outgoing cattle	Nodes receiving	Nodes sending
Sale yard 1	825	75	7,727	2,037	29	248
Sale yard 2	1,230	89	20,872	342	32	261
Total	2,055	164	28,559	2,379	61	509

CHAPTER 4 - PATTERNS OF CATTLE FARM VISITATION BY WHITE-TAILED DEER IN RELATION TO RISK OF DISEASE TRANSMISSION IN A PREVIOUSLY INFECTED AREA WITH BOVINE TUBERCULOSIS IN MINNESOTA, US

The objective of this study was to characterize spatial patterns of white-tailed deer (*Odocoileus virginanus*) movement related to bovine tuberculosis (BTb) transmission risk to cattle in northwestern Minnesota (MN). Twenty-one adult deer (16 females and 5 males) were captured during winter (January-March) 2011 in an area adjacent to the region in northwest MN were a recent (2005-2009) outbreak of BTb occurred in deer and cattle. The captured animals were fitted with GPS collars that were programmed to collect deer location information every 90 minutes for a 15-month period. The exact locations of cattle, cattle feeding areas, and feed storage areas on farms that were available to collared deer were assessed seasonally. Deer mortality was unexpectedly high during this study, primarily due to wolf-kills; 47% (n = 9) of collared deer survived to the study's end date. Causes of mortality for deer included wolves (n = 7), hunters (n = 1), and unknown cause (n = 2); additionally, 2 deer were censored due to collar malfunctions. Our results indicated that 5 deer (25%) had locations within 6 cattle farms (20%). Most (77%) of the deer visits occurred in areas where cattle were present. Two deer visited only one farm, two deer visited two farms, and one visited three farms; most visits (60%) occurred from 12am to 6am. The spring months, March to May, had the

most farm visitations by deer (37%). This study provided baseline information regarding cattle-deer interactions critical to transmission of BTb in this region and suggested that risk mitigation practices should be implemented to separate wildlife and domestic livestock when feasible.

4.1. Introduction

Mycobacterium bovis (M. bovis), the causative agent of bovine tuberculosis (BTb), has a wide host range, including domestic animals, wildlife, and humans. Among domestic animals, cattle, goats, sheep, pigs, horses, farmed deer, and also dogs and cats have been identified as susceptible to *M. bovis*, though with different levels of occurrence and virulence [4, 11, 35, 108]. Two recent review articles identified more than 40 species of wildlife with global distribution from which *M. bovis* was cultured [11, 35].

Several wildlife species have negatively impacted BTb control and eradication efforts due to their confirmed role as maintenance hosts of BTb, thus being able to self-sustain and spread the disease; whereas spillover hosts are unable to maintain infection [4, 17]. The European badger (*Meles meles*) in the United Kingdom, brushtail possum (*Trichosurus vulpecula*) in New Zealand, elk (*Cervus elaphus*) in Canada, African buffalo (*Syncerus caffer*) in South Africa, wild boar (*Sus scrofa*) in Spain, and white-tail deer (*Odocoileus virginianus*) in the United States are examples of wildlife species that have been shown to undermine efforts to control the disease in cattle populations because

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the wildlife populations both maintain the disease and have continuous interaction with domestic cattle [9, 14, 15, 36, 37].

White-tailed deer, based on the most recent outbreaks of BTb, have been identified as the most important wildlife species for transmission of *M. bovis* to cattle in the United States. Analysis of the genetic fingerprinting of *M. bovis* isolates in Michigan (MI) and Minnesota (MN) indicated a relationship between infected wildlife and cattle [2, 40]. Furthermore, there is strong support to the role of indirect contact, through consumption of contaminated feeds, as an important route of *M. bovis* transmission [30, 46, 187].

Bovine tuberculosis re-emerged in cattle in MN in 2005 and by 2009, a total of 12 cattle herds were infected with BTb. Further, the disease had spilled into the white-tail deer population, affecting at least 27 deer [40, 63]. Minnesota, having lost its BTb free accreditation in 2006, was granted *split-state* status by the United States Department of Agriculture (USDA) in 2008, with most of the state upgraded to modified-accredited advanced (MAA) and only a small area (6,915 km^2) in northwestern MN remaining as modified accredited (MA). These areas are established by USDA based on herd-level prevalence for BTb after intense animal-level testing [188]. In October 2011, the state regained its BTb-free accreditation. The success of this state's BTb eradication effort, particularly in avoiding the establishment of a wildlife reservoir, was driven by implementing immediate and very aggressive control measures, including a ban of

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recreational feeding of wild cervids and aggressive deer removal efforts to reduce deer

densities [63]. Further, 6,200 cattle were removed from the endemic area in 2008

through a cattle buy-out process and mandatory fencing of stored forage and cattle

feeding areas was instituted on all remaining farms.

A thorough understanding of deer-cattle interactions in a previously BTb-infected

area, focusing on the "when" and "where" of those events, is essential to implement cost-

effective control strategies that will reduce the potential for disease to spillover to the

wildlife population, or mitigate transmission to a susceptible cattle population. Although

other studies have been conducted that describe the contacts between deer and cattle,

there has been no prospective study in the US on the frequency and spatial-temporal

distribution of contacts between these two species. Our goal with this study was to infer

the potential risk of BTb transmission to cattle in northwestern MN by quantifying how

deer movements overlap with the exact locations, through time, of cattle, stored feed and

feeding sites on the farm landscape.

4.2. Material and Methods

4.2.1. Study area

The study area was 360 km^2 and included a mix of state forest and wildlife

management lands, private recreational lands, and private farms (including row-crop

agriculture, farmsteads, and stored forage) with 25 farms actively owning cattle. Farms in

the study area were mostly small beef cow-calf operations (< 200 head). The study area

was located within the BTb MA area and just south of the BTb Management Zone, which

was an area established by MN Department of Natural Resources (MNDNR) based on a

buffer delimitation of 16 km around the initial 7 BTb infected deer in 2006 [63]. The

study area contained 2 formerly BTb-infected cattle farms; however, the disease had not

been detected in wild deer in this area (Figure xix).

Deer densities, before control measures were implemented to respond to the 2005

outbreak, ranged from 15-20 deer/km^2. Major predators include gray wolves (*Canis*

lupus), black bear (*Ursus americanus*), coyote (*Canis latrans*), and bobcats (*Felis rufus*).

The 2 winters encompassed in this study were moderately severe (2011) and

historically mild (2012). To gauge winter severity, the MNDNR calculates a winter

severity index (WSI) by accumulating 1 point for each day with an ambient temperature

$\leq -17.8°C$ (temperature-days) and 1 point for each day with snow depth ≥ 38 cm (snow-

days) from November 1–May 31. The maximum WSIs for winter 2011 and 2012 were

159 and <20, respectively.

4.2.2 Deer capture and handling

In January 2011, 16 deer (4 males, 12 females) were captured by helicopter

netgunning (Quicksilver Air, Inc., Fairbanks, Alaska) within ($n = 11$) and slightly

northeast ($n = 5$) of the study area (Figure xix). Capture locations were driven by farm

densities, deer distribution at the time of capture and access to private land to process deer.

Captured deer were chemically immobilized (100 mg xylazine, 400 mg ketamine HCl) after capture. Blood, urine, and fecal samples were collected for health screening, rump fat was measured by ultrasound and a last lower incisor was extracted to determine exact age by cementum annuli (Mattson's Laboratory, Milltown, Montana). Deer were ear-tagged and fitted with a satellite-linked radiocollar (ARGOS, SirTrack, Hawkes Bay, New Zealand). Body temperature was monitored at 5-min intervals throughout the processing period. A long acting antibiotic (LA-200, oxytetracycline) was administered intramuscularly (1 mL / 10 kg body weight). Before release, anesthesia was reversed by intravenous injection of 15 mg / deer of yohimbine HCl. An observer monitored each deer's recovery and recorded the time deer were standing and moving away from the recovery area.

A second capture effort, using Clover-traps [189], added 5 deer (1 male, 4 females) to the study in March 2011 to compensate for high early-winter mortality (Table xvi). One deer (ID 577) was fitted with a test collar provided by SirTrack (Iridium satellite system prototype).

4.2.3. Data collection

4.2.3.1. GPS location data

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Radiocollars were programmed to record locations every 90 minutes and
transmitted these "fixes" every 3 days through the ARGOS satellite system. Collars were
fitted with timed, remote blow-offs and discharged on April 15, 2012; stored data were
directly downloaded from all recovered collars. During the study, fixes were downloaded
from the satellite system weekly and examined for temporal and spatial movement
patterns to determine mortality, movements, and habitat use. For any study animals that
died during the study period, MNDNR wildlife staff investigated the cause of mortality,
recovered the collar, and collected cranial lymph node samples from the deer (when
possible) for BTb culture.

4.2.3.2. Disease screening of deer

Captured deer were screened for exposure to diseases common to domestic
livestock in the United States. Serum collected was tested for malignant catarrhal fever
(MCF) via peroxidase-linked assay (PLA), with positive PLA tests further tested with a
virus neutralization test (VN) at the National Veterinary Services Laboratory (Ames,
Iowa). All other serology was conducted at the UMN's Veterinary Diagnostic
Laboratory (VDL) in St. Paul, MN, which included screening for leptospirosis (6
serovars, microscopic agglutination test), anaplasmosis (card test), brucellosis (card test),
and bovine parainfluenza 3 (PI3, hemagglutination inhibition test). Exposure to
bluetongue virus and neosporosis were determined by enzyme-linked radioimmunoassay

(ELISA). Exposure to bovine viral diarrhea (BVD, Types 1 and 2) and infectious bovine rhinotracheitis (IBR) were confirmed using serum neutralization tests (SN); titers ≥ 8 were considered positive. Fecal samples from 20 deer were screened for evidence of parasites by fecal floatation.

4.2.3.3. Cattle farms

Exact location of cattle, stored feed, and feeding sites within the study area were surveyed and delineated using ArcGIS®. Ground-truthing of these areas was performed 4 times during the study period for all farms in the study area: immediately prior to the beginning of the study (January 2011), May 2011, October 2011 and January 2012. The choice for the 4 time periods was based on seasonal weather changes that impact management on the farm and consequentially changes in the locations of cattle.

The Board of Animal Health (BAH) performed wildlife risk assessments (RA) of the farms in the BTB MA area in 2009 ($n = 309$) based on a template previously developed [142]. In order to evaluate risk for deer interactions with cattle that could eventually result in disease spread, this RA evaluated four main areas including feed storage, feeding practices, cattle housing and water management. For each item evaluated, the farm received a score ranging from 0 to 180 points where higher scores indicate greater risk for deer-cattle interaction in the farm landscape.

4.2.4. Analysis

4.2.4.1. Deer visitation to farms

A buffer of 30 meters (estimated error for each collar location) was defined around each identified area of cattle, cattle feeding site, and stored feed (hay bales). Deer point data and polygons of the specified areas within the farm were spatially joined in order to identify specific deer locations within key areas. This analysis was performed for each time period in the study. Data was exported to Microsoft Excel® for graphical representation.

4.2.4.2. Board of Animal Health risk assessment scores

Farms were ranked, from highest to lowest, based on the score of the BAH RA, and this number was used as an identifier in the analyses. The results of those RA for farms within the study area were evaluated in relation to the deer location data, in order to describe the relation between RA score and the deer visitation data on the identified areas in the farm landscape.

4.2.4.3. Home ranges

Home ranges for each deer were calculated, using the minimum bounding geometry tool in ArcGIS®, by delineating a convex hull around each individual deer location data for the duration in the study.

4.3. Results

4.3.1. Fix Success

The fix success rate, calculated by number of locations received through satellite divided by number of locations available (assuming 16 locations a day) was 98%, which indicates the high quality of the data and the low impact of landscape features that could bias the data collection. Approximately 80,000 total locations were collected throughout the study.

4.3.2. Deer Population

A total of 21 deer (16 females, 5 males) were radiocollared during the study. Immediately after the 1st capture, deer 519 was censored from the study, due to collar failure. Another deer (ID 577), fitted with the test collar, was also censored due to collar malfunction. Of the 19 deer remaining, only 47% (n=9) reached the end of the study and 53% (n=10) died. Mortality due to predation by wolves (including the deer with test collar from 2nd capture) accounted for 70% ($n = 7$) of the deer mortality, which occurred mostly during winter 2011; 10% (n=1) of mortality was attributed to hunter-harvest, and 20% (n=2) to unknown cause (Table xvi).

4.3.3. Risk factors for cattle farms

The RA's performed by BAH in 2009 showed a high variability in the risk score for farms within study area; however, the three farms with highest risk score had deer locations within the identified areas. Size of cattle, feeding and feeding storage areas within farms varied, as expected, between farms (Table xvii). Between seasons this area also varied, with more area available during the spring, summer and fall months although overall it represented only about 1% of total study area.

Six farms (24%) had deer visits out of fifteen farms that were eligible for deer visitation, based on home ranges for the radiocollared deer (Figure xxiv). The farms not eligible were located in the northwest section of the study area, which was dominated by private agricultural land and had minimal deer habitat (e.g., wildlife management area or forest lands). Farms with lowest risk scores, even those with more than one deer homerange overlapping with their farm areas, did not have any deer visits (Table xvii).

4.3.3. Descriptive analysis of farm visits by white-tail deer

Only 1% of total locations were within identified farm areas. Deer visits to identified cattle, feeding site, and stored feed areas occurred in six farms in the study area, with one farm accounting for 61% of the visits. Five deer accounted for all farm visits, including two deer visiting only one farm, two deer visiting two farms and one deer visiting three farms (Figure xx). Regarding the latter, the three farms were neighboring farms in the southeast section of the study area and deer 578 was captured in

that same area, and remained there for most of the study period. Deer 336 and 497 each

visited two farms (Figure xx) and, these farms were also in the same section of the study

area; southeast and northwest respectively (Figure xxiv). However, deer 497 migrated

more than 25 km east to winter habitat (e.g. forest) and later returned to the same area

closer to farmland. Deer 578 covered an area similar to 336 (i.e., the southeastern portion

of the study area; Figure xxiv).

Over 75% of deer visits occurred in areas where cattle were present, either on a

pasture or in an area with a feeding site and/or stored feed (hay bales) (Figure xxi). Most

of the farm visits happened during the spring (March– May) and in October (Figure xxii).

Deer visits increased during the crepuscular period achieving its maximum during the

night (from 12am to 6am), only 5% occurred between 9am and 6 pm. (Figure xxiii).

4.3.4. Home ranges

Mean home range size for deer ($n = 9$) surviving through the end of the study was

46.7 km^2 (SE = ±10.1; Figure xxiv). Deer that died (or slipped their collar) during the

study had significantly smaller home range sizes than survivors (13.9km^2 ± 5.3). This

apparent difference in home range size might be due to the fact that surviving deer were

tracked over a longer time period.

Given the timing of deer capture (mid-January and early March), we assumed

these animals were on their winter range (if migratory) or possibly year-round residents

at the start of the study. This was an incorrect assumption, as movements to distinct winter ranges did not occur until late-January or February 2011. Seven deer had home ranges > 40 km^2 and can be attributed to a few long-distance movements from one end of their range to the other. These movements began in late January for 5 deer, moving 4-20 km in a 2-3 day period. The other 2 deer moved 14-19 km in mid to late March, again over a 2-3 day period. Of these 7 deer, 2 were killed during winter, but the other 5 returned the same distance (in a 2-3 day period of travel) to the area they were originally captured in late March or early April. Interestingly, only 3 of the 5 surviving migratory deer returned to their winter ranges during the mild winter of 2012; however, the start of their movement was much later (late February-early March) and they returned to their spring-summer-fall ranges sooner (mid-March).

4.3.5. Deer health data

Serological results indicated deer were exposed to bovine parainfluenza 3 virus (PI3 = 24%), malignant catarrhal fever (MCF = 19%), and infectious bovine rhinotracheitis (IBR = 9%). There was no evidence of exposure to anaplasmosis, bluetongue virus (BTv), bovine viral diarrhea (BVD types 1 and 2), brucellosis, leptospirosis, and neosporosis. These tests only indicated deer had been exposed to these pathogens and thus developed an immune response in which the antibodies were detected

through testing. We were not able to confirm current infection or clinical illness from any of these diseases in these deer.

Thirteen (65%) of deer had evidence of liver fluke (*Fascioloides magna*) infection and strongyle-type ova was detected in 4 (20%) deer. Also, culture of fecal samples did not detect any evidence of *Mycobacterium paratuberculosis* (MAP) in these deer. Negative results in the fecal exam do not necessarily mean the animal was parasite-free, only that it was not actively shedding at the time the feces were collected, which also applies to MAP.

4.4. Discussion

4.4.1. Deer as a maintenance host

One of the most important steps necessary for assessing the risk of wildlife populations infecting cattle with pathogens that can cause disease is understanding the spatiotemporal factors that influence either direct or indirect interactions between the infected wildlife and cattle [17]. With regards to BTb, the transmission initially occurs in cattle, with spillover to wildlife. Although still possible to transmit disease, a greater risk exists if the spillover host becomes a maintenance host, capable of perpetuating the infection in its own species and increasing the risk for inter-species transmission [60].

In Michigan, the most likely factors that originated a maintenance white-tailed deer host for BTb were high deer densities and unnatural congregations associated with

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baiting and feeding [69]. These two factors created ideal conditions for intraspecies transmission of BTb. Bovine tuberculosis has been a problem in MI since 1995, and continues to burden farmers, hunters, and state-federal animal health agencies. Minnesota, having learned from the recent MI experience, implemented aggressive measures to dramatically reduce the deer densities and restrict recreational feeding. Consequentially, these steps reduced the "force of infection", and as result, white-tailed deer in MN did not become a maintenance host [63, 145].

4.4.2. Deer cattle-interactions

The current study aimed to enhance our understanding of the role of deer in BTb disease transmission. Despite a small sample, the results are strengthened by previous knowledge of deer behavior patterns. The temporal trends in deer visitation, more likely to occur after dark, were similar to what is known about deer activity. This shows that even when exposed to farm level management factors, such cattle feeding times, deer did not change their normal behavior. The increased deer use of farms during the spring is likely associated with nutritional restriction and negative energy balance after a severe winter, augmented in female deer by the approaching fawning season [190, 191].

Deer densities have decreased in northwestern MN to an estimated 1-3 deer/km^2, in response to aggressive BTb management efforts that occurred from 2005 to 2010. With deer densities even lower than previous years, our study likely underestimates deer use of

cattle and feed locations on farms, particularly those farms with higher risk for wildlife interaction. The visits of the study deer to multiple farms or the farms visited by multiple deer indicate the risk for disease transmission, if present. In the event of BTb again spilling over into the deer population, the outbreak could spread to other farms since deer function as a vector for disease transmission. Furthermore, the increase in deer densities in some farm areas and also on winter range, where several deer migrated, could enhance the potential for a maintenance host to emerge.

Four of the six farms visited by deer were located in the east side of the study area, between a mix of forest and wildlife management areas, in contrast with farms on the northwest side of the study area that were not visited by study deer and are located in an area with more agricultural land (thus less viable deer habitat). A cross-sectional study in MN evaluated the land surrounding cattle farms and found that landscapes with a greater percentage of land classified as woods, swamp, or other wildlife habitat had increased risk of deer accessing stored feeds on the farm [142]. Furthermore, a recent risk assessment carried out by USDA-Animal and Plant Health Inspection Service-Veterinary Services evaluated the spatial association between infected deer and cattle herds in northwestern MN, and found that positive deer were significantly clustered with one another, and all positive and suspect deer were within 10.9 kilometers of positive cattle herds [144].

The extension of the home ranges in combination with the information regarding overlap of home ranges and farm areas indicates the potential for the study deer to have visited other farms. This data could not have been collected as a consequence of the time interval for data collection programmed in the GPS collar.

4.4.3. Deer mortality

The mortality rate observed from February to April 2011, specifically due to wolf predation, was higher than expected. Winter conditions were moderately severe in the study area, with a WSI of 159, primarily due to prolonged snow cover of > 14 inches from late-January through early April. In MN's forest zone, researchers reported a 37% winter mortality rate for adult deer during the historically severe winter of 1995-1996 (WSI = 195), with wolves accounting for 63% of those deaths [192]. Additionally, during more moderate winters (WSI = 124 to 159) in north-central MN, winter mortality rates ranged from 7 to 19%, with wolf predation accounting for 50-80% of the deaths [192]. In contrast, the winter mortality rate for adult female deer in MN's farmland zone has been reported as only 5%; however, there was an absence of wolves and typically more mild winter conditions [193]. On the other hand, both the BTb MA area and the study area consist of a transitional habitat zone that divides forest and agricultural land; little is known about deer or wolf behavior in this area. As a result, little information exists on winter mortality rates for deer in the transition zone of Minnesota, and although the

sample size was limited in this study, our preliminary findings suggest there might be some unique attributes in northwestern MN that make deer population dynamics different in both farmland and forest zones. Also, the combination of winter severity and extensive deer population control in previous years could have led to changes in wolf behavior. Interestingly, during the historically mild winter 2012 (WSI < 20), none of the remaining study animals were killed by wolves. The potential role of wolves to prevent the establishment of a maintenance host needs to be further explored. In general, carnivores are more likely to kill sick animals, since they are more vulnerable; thus increasing their exposure to highly infective material, but at the same time removing those infective individuals from the population [194].

4.4.4. Deer ecology

White-tailed deer in MN farmland zone have been reported as migratory (43% obligate and 35% conditional migrators), with a mean migration distance of 10 km and with mean winter and summer home ranges (95% MCPs) of 5.2km^2 and 2.6km^2, respectively [193]. Conversely, forest zone deer in northeastern and north-central MN were 89% and 68% migratory, respectively [195, 196]. Migration distances were typically 10-14 km, but ranged from 2-135 km; onset of migrations varied annually, but ranged from early November to January [196]. In both studies of forest zone deer, severe winters coincided with a higher number of conditional migrators making movements to a

distinct winter range [195, 196]. This could increase transmission, if disease is present, between the deer that migrate and share the same winter habitat despite the fact that the rest of the year returned to distinct spring-summer-fall ranges.

4.4.5. White-tailed deer health results

The serological results for exposure to cattle diseases in the study deer provide further evidence for potential interaction between cattle and the white-tailed deer and for the likelihood for inter-species disease transmission. Exposure to PI3 in this study was not unexpected, as our prevalence was similar to the 20% previously reported for deer throughout MN [197]. Little is known about PI3 infection in white-tailed deer, although it has been demonstrated to be infective in both captive and free-ranging pronghorn (*Antilocapra americana*) in Alberta [198]. In a serologic survey of wild cervids in national parks in the US, 58% of mule deer (*Odocoileus hemionus*) and 57% of elk (*Cervus eleaphus*) were exposed to PI3 [199].

Our findings of 19% prevalence for MCF in deer is lower than what has been recently reported for northwestern wild elk and northwestern moose, 29 and 35% respectively [200, 201]. Malignant catarrhal fever affects many species in the family *Artiodactyla* (even-toed ungulates) including cattle, bison, deer, moose, exotic ruminants and pigs. The deer strain of MCF is typically carried asymptomatically, but it can cause disease in other susceptible species or in rare cases, in the maintenance host itself.

Infectious bovine rhinotracheitis is a highly contagious, primarily respiratory, infectious disease of cattle that is caused by Bovine Herpesvirus-1. Little is known about IBR virus in deer. While we report a 9% prevalence, a higher prevalence (15%) was noted in a statewide serologic survey of MN deer [197]. Exposure to IBR has also been reported in MN's moose (*Alces alces*) [202].

Besides providing more evidence for cattle-deer interactions, this information also highlights the risk for other high impact diseases, other than BTb, that if introduced could spread among domestic and wildlife populations.

4.4.6. Implications in disease control and surveillance

These data inform future delineation of surveillance areas when responding to an outbreak. National and international animal health agencies (USDA and the World Organization for Animal Health- OIE) have highlighted the need to implement science-based zoning approaches when controlling disease. However, one of the main issues with disease surveillance for BTb is the timeliness of the program with very lengthy periods (more than five years) from disease introduction until detection [23]. The implementation of zoning approaches, based on known risk factors for disease introduction and spread, before an outbreak occurs in order to detect disease earlier and prevent its spread could be extremely useful.

Our study highlights the need for different approaches to be considered regarding both surveillance and disease control efforts, particularly at regional and farm scales. At the farm level, these data illustrate the importance of educating farmers about the consequences of their management practices, particularly when deer are a host for a disease like BTb if risk management is not taken seriously. Our results should reinforce the need to implement strategies shown to be effective for mitigating cattle-deer interactions, such as building fences to protect stored feed and the use of farm dogs [203, 204].

The temporal patterns of white-tailed deer locations on farms highlight the potential for target interventions on the deer population when aiming to reduce population densities to prevent the establishment of a wildlife reservoir while controlling disease spread. This could be expressed by implementing control measures directed towards high risk locations and times (i.e., during the spring season and nighttime). Also, the wildlife state regulatory agency could target deer harvesting around agricultural lands. The rationale for such approaches is that risk for disease is not random but is rather based on a combination of magnifying risk factors.

This study is novel since it was designed to identify the exact areas within the farms where deer could be visiting and how those change seasonally. This allowed to identify risk factors that could be used in a targeted surveillance system where resources are focused on the subsets of the population that are more likely to play a role in disease

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spread. Furthermore, the results are extremely helpful as a resource to educate farmers on

the risks posed to their own cattle by certain farming practices or by the absence of

biosecurity measures that mitigate the interactions between deer and cattle.

4.5. Figures
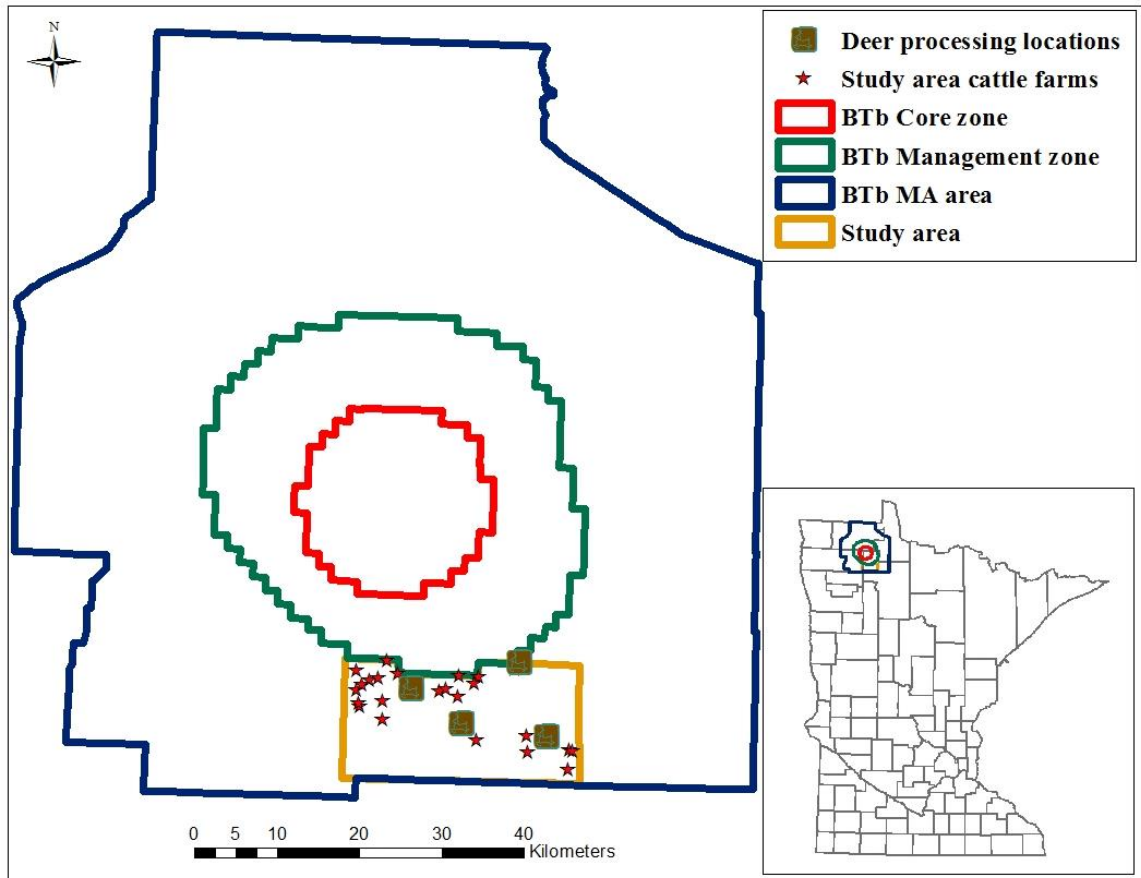
Figure xix. BTb zones from the 2005 outbreak and study area with deer processing

locations and farm locations.

The processing locations corresponds only to deer captured using a helicopter however,

the three clover traps were in approximate locations with the further west and the 2

further east processing locations.
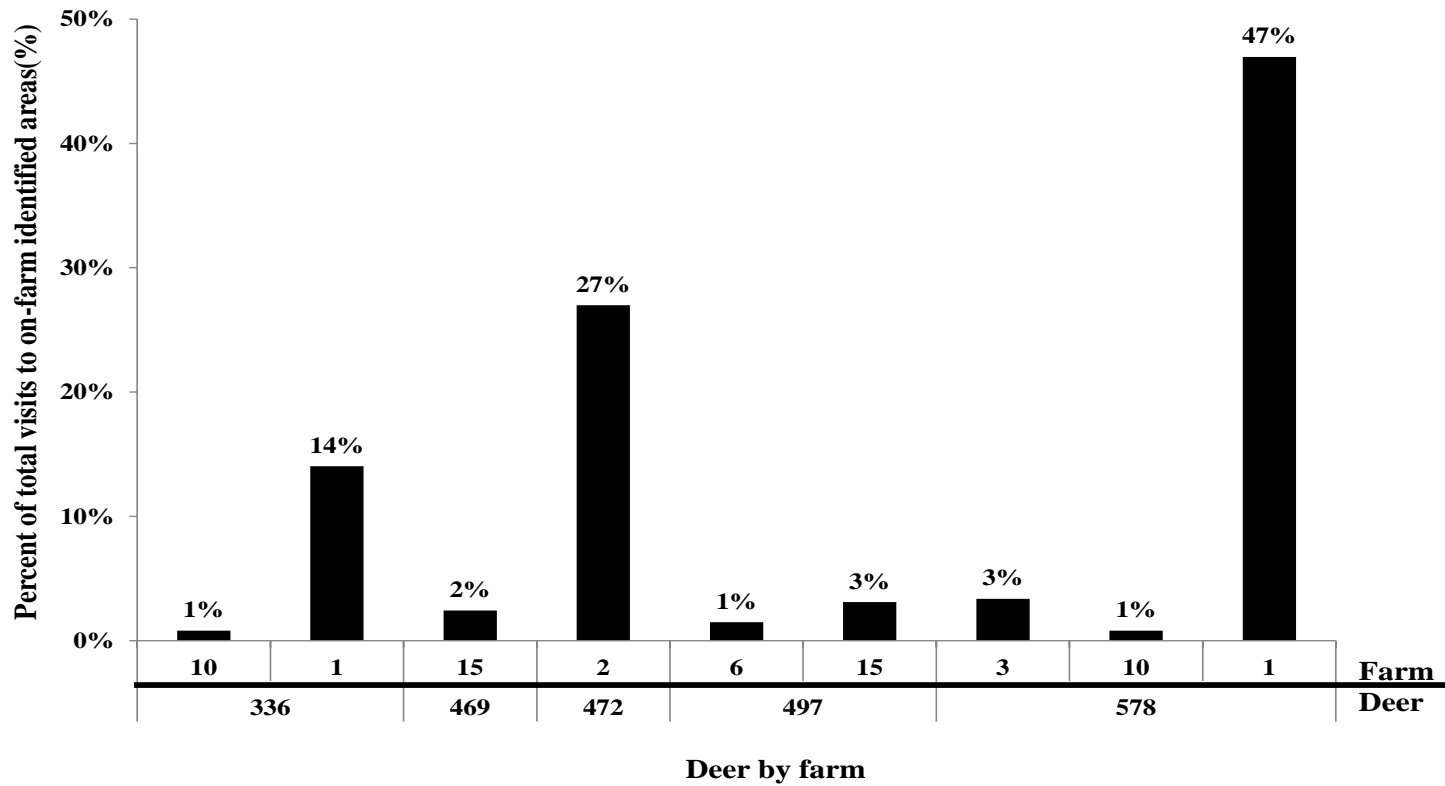
Figure xx. Deer visits, as percent of total visits to cattle, feeding site and stored feed areas by each deer and farms visited.
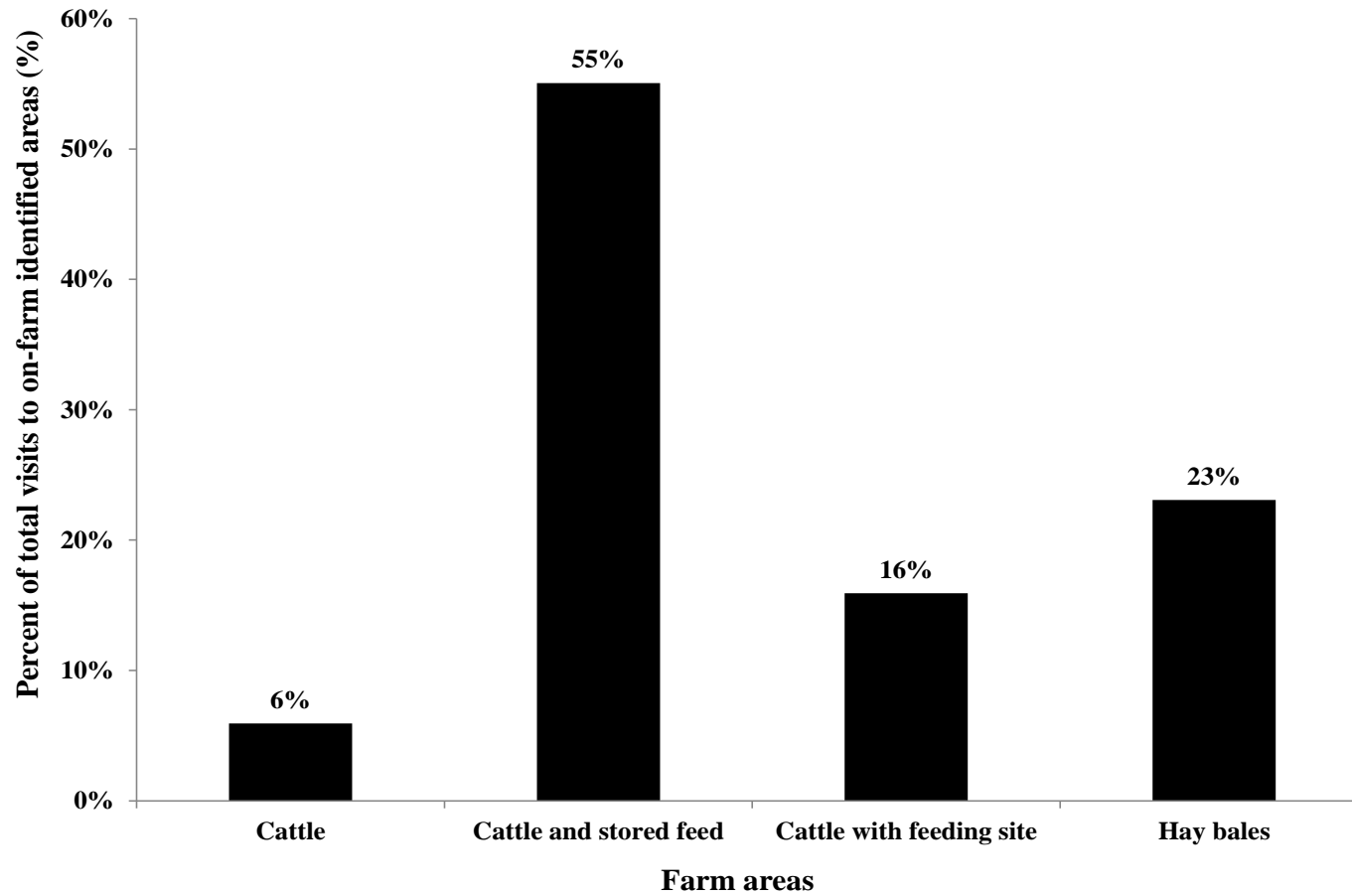
Figure xxi. Deer visits, as percent of total visits to cattle, feeding site and stored feed areas, by location within farm.
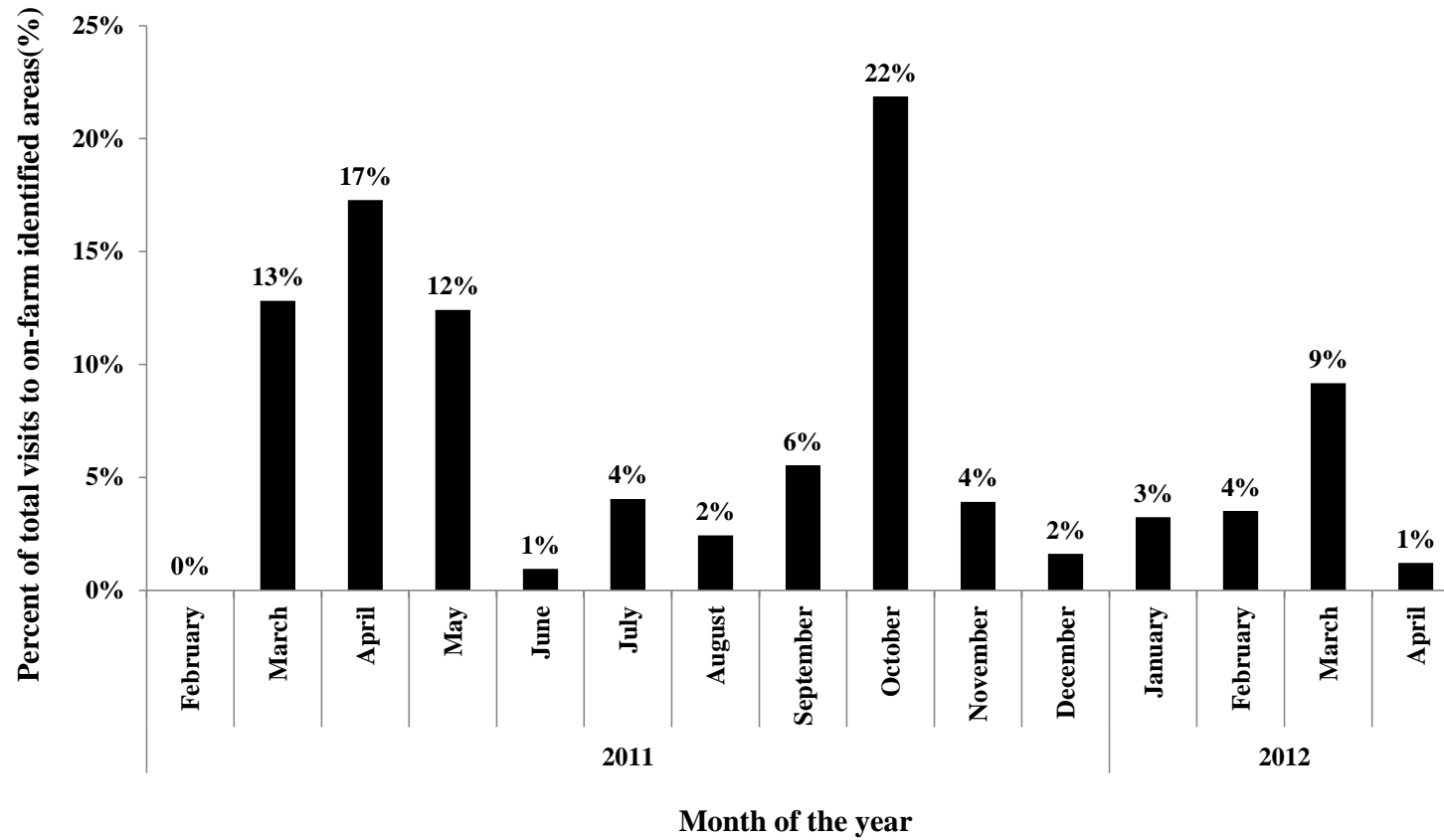
Figure xxii. Deer visits, as percent of total visits to cattle, feeding site and stored feed areas, to farms by month of the year.

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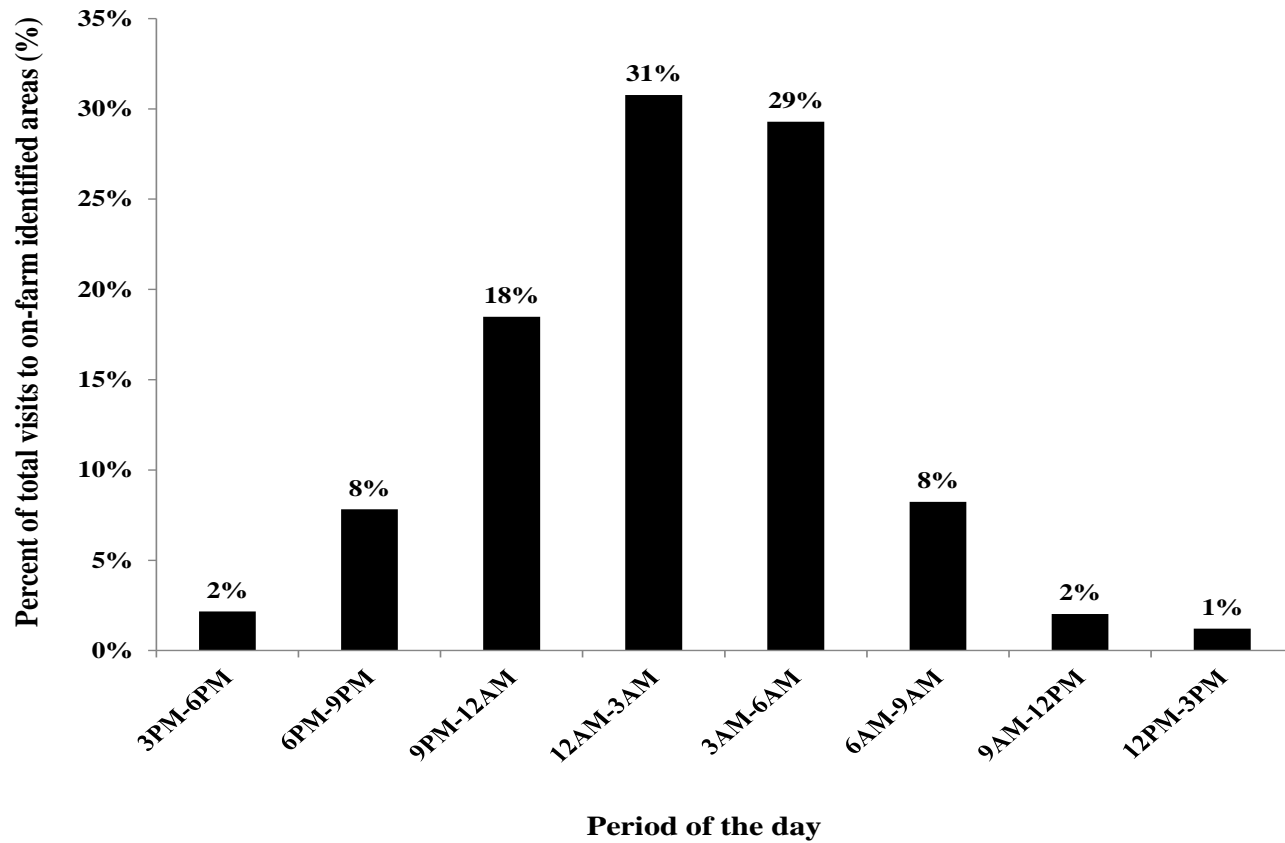
Figure xxiii. Deer visits, as percent of total visits to cattle, feeding site and stored feed areas, to farms by period of the day.
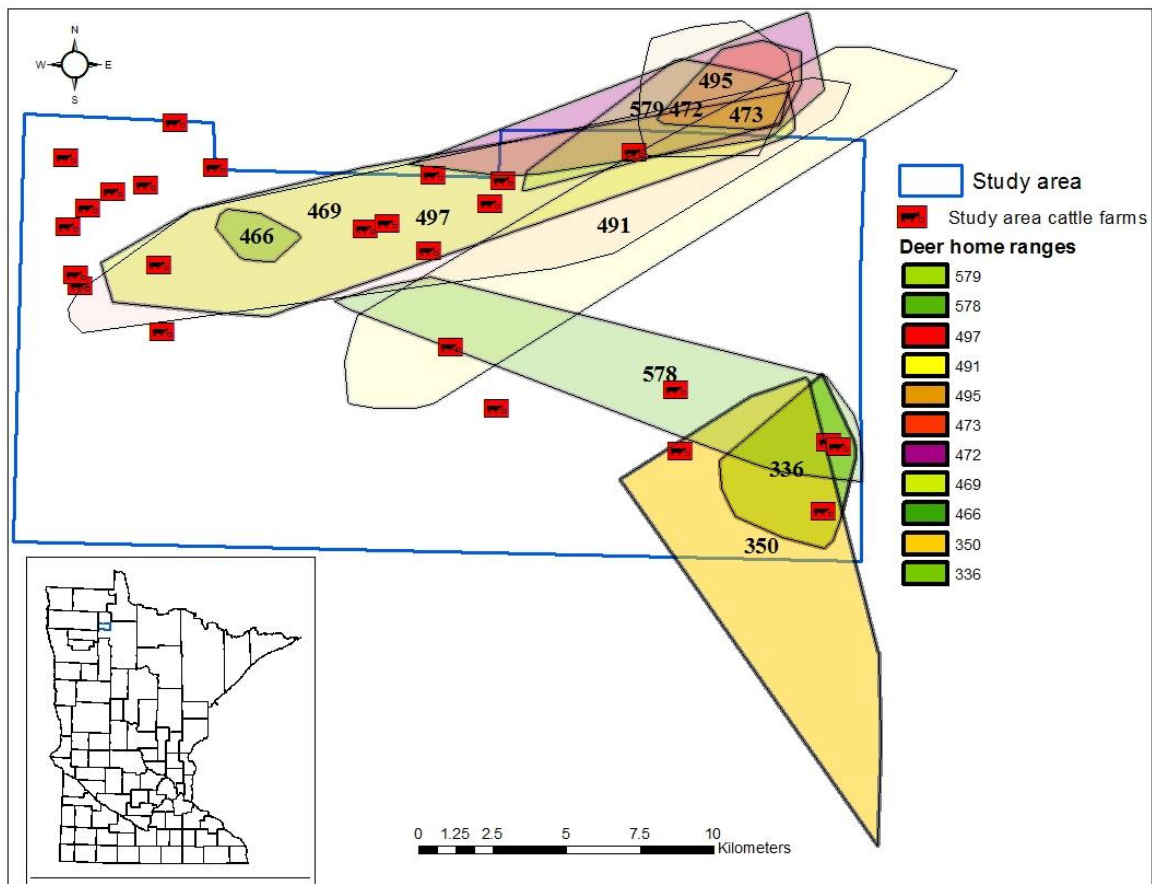
Figure xxiv. Home ranges, determined by minimum convex polygons (MCP), for white-tailed deer ($n = 11$; 91.5% of all locations) that survived ≥ 200 days of the study. Home ranges were calculated using the minimum convex polygon tool in ArcGIS®.

4.6. Tables

Table xvi. ID's and demographics of white-tail deer captured for the study

Deer ID	Capture date	Capture method[1]	Age group[2]	Age[3] (years)	Sex[4]	Estimated removal date	Days in the study	Cause of removal
348	01/15/2011	H	A	9.5	F	2/12/2011	28	Died – wolf kill
350	01/15/2011	H	A	11.5	F	4/15/2012	455	End of study
460	01/15/2011	H	A	2.5	F	2/10/2011	41	Died – wolf kill
461	01/15/2011	H	Y	1.5	F	3/31/2011	77	Died – wolf kill
466	01/15/2011	H	A	8.5	F	4/15/2012	456	End of study
467	01/15/2011	H	Y	1.5	M	2/18/2011	43	Died – wolf kill
469[*]	01/15/2011	H	A	4.5	F	4/15/2012	456	End of study
471	01/15/2011	H	Y	1.5	F	4/5/2011	88	Died – wolf kill
472[*]	01/15/2011	H	A	5.5	F	4/15/2012	456	End of study
473	01/15/2011	H	A	4.5	M	7/28/2011	200	Died – unknown cause
491	01/15/2011	H	Y	1.5	F	4/15/2012	455	End of study
495	01/15/2011	H	A	2.5	M	4/15/2012	456	End of study
496	01/15/2011	H	A	2.5	F	2/23/2011	42	Died – unknown cause
497[*]	01/15/2011	H	Y	1.5	F	4/15/2012	456	End of study
519[5]	01/15/2011	H	A	3.5	M	4/15/2012	-	Collar malfunction
524	01/15/2011	H	A	6.5	F	3/10/2011	61	Died – unknown cause
336[*]	3/7/2011	CT	Y	1.5	M	11/5/2011	244	Hunter- harvested
577[6]	3/8/2011	CT	A	11.5	F	4/10/2011	-	Died – wolf kill
578[*]	3/8/2011	CT	A	4.5	F	5/15/2012	404	End of study
579[7]	3/8/2011	CT	A	-	F	5/15/2012	404	End of study

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447	3/10/2011	CT	A	3.5	F	5/22/2011	89	Collar mal function

[1]H = helicopter; CT = clover trap.

[2]A = adult; Y = yearling.

[3]Age (in years) at capture was determined by *cementum annuli*.

[4]M = male; F = female.

[5]Deer 519's collar failed to transmit immediately after capture. No location data were obtained for this deer; however, it did survive through the study period.

[6]Deer 577 was fitted with a SirTrack test-collar (Iridium satellite system) and no movement data was recovered; mortality date is based on a public report of a severely injured deer and carcass remains.

[7]Deer 579 was unable to be aged due to a broken tooth with missing cementum.

*Deer with locations within identified areas of cattle, feeding site and stored feed on the farm landscape.

Table xvii. Summary of farm level risk factors and deer visitation data.

Farm number	# of cattle[1]	Polygon area (m²) (mean ± SD)[1,2]	Risk score[1] (% of total risk)	Deer locations[3]	Total deer[4]	Deer home ranges (# of deer)[5]
1	35	568,197 ± 258,978	111 (61.7)	Yes	2	Yes (2)
2	90	88,677 ± 150,367	91 (50.6)	Yes	1	Yes (8)
3	70	112,194 ± 115,890	91 (50.6)	Yes	1	Yes (2)
4	54	59,632 ± 69,180	91 (50.6)	No	-	Yes (1)
5	183	29,467 ± 26,123	90 (50.0)	No	-	No
6	70	216,609 ± 183,823	89 (49.4)	Yes	1	Yes (1)
7	6	92,081 ± 43,055	81 (45.0)	No	-	No
8	39	172,293 ± 168,529	81 (45.0)	No	-	No
9	60	62,519 ± 56,741	72 (40.0)	No	-	Yes (3)
10	40	18,277 ± 22,862	69 (38.3)	Yes	2	Yes (2)
11	50	35,835 ± 55,293	67 (37.2)	No	-	Yes (4)
12	35	51,089 ± 31,336	67 (37.2)	No	-	Yes (4)
13	30	85,550 ± 68,445	67 (37.2)	No	-	No
14	13	119,292 ± 120,731	66 (36.7)	No	-	No
15	168	59,368 ± 32,160	63 (35.0)	Yes	2	Yes (2)
16	30	57,151 ± 9,041	61 (33.9)	No	-	No
17	78	7,139 ± 5,094	61 (33.9)	No	-	No
18	50	1, 754 ± 1,865	60 (33.3)	No	-	No
19	2	192,431 ± 186,756	55 (30.6)	No	-	No
20	70	36,055 ± 37,811	51 (28.3)	No	-	Yes (3)
21	47	157,944 ± 137,595	49 (27.2)	No	-	Yes (3)
22	70	728,769 ± 451,259	48 (26.7)	No	-	Yes (3)
23	60	14,190 ± 13,693	46 (25.6)	No	-	No

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| 24 | 18 | 57,550 ± 48,719 | 41 (22.8) | No | - | Yes (1) |
| 25 | 14 | 197,496 ± 126,819 | 21 (11.7) | No | - | Yes (4) |

[1]Data obtained from the Board of Animal Health Wildlife Risk Assessments. This RA evaluated 4 main areas, feed storage, feeding practices, cattle housing and water management, in order to evaluate risk for deer interactions with cattle that could eventually result in disease spread. For each item evaluated the farm received a score, thus the higher the score the greater the risk for deer interaction in the farm landscape.

[2]Area per farm represents the sum of areas with cattle and/or stored feed (hay bales) and/or feeding site averaged across the 4 time periods of data collection.

[3]Yes – farm had deer location within identified areas; No – farm did not have deer locations within identified areas.

[4]Number of deer with locations within identified areas for each farm.

[5]Yes – home ranges of deer overlapped with farm areas with specific number of deer; No – home ranges of deer did not overlap with farm areas.

CHAPTER 5 - LANDSCAPE CONTEXT AND DEER-CATTLE

INTERACTIONS: A TARGETED APPROACH TO BOVINE TUBERCULOSIS

SURVEILLANCE

Wildlife that spatially overlap with livestock operations represent an additional source of infection and a susceptible population for infectious disease transmission. The objective of this analysis was to evaluate the impact posed by the landscape surrounding any given cattle farm on the risk for cattle-deer interactions. White-tailed deer (*Odocoileus virginianus*) were captured (n=19) and fitted with satellite-linked radio collars to collect GPS data every 90 minutes. The study area (360 km^2) contained 25 private cattle farms and included a mix of state forest and wildlife management areas in addition to private recreational lands. A step selection function (SSF) was estimated using a standard conditional logistic regression (50 controls per deer location). Model selection for data from individual deer, was based on AIC, where models with ΔAIC $\leq$ 3 were considered. The best fitting model showed that for five of the six cattle farms with deer visits, farm locations overlap with higher resource selection areas. On average, there was a tendency for stronger selection for open areas outside forest in early Spring and Fall. In the Summer months, the selection was stronger for within forest areas, while in the Winter deer preferentially selected for the edge of forest. Broad-scale patterns in the distribution of RSF values show that the eastern portion of the study area had higher

values, corresponding to higher densities of forest land. In this study, we show that the type of landscape surrounding a cattle farm can predict risk of deer-cattle interactions. Understanding factors that drive habitat use by deer will aid in the implementation of targeted approaches for disease surveillance and control.

5.1. Introduction

Wildlife that spatially overlap livestock operations represent a potential source of infection and a susceptible population for infectious disease transmission [9, 141]. This occurs because there are many pathogens that are shared between wild and domestic species [205]. Furthermore, when a cattle disease such as bovine tuberculosis (BTb), spills into a wildlife population, a disease reservoir could emerge, making the implementation of an effective disease control program extremely difficult [19, 206].

Bovine tuberculosis recently emerged in two states of the USA, Michigan (MI) and Minnesota (MN), and impacted both cattle (*Bos taurus*) and white-tailed deer (*Odocoileus virginianus*) populations [9, 145]. In the state of MI, white-tailed deer became a maintenance host and state agencies have not yet succeeded in controlling the spread of the disease [20]. As a result, when BTb was diagnosed in MN cattle and deer in 2009, an aggressive plan was implemented to control the establishment of a wildlife maintenance host and to avoid further spread among cattle farms. The state agencies were

successful in returning MN to disease-free status; however, this came at great financial cost and with huge reductions of local deer population densities [63].

White-tailed deer are considered to be "edge species" (i.e., a species that thrives at the interface of forested and open land cover types), and thus well-adjusted to fragmented forest landscapes where food resources and forested cover are contiguous [207]. The Midwest US, with its mixture of forest and agricultural land covers, provides ideal habitat for white-tailed deer. Unfortunately, because deer are attracted to cattle feedstuffs and agricultural fields (and in some cases, bait piles placed by landowners to supplement the deer population for subsequent hunting), the frequency of deer-cattle interactions in farm yards increases and thus magnifies the risk of wildlife-livestock disease transmission [9].

Bovine tuberculosis transmission can occur both by direct contact, through aerosol droplets, and by indirect transmission, when an infected animal contaminates shared food resources [46, 66, 68]. The latter poses a great risk for broad-scale disease transmission due to the high survivability of *Mycobacterium bovis* in the environment, particularly under the temperature and humidity conditions typical of northern Midwestern states [30].

Several strategies to reduce livestock-wildlife contact risk have been tested. These include setting up fences, use of farm dogs, and, in response to an outbreak, intense reductions in the deer population to decrease the force of infection by reducing the

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susceptible population [63, 203, 204]. All of these measures can be effective but also extremely costly when applied indiscriminately in a large region.

Many factors are expected to affect the risk of interaction between white-tailed deer and cattle; however, a critical driver of this risk will be the relative probability that deer use the landscape surrounding any given farm. In this study we developed fine-scale resource selection models to better understand which landscape features drive deer movements. We then tested whether the landscape context of individual farms affects the probability of deer visitation. Understanding what drives habitat use by deer will aid in the implementation of targeted approaches for disease surveillance and control, particularly in response to an outbreak.

5.2. Material and Methods

This study uses the deer data generated in the previous chapter. Therefore, further details regarding study area, deer capture, deer location data and farm level data collected are available in the material and methods section of chapter 4 - "Patterns of cattle farm visitation by white-tailed deer in relation to risk of disease transmission in a previously infected area with bovine tuberculosis in Minnesota, US".

5.2.1. Study area

The study area covered 360 km^2 in NW MN. This area is defined as a landscape transition zone that includes a mix of State Forests and Wildlife Management Areas, private recreational lands, and 25 private cattle farms. The area is also located within a region where cattle and white-tailed deer were previously infected with BTb [63].

5.2.2. Data collection

5.2.2.1. Deer location data

In January 2011, 16 deer (4 males, 12 females) were captured by helicopter netgunning (Quicksilver Air, Inc., Fairbanks, Alaska) and in March 2011, the study population was augmented by 5 deer (1 male, 4 females) to compensate for high winter mortalities. Deer capture locations were driven by farm densities, deer distribution at the time of capture and access to private land to process deer. All deer were fit with satellite-linked radio collars (ARGOS, SirTrack, Hawkes Bay, New Zealand) that collected GPS locations every 90 minutes. The study ended in April 2012 and yielded a total of ~ 80,000 relocations, with a fix rate of 98% and spatial error of 30 m.

5.2.2.2. Landscape data

Spatial data for the study area were obtained from the MN Department of Natural Resources (MNDNR; http://deli.dnr.state.mn.us/). The data collected included land cover types (based on classified Landsat imagery collected in 2006) as well as the location and distance to roads, streams, and rivers (Table xviii).

Exact location of cattle, stored feed, and feeding sites within the study area were surveyed and delineated using ArcGIS®. Ground-truthing of these areas was performed four times during the study period for all farms in the study area: immediately prior to the beginning of the study (January 2011), May 2011, October 2011 and January 2012. The choice for the four time periods was based on seasonal weather changes that impact management on the farm and consequentially changes in locations of cattle.

5.2.3. Covariate building

We described the landscape context around any given location by calculating the proportion of key land-cover types within a fixed radius of 500m. To do this, we created a 500-m buffer around the center of every 30-m pixel of the land-cover map. We calculated the proportion of each land-cover type within these buffers by applying the function "isectpolyrst" of the Geospatial Modeling Environment software package (GME version 0.7.2.1 RC2; http://www.spatialecology.com/gme/). The proportions of land-cover types were then combined based on a coarser classification (Table xviii). The table of this polygon file was spatially joined to the original point data file and then the proportion of each combined cover type was converted into raster format for analysis (i.e., the value within any pixel represented the proportion of a given land-cover type within a 500-m buffer centered on that pixel). Distance variables were obtained by calculating distance of each pixel in the map to the nearest road, lake, and stream. Roads

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were separated into high, medium, and low density (this corresponded to state highways, county, and township roads, respectively). Distance to forest edge was constructed by calculating the distance of each pixel to the nearest pixel of forest and by subtracting this value from the distance to the nearest non-forest pixel.

5.2.4. Model building and selection

To model resource selection, we estimated a suite of step selection functions (SSF) for the relocation data of each deer [208]. The SSF includes a resource selection function (RSF) that is based on availability defined by a resource-independent movement kernel. These models use animal "steps" as the experimental unit, where the step is defined as the movement from a starting point to an ending point over some fixed temporal interval (in this case the step interval was180 minutes, with observations collected every 90 minutes). To fit these models, we used standard case-control logistic regression where for the end point of each observed deer step ("case" or "use"), 50 random locations ("control" or "available") were sampled using an exponential distribution centered on the starting location of that step ($\lambda = 1/(2*\mu)$, where μ is the average 180-minute step length measured across all animals). These models were fit using the "coxph()" function in the "survival" package in R [209, 210]. Multiple a priori models, fit separately to data from each deer, incorporated different combinations of landscape covariates (Table xviii) and interactions with four harmonics of time of year

and time of day. The temporal harmonics accounted for variability of resource selection through time but were only considered for "Distance to forest edge" and "Proportion Agriculture" because we had an a priori hypothesis that these covariates would be sensitive to time of year due to the phenology of agricultural crops and the timing of the hunting season. We added linear splines in all models to reduce the effect of sampling bias on our coefficients (spline knots were located at the first, second, and third quartiles of movement distance, calculated across animals) [208]. The models evaluated had distance to forest edge and agricultural land as baseline variables in all models and then different combinations of the remaining variables were added.

Model selection was performed for the model sets of each individual deer using ΔAIC, where all models within ΔAIC ≤ 3 were considered [211]. The final model chosen was the model that was retained a greatest number of times. To obtain a weighted average of the coefficients for each variable of the final model, weights were calculated by dividing the inverse of the variance (σ^2) of each coefficient by the total sum of the inverses of the σ^2 for that variable; these weights were multiplied by the respective coefficient and averaged. Relative probability of selection was obtained by exponentiation of the averaged regression equation of the final model applied to each pixel in the raster files.

5.3. Results

After evaluating a suite of models that included up to 14 covariates (plus interactions with temporal harmonics), the final model was composed of 7 main effects, as well as a quadratic term and interactions with time of year for "distance to forest edge" (Table xix). This model was retained for 12 of 19 deer, based on the criteria ΔAIC < 3. Of the seven deer for which this model was not retained, six were animals with very few locations that were only in the study for a short period of time due to early removal (<2 months). We collected many relocations for the seventh deer, but that animal had a very restricted home range and thus experienced less variation in covariates compared to other animals; however, this animal's "best" model was a subset of the model chosen for the other 12 animals with the addition of proportion of forest land and it's interaction with agriculture land. When interpreting the magnitude of the weighted coefficients, the proportion of agriculture within a 500–m radius was the variable with the largest coefficient, having a negative impact; however this does not correspond to a greater effect on resource selection since it depends on the value of the covariate, which for distance variables could much larger. Weighted coefficients indicate a tendency for stronger selection for open areas, ~200m maximum from forest edge in early Spring and ~ 50m maximum in Fall (this was estimated by calculating the vertex of the quadratic function for distance to forest edge where distances within forest are positive, and those within open areas are negative). In the Summer months, the selection was stronger for

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forest areas (with a maximum of 400 m within forest) while in the Winter, deer seem to select for the edge of forest (Figure xxv).

Broad-scale patterns in the distribution of RSF values show that the eastern portion of the study area has higher values. This area has a greater density of forest land compared to the western portion of the study areas where agriculture land is more dominant and forest patches are smaller and more distributed (Figure xxvi).

The cattle farm areas in Figure xxvii correspond to areas where cattle and cattle feed were present. The six farms displayed correspond to farms where collared deer visited areas of cattle and cattle feed. For five of the six cattle farms, based on the relative probability of selection, deer locations their locations overlap with higher resource selection areas, thus the model prediction matches the empirical data. The sixth farm had a low number of deer locations and was on a path of a deer moving from east to west and vice-versa. Although many deer migrated from east to west, crossing areas where agricultural predominates; the farms in the north-central section of the study area were not visited by collared deer.

5.4. Discussion

The implementation of effective measures of disease control for multihost pathogens, such as BTb, requires a thorough understanding of the epidemiologic triad and its interactions: pathogen, host, and environment [212]. Minor differences in any of the

components of the triad, particularly cattle and wildlife densities and the type of ecosystem surrounding a farm (i.e., the landscape context), could yield very different results [145]. This is even more important when local farm-level measures to either increase immunity (e.g., vaccination), or reduce contact in order to mitigate inter-species transmission (e.g., building fences) are not readily available or are difficult to implement.

In the current study, we aimed to understand the role of landscape context on the risk for cattle-deer interactions at the farm level. Although the deer sample size was small and half of the collared individuals were lost during the study, the modeling approach implemented had the goal of describing behavior at the individual level that is common among the study deer and thus allow for population-level predictions. Furthermore, the method to select control locations allowed us to reduce sampling bias because our control locations were selected in areas that could reasonably be considered available by the deer at any given step.

Our results provide a causal explanation to empirical data previously described. In a study using a survey approach, performed in a geographical area within which our study site is nested, the authors concluded that increased proportion of non-agricultural lands around farms could predict risk for deer damage to store feeds [142]. This tool was further used by the MN Board Animal Health (BAH) as a risk assessment tool (RA) to score farms based on risk for wildlife interaction with cattle farms. In our study area, the three farms with the highest RA score were visited by the study deer. The RSF values,

when compared with the survey approach and knowing that a total of six farms had deer locations in cattle and feed areas, provide an improved prediction of risk for cattle-deer interactions on the farm landscape.

The study area had a greater density of forest land towards the east and more agricultural land towards the west, which explains the low level of selection in the west (area with more green in the map for Figure xxvi). However, smaller areas in the west side still have deer-friendly habitat available in the edge of cattle farms, which explain how two of the deer migrated 30 km from winter habitat in the northeast corner of the study area to a location in the opposite side surrounded by agricultural lands. From the total farms visited (n=6), four were located in the eastern part of the study area, closer to larger areas of dense forest land.

The objective of the "distance to forest edge" covariate was to evaluate the behavior of deer as an "edge species" and also to identify if there was spatio-temporal variability in resource selection, particularly for open areas outside forest land and close to pastures. The increase in selection for open areas outside forest in late Winter and early Spring is very likely, as shown by other authors, associated with a negative energy balance as a consequence of the long Winter Season augmented by pregnancy for female deer, a majority of our study population [190]. In the Summer, the abundance of food resources in the forest and the higher ambient temperatures were likely the factors that caused a greater selection for areas within forest land. On the other hand, in the Fall

these food resources are more scarce and crops are mature in the fields, which potentially explains the greater selection for open areas outside forest land. This is supported by the previously described empirical data, where more deer locations within farms occurred in the Spring and Fall months. In a study looking at similar outcomes in elk, there was an increase of pasture use during the Spring season [213].

The negative sign for the quadratic term for distance to forest edge indicated that deer select for open areas until a certain distance. This increases the risk for deer location in farm areas located closer to forest land. Other authors have shown that forest cover around cattle feeding areas increases the risk for white-tailed deer locations within those areas [141].

Control efforts for BTb have been undermined in other countries by the presence of wildlife, particularly the UK and New Zealand where the presence of high-quality habitat for badgers (European badger - *Meles meles*) and possums (brushtail possum - *Trichosurus vulpecula*), respectively, have been shown to increase the risk for disease transmission [19, 134]. In addition, studies performed in the UK, with the goal of reducing badger densities, yielded variable results in BTb prevalence, to the point of questioning the effectiveness of a badger culling strategy [214]. However, proactive approaches that included badger culling across larger areas had a stronger effect in reducing BTb prevalence when compared with reactive approaches [215]. Such broad-scale culling efforts are costly, so understanding what areas are at greater risk for

wildlife-cattle farm interactions could allow for a more cost effective approach to mitigating disease spread. The identification of targeted areas is also very useful in the application of farm-level mitigation strategies (e.g., protective fences for animals and feed), which while effective are also very costly [141, 203]. This approach can also now be implemented on cattle farms located in high risk areas based on the profile of the surrounding landscape.

In the context of risk analysis, there are two important components: the risk of occurrence and the impact of the event [181]. The former, in the context of BTb, is associated with introduction of disease through cattle movements, while the latter has been magnified by the presence of a wildlife maintenance host. To control a disease such a BTb, a holistic approach based on the epidemiologic triad is essential in order to be successful. Thus, to ignore the impact of the ecosystem in the risk for disease transmission would increase the likelihood of failure and also limit the possibility to apply more cost-effective, science-based targeted approaches to disease control [216]. Our results suggest that the landscape context surrounding cattle farms impacts the risk for deer-cattle interactions on the farm landscape and can be used to inform disease management and mitigation strategies.

5.5. Figures
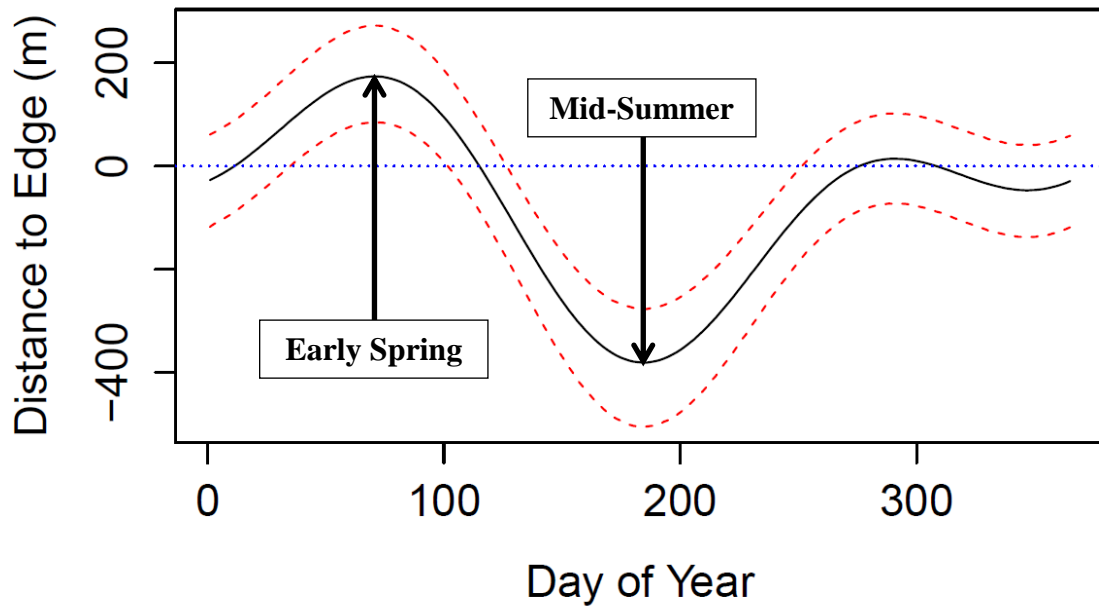
Figure xxv. Location of selection vertex for the interaction term between distance to forest edge and temporal harmonics.

The y-axis corresponds to distance to forest edge, where a positive value indicates selection for open areas outside forest and a negative value selection for areas within forest; x-axis corresponds to day of the year.
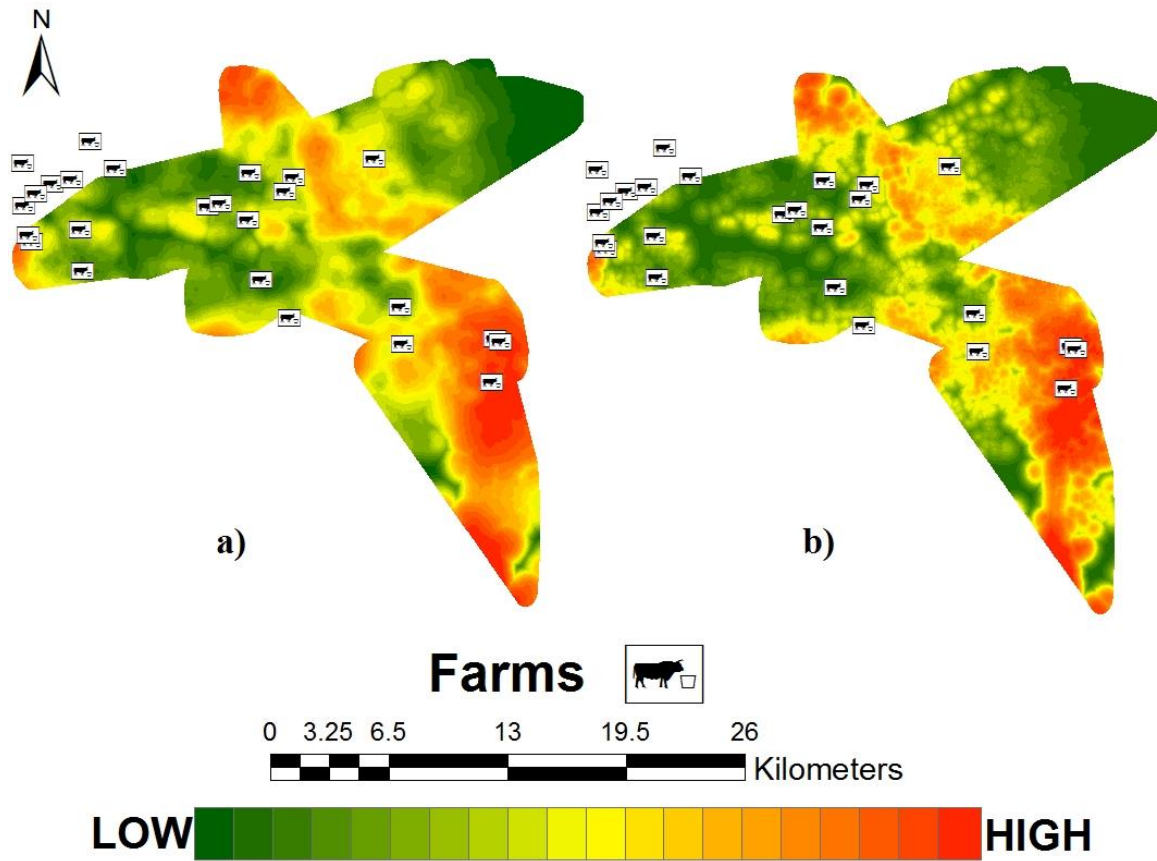
Figure xxvi. Map with relative resource selection (red=higher selection) from the best model based on AIC and ΔAIC.

a) Resource selection values for day 75 (March 15th - highest vertex in Figure 5.1. corresponding to highest selection outside forest edge;

b) Resource selection for day 188 (July 14th - lowest vertex in Figure 5.1. corresponding highest selection for within forest areas).

The area represents the combination of home ranges for study deer plus 1000 meters of buffer area.

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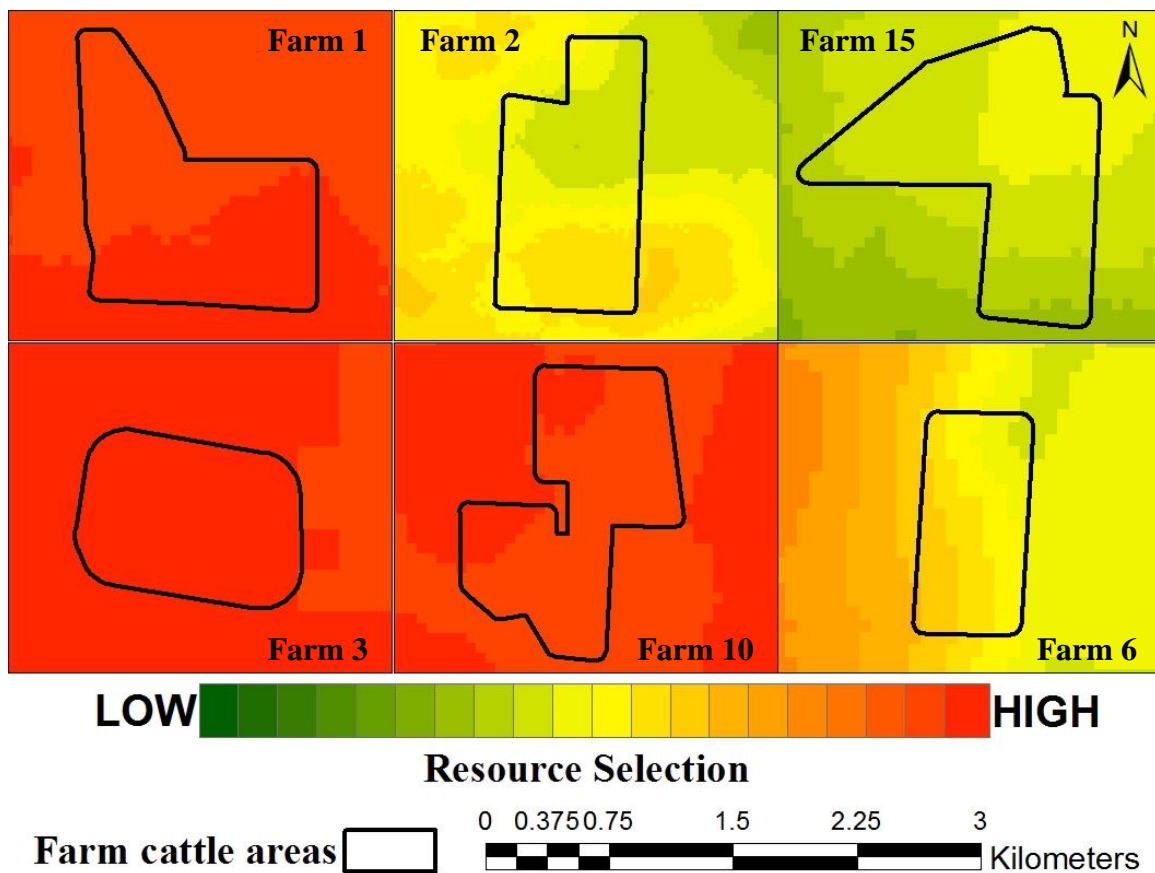
Figure xxvii. Map with relative resource selection (red=higher selection) for the Spring

Season from the best model based on AIC and ΔAIC with farm areas of cattle and feed

for the farms visited by the study deer.

Farms are ordered from left to right and top to bottom based on number of deer locations

within identified farm areas;

Farm numbers correspond to ranking of the 25 farms in the study area based on the

Minnesota Board of Animal Health Risk assessment for cattle-deer interactions.

5.6. Tables

Table xviii. GIS data collected for covariate building to use in the data analysis.

GIS Dataset[1]	Description	Variable
MNDOT roads[2]	All types of roads in MN	Distance to road
		Road density
24K lakes[3]	Lakes location in MN	Distance to lakes
24K streams[4]	Streams location in MN	Distance to streams
NLCD2006[5]	National land cover data	Proportion forest[6]
		Proportion agriculture[7]
		Proportion wetlands[8]
		Proportion developed[9]
		Proportion water[10]
		Distance to forest edge[11]

[1]Files were clipped to fit the combined area of home ranges of all deer with a buffer of
1000 meters around it.

[2]Includes state highways, county roads and township roads, which characterize 3
different variables for both density and distance.

[3]Includes location of all lakes in MN

[4]Includes location of all streams in MN

[5] National Land Cover Database based on classified Landsat imagery collected in 2006. Proportion of each cover type were calculated within a 500 meter buffer around each point and after intersecting the polygon file with the raster file with the cover types.

[6] Includes a combination of deciduous, evergreen and mixed forest.

[7] Includes a combination of cultivated crops, grasslands and herbaceous vegetation and pasture.

[8] Includes emergent herbaceous wetlands and woody wetlands

[9] Includes areas of open space and low and medium intensity development.

[10] Includes areas of open water, with less of 25% cover of vegetation or soil.

[11] Distance to forest edge for each point in the raster file, for both within and outside forest.

Table xix. Summary of weighted and averaged coefficients for best fitting model

Variables	Regression Coefficients ±SE
Distance to forest edge[1]	-2.12e-04 ± 9.91e-05
Distance to forest edge (quadratic)	-1.53e-06 ± 1.45e-07
Distance to forest edge *s1y[2]	2.11e-04 ± 6.35e-05
Distance to forest edge *c1y[2]	5.33e-04 ± 6.85e-05
Distance to forest edge *s2y[2]	6.95e-05 ± 6.38e-05
Distance to forest edge *c2y[2]	-4.10e-04 ± 6.37e-05
Agriculture[3]	-1.17e+00 ± 8.63e-02
Distance to lakes[4]	-4.60e-04 ± 2.70e-05
Distance to streams[4]	-1.21e-04 ± 3.87e-05
Distance to high density roads[4]	-2.82e-04 ± 2.72e-05
Distance to medium density roads[4]	3.79e-04 ± 2.62e-05
Distance to low density roads[4]	-2.42e-04 ± 2.98e-05

[1]Obtained by calculating the distance of each pixel to forest and by subtracting this value by the distance to all other cover types.

[2]Interaction term between "distance to forest edge" and harmonics for time of the year. Where the middle point for each harmonic, S1y, C1y, S2y and C2y, corresponds to Winter, Spring, Summer and Fall.

[3]Proportion of each cover type were calculated within a 500 meter buffer around each point and after intersecting the polygon file with the raster file with the cover types.

[4]Distance variables were obtained by calculating distance of each pixel in the map to a road, lake and stream. Roads were divided into state highways (high density), county roads (medium density) and township roads (low density).

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CONCLUSION

The central objective of this doctoral dissertation was to develop approaches for a risk-based surveillance program for bTB. Initially; this undertaking was performed by identifying from the published literature all potential pathways of transmission of bTB to cattle under the framework of the bTB "epidemiologic triad" - pathogen, host and ecosystem. Further understanding of the risk factors that modulate the relation between the components of the triad is essential in order to better inform surveillance. In this context, the description of outbreaks in the US, in terms of potential cause, characteristics of herds affected and areas affected is extremely important to identify critical factors for the cattle population as a whole, for the entire country, and at the state level. The initial study provides a holistic view of bTB transmission risk and shows the importance of looking at the mechanistic factors for disease introduction instead of focusing on mere associations (sometimes spurious) that reflect characteristics of animals, herds or regions affected but not the causes for outbreak occurrence. Thus, the profile of import cattle movements and presence of a susceptible wildlife species in the same ecosystem as cattle farms are shown to be the fundamental components that need to be better understood and incorporated into a novel surveillance system. The studies here presented follow an outline to evaluate:

1. Risk for disease introduction at the state level (Chapter 2)

2. Risk of introduction and spread of bTB at the regional level using a network

   analysis approach (Chapter 3)

3. Risk posed by white-tailed deer as a potential vector for disease transmission

   (Chapter 4)

4. Risk from posed by the ecosystem (as expressed by landscape cover types) to

   predict risk for cattle-deer interactions on the farm landscape (Chapter 5).

Using this approach, by incorporating the current knowledge about *M. bovis* and

transmission pathways to cattle, we explore the risk posed by both the domestic and

wildlife host and also the surrounding ecosystem that impacts disease dynamics. In

chapter 2, the method described developed a framework for a targeted surveillance

approach to bTB, using data available from a state animal health agency and also

available in all other US states. The fundamental problems with bTB slaughter

surveillance are the long period of time between an animal is infected and when it is

detected by an inspector and also the unequal coverage of different types of farms by

herd size and cattle type. The approach suggested here overcomes both problems by

focusing on mechanistic factors that increase risk of disease being introduced into a cattle

farm and not on unrelated characteristics of the farm itself. Furthermore, by increasing

testing intensity in those herds more likely to be the index herds of an outbreak, time

from infection to detection will consequentially be reduced which can mitigate the impact

of disease by limiting its spread in the MN cattle population. Also, by limiting the impact

of disease, the cost will be reduced either in scenarios of depopulation or test and removal.

In chapter 3, the application of network analysis to understand the heterogeneous structure of contacts in a population has been extremely important in identifying the high risk players, or "superspreaders", that play a key role in the spread of infection. The benefits of the risk score proposed are the ability to score farms and to identify those at high risk for disease introduction, which could greatly improve the effectiveness of disease surveillance by finding disease faster while reducing cost. In addition, the network approach allows, in contrast with the analysis in the previous study, to evaluate the impact of each node (i.e., farm) on the network of cattle movements, thus adding extremely important information in the context of disease surveillance.

Both approaches (Chapters 2 and 3) do not exclude the standard slaughter surveillance applied to every animal, but only suggests the implementation of additional higher levels of surveillance, expressed by more field testing or increased submission of tissues from slaughter, for farms identified as higher risk in order to increase the surveillance system sensitivity. Furthermore, the identification of such farms and the higher level of surveillance implemented would allow a faster response when performing contact tracing. The main objective was to perform targeted surveillance of farms that are more likely to become infected with bTB and consequentially mitigate bTB spread into the overall cattle population and eventually to the wildlife population. The number of

farms to test will depend on availability of resources, but can be based on risk classification instead of being a random selection process. The objectives of animal health surveillance can be greatly enhanced by using targeted approaches, particularly in areas that are disease-free, in order to act more effectively and avoid the high costs associated with investigating and controlling a disease outbreak.

Bovine tuberculosis affecting wildlife creates additional problems beyond control of the disease in the cattle population. The transmission of bTB initially occurs in cattle, with spillover to wildlife. Although still possible to transmit disease, a greater risk exists if the spill-over host (i.e., deer) becomes a maintenance host, capable of perpetuating the infection in its own species and increasing the risk for inter-species transmission.

In chapter 4, the study approach was novel since it was designed to identify the exact areas within the farms where deer visit and how these change seasonally, thus allowing us to identify risk factors that could be used in a targeted surveillance system, by again focusing resources towards subsets of the population that are more likely to play a role in disease spread. Furthermore, the results are extremely helpful as a resource to educate farmers on the risks posed to their own cattle by certain farming practices or by the absence of biosecurity measures to mitigate interactions between deer and cattle. The temporal patterns of white-tailed deer locations on farms highlight the potential for target interventions in deer populations when aiming to reduce population densities to prevent the establishment of a wildlife reservoir and control disease spread. This could be

expressed by implementing control measures directed towards high risk locations and times: during the spring season and the night time.

In chapter 5, the results show an impact of the landscape on the risk for deer-cattle interactions at the farm level, by confirming the results of chapter 4. This knowledge is also very useful in the application of farm level mitigation strategies, as building fences to protect animals and feed, while effective, is extremely costly. This approach can now be implemented in cattle farms located in high risk areas based on the profile of the surrounding landscape. Also, an understanding of the areas at greater risk for deer-cattle farm interactions could be of extreme importance when responding to a disease outbreak, in order to prevent inter- and intra-species disease transmission, by implementing population control measures to reduce the number of infectious and susceptible individuals in the wildlife population, using a targeted approach.

The studies here presented were developed in the absence of disease and with the aim of measuring exposure to risk at farm and regional level. In the future, would be extremely beneficial to evaluate a similar approach in endemic areas of disease (bTB or other) in order to link the risk measurements with disease occurrence.

In the context of risk analysis, there are two important components: the risk of occurrence and the impact of the event. The former, in the context of bTB, is associated with introduction of disease through cattle movements, while the latter has been magnified by the presence of a wildlife maintenance host. To control a disease such a

bTB, the systems approach here presented based on the epidemiologic triad is essential in order to minimize failure and maximize success in detection and control. Thus, no essential component can be ignored when implementing targeted approaches to the control of zoonotic diseases.

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