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Climatic Factors Limiting the Distribution of Dermacentor andersoni in Montana's Rocky Mountain Region

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Climatic Factors Limiting the Distribution of *Dermacentor andersoni* in Montana's Rocky Mountain Region

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This thesis for honors recognition has been approved for the Department of Life and Environmental Sciences.

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Abstract:

The Rocky Mountain Wood tick, *Dermacentor andersoni*, serves as the primary vector for the Colorado tick fever virus in Montana's Rocky Mountain region. This disease presents symptoms such as fever, headaches, and myalgia; and generally lasts for approximately three weeks. Factors affecting the distribution of *D. andersoni* in Montana are not well known, limiting our ability to locate areas endemic to the CTF virus. To determine if various climatic factors influenced the distribution of *D. andersoni* Montana's Rocky Mountain region, tick samples from Western Montana were collected and relative tick abundance was tested for association with six different climatic variables using ANOVA. CTF infection rate was also measured at each sampled site through RT-PCR. I hypothesized that increasing moisture content will decrease the relative abundance of *D. andersoni* ticks. I also hypothesized that the infection rate would fall within the range of 10-15% in accordance with previous findings. The results of this study indicate that soil temperature, precipitation, and average annual air temperature may influence the distribution of *D. andersoni* in Montana's Rocky Mountain Region. Elevated soil temperature and precipitation correlated with high relative tick abundance, while decreased average air temperature correlated with high relative tick abundance. The results also show an overall CTF infection rate of 1.05% for sampled sites.

Introduction:

Many tick-borne zoonotic diseases are prevalent in the United States such as Lyme disease, Rocky Mountain spotted fever, tularemia, anaplasmosis, and Colorado tick fever (CTF) (Choi et al. 2016). These diseases are caused by pathogens such as bacteria and viruses that utilize ticks as a vector (Choi et al. 2016). In the Rocky Mountain region, the Rocky Mountain wood tick, *Dermacentor andersoni*, serves as the primary vector to pathogens causing Rocky Mountain spotted fever, tularemia, and CTF (Eisen 2007). CTF is caused by a non-enveloped, double stranded RNA virus present in mature red blood cells after infection (Philipp 1993). The virus causes a febrile illness which presents symptoms such as fever, headaches, and myalgia or muscle pain (Brackney et al. 2010). It generally lasts for three weeks or more with approximately 20% of patients being hospitalized (Brackney et al. 2010). Because these symptoms are so general, this disease is often misdiagnosed (Brackney et al. 2010).

The factors influencing CTF infection risk are not well known in Montana, and extrapolations on previous surveillance data may provide inaccurate estimations for CTF infection (Eisen et al. 2013). Thus, documentation of different environmental variables and their influence on the distribution of *D. andersoni* populations would be useful in assessing endemic areas. Rising human populations and encroachment of tick habitat increase the level of contact between humans and the CTF vector *D. andersoni* (Eisen 2007). Thus, the importance for developing a risk model for CTF in Montana continues to grow. Recognizing regions that are endemic to CTF will greatly improve diagnosis for persons presenting with symptoms, and knowing infectious regions will provide a form of prevention that could decrease the occurrence of CTF (Brackney et al 2010). In

addition to rising human population in tick inhabited areas, there is evidence for range expansion of *D. andersoni* in the northwestern United States and Canada (Dergousoff et al. 2013). The synchronized expansion of both tick and human populations may increase exposure to infectious ticks, and furthermore illustrates the necessity of a risk-model for prevention of CTF.

Given that CTF is principally spread through the vector, *D. andersoni*, understanding which variables influence tick distribution and infection risk is of importance. Eisen (2007) found peak tick density to be between the months of April and June in Colorado, where daily maximum temperatures are between 16-19°C with relative humidity below 20%, between elevations of 1800-2500m, and in areas where sagebrush is present. Eisen et al. (2008) also found that large elk populations negatively correlate with tick density. Geissler et al. (2014) confirm these variables as indicating increased tick density, but also suggest that greater infection risk occurs while at elevations above 2100m and in areas where sagebrush is present. Although sagebrush and elevation are possible indicators of elevated infection risk, other factors may have significant influence (Burgdorfer and Eklund 1959).

According to Yoder et al. (2007), water availability, most notably in the form of water vapor, may be a critical environmental factor limiting the distribution of *D. andersoni* populations. *D. andersoni* is classified as xerophilic, meaning it survives in environments with minimal water availability, and must rely on water vapor as they are unable to drink free water (Yoder et al. 2007). For this reason, Yoder et al. (2007) recognized overhydration as the principal environmental variable impacting the distribution of *D. andersoni* in the Rocky Mountain Region. Although overhydration

could limit the distribution of *D. andersoni*, there is evidence that *D. andersoni*'s tolerance of environmental factors could vary between populations (Owen et al. 2014). Thus, continued research into the factors affecting the distribution of novel and historic populations of *D. andersoni* is of importance.

The objective of this research was to test whether climatic variables that impact water vapor content such as relative humidity, air temperature, soil temperature, and precipitation influence the relative abundance of *D. andersoni*. Also, each tick captured was analyzed for presence of the CTF virus through real time RT-PCR to determine an overall infection rate. This will help to recognize areas endemic to the CTF virus. I hypothesized that increasing moisture content will decrease the relative abundance of *D. andersoni* ticks. I also hypothesized that the infection rate would fall within the range of 10-15% in accordance with previous findings (Dotson 2015; Eads and Smith 1983)

Methods:

Sampling:

Ticks were collected from late April-mid June in 2016 as this time period has shown increased host seeking activity of *D. andersoni* in Colorado (Eads and Smith 1983). They were collected at local and novel sites in Montana's Rocky Mountain region (Figure 1.). Questing ticks were captured using a drag net sampling technique which has been shown to be an effective representation of human risk of tick exposure by Eisen (2007). Drag net sampling was performed by constructing a T-bar out of PVC pipe and attaching a one meter by one-meter piece of flannel to the bar. The flannel cloth was then dragged over ground cover and the flannel was checked for ticks every 15s-30s. This

method was performed for one-person hour at each sampled site. Ticks found on the body within the person hour were also included. Topographical, climatic, and vegetation data were recorded at each sampled site. The onsite climatic data collected included relative humidity, air temperature (°C), and soil temperature (°C). Sampling was not performed on rainy days due to significantly low tick abundance. Previous tick data from years 2013 and 2014 were sampled in the same manner.
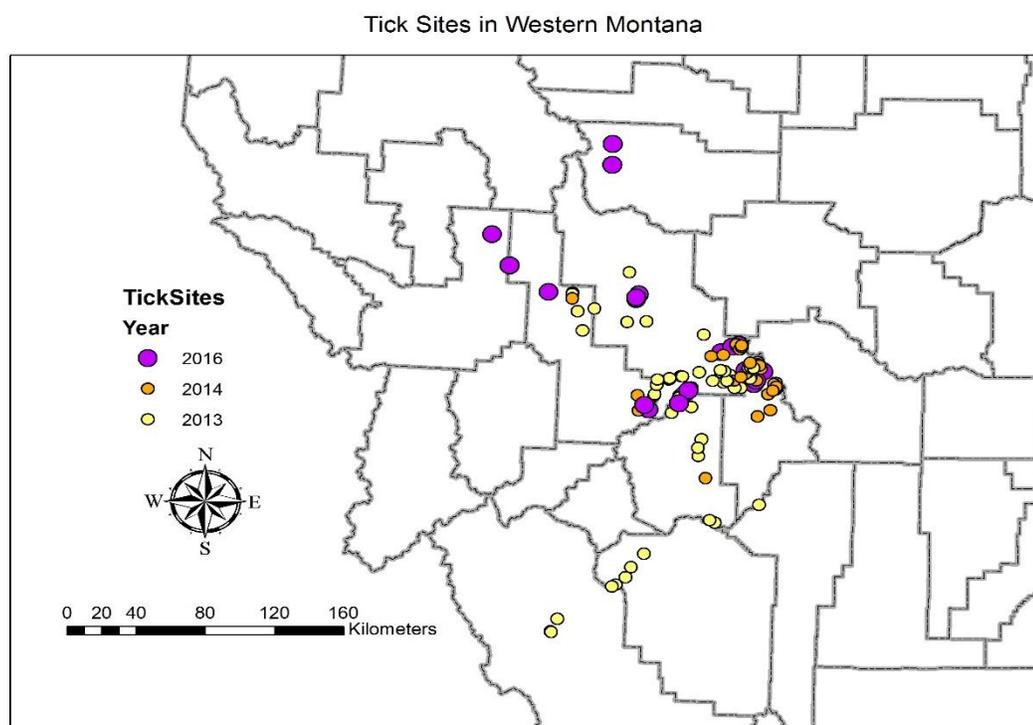

Figure 1. Map of tick sites sampled in Montana's Rocky Mountain Region.

Statistical Analysis:

The relative abundance of *D. andersoni* was tested as the dependent variable using single factor analysis of variance (ANOVA). The relative abundances were grouped into three categories: none (0 ticks), low (1-5 ticks), and high (6< ticks). These three groups were tested for associations with six independent climatic variables: relative humidity, soil temperature (°C), air temperature (°C), 30-year average annual vapor pressure deficit, 30-year average annual precipitation (mm), and 30-year average annual air temperature (°C). The 30-year average annual data were collected from the PRISM data source (PRISM 2016). Relative humidity 30-year average data were unavailable, statistical analysis was instead performed on the 30-year average annual data for vapor pressure deficit (VPD). VPD is the difference between the amount of moisture the atmosphere can hold and the actual average observed moisture (vapor) content, thus when relative humidity is high VPD is low. Statistical analysis was performed on preexisting tick data recorded by previous Carroll College research students from 2013 and 2014 (Figure 1) in addition to the data recorded in 2016.

Infection Rate:

Ticks were homogenized using a QIAgen RNeasy blood and tissue kit following manufacturers protocol with modifications as described by Dotson (2015). RNA was then extracted from tick homogenate using the QIAcube and analyzed for presence of the CTF virus through real time RT-PCR following the protocol of Dotson (2015) with modifications to contents of master mix (Table 1.) as well as RT-PCR well amounts (Table 2). BioRad IQ500 thermocycler conditions followed the protocol of Dotson (2015) with modifications to number of cycles. The RNA samples were analyzed with

two primer and probe sets shown to be effective by Lambert et al. (2007). Samples collected in 2016 were analyzed for infection rate.

Table 1. Real time RT-PCR master mix ingredients for detection of CTF virus.

Reagents	Amount (µL)
4X TaqMan	5
Sequence 1 or 2 Forward Primer (1:5)	1
Sequence 1 or 2 Reverse Primer (1:5)	1
Sequence 1 or 2 Probe (1:10)	1
RNAse Free Water	9.5

Table 2. Real time RT-PCR well amounts for detection of CTF virus.

Reagents	Amount (µL per well)
Master Mix	12
RNA Sample	8

Results:

Statistical Analysis:

ANOVA analysis showed statistically significant results for soil temperature (°C), 30-year average annual air temperature (°C), and 30-year annual average precipitation (mm) (Table 3). Elevated soil temperature indicated a higher relative tick abundance (Figure 2). Elevated average annual air temperature indicated a lower relative tick abundance (Figure 3). Elevated average annual precipitation indicated a lower relative tick abundance (Figure 4). Although the data suggest that soil temperature, air temperature, and precipitation influence tick distribution, the results are only significant in the range of independent variables tested and for our geographic region.

Table 3. ANOVA analysis for relative abundance of *D. andersoni.*

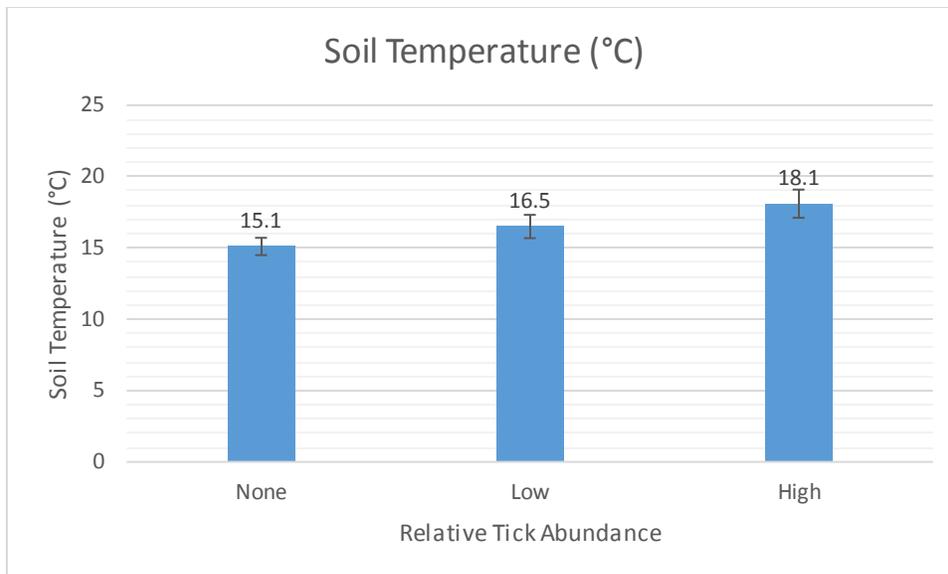| Variable	F	df	P-value
Soil Temperature (°C)	3.05	145	0.05
Air Temperature (°C)	1.12	145	0.328
Relative Humidity (%)	2.30	145	0.104
Air Temperature 30 yr. Ave. (°C)	2.97	144	0.055
Precipitation 30 yr. Ave. (mm)	4.74	144	0.01
Vapor Pressure Deficit	2.70	144	0.071

Figure 2. Graph of the mean soil temperature (°C) for each category of relative tick abundance. High relative tick abundance was present at sites with significantly elevated soil temperature, indicating that high soil temperature positively influences *D. andersoni* density.
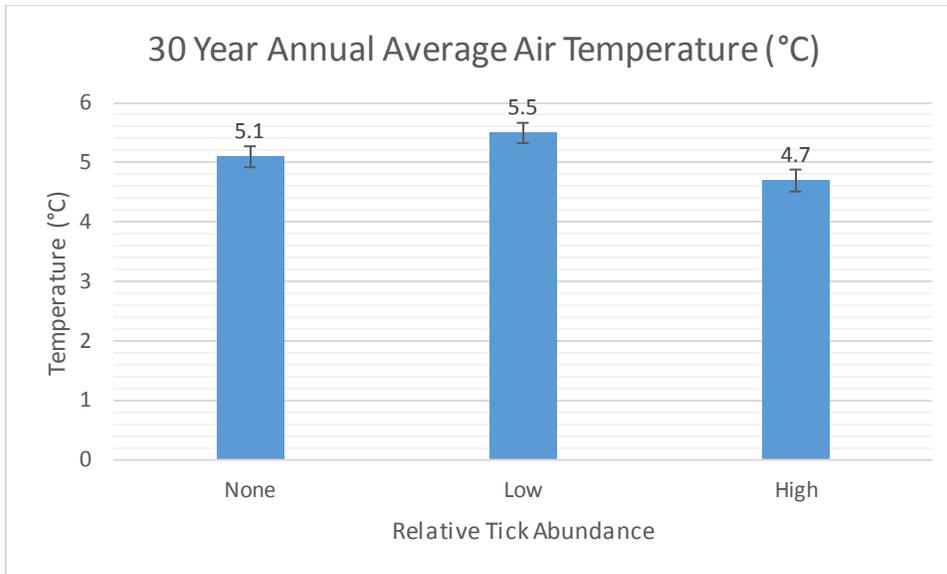
Figure 3. Graph of the mean 30 yr. average annual air temperature (°C) for each category of relative tick abundance. High relative tick abundance was present at sites with lower average annual air temperature, indicating that low air temperature positively influences *D. andersoni* density.
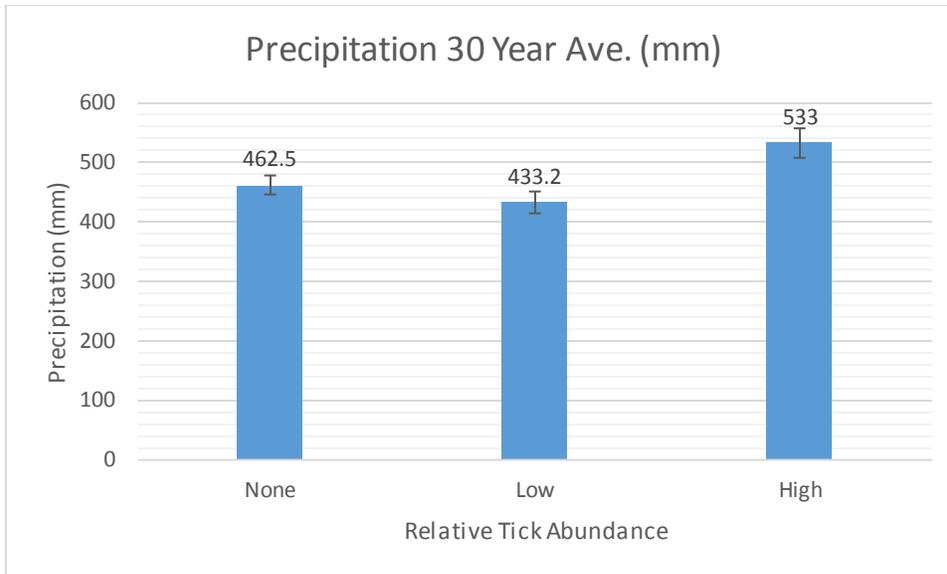
Figure 4. Graph of the mean 30 yr. average precipitation (mm) for each group of relative tick abundance. High relative tick abundance was present at sites with elevated annual average precipitation, indicating that high precipitation positively influences *D. andersoni* density.

Infection Rate:

Four of the 380 samples of *D. andersoni* tested positive for the CTF virus indicating an infection rate of 1.05% for samples collected in 2016. Sites with positive samples are shown (Figure 5).
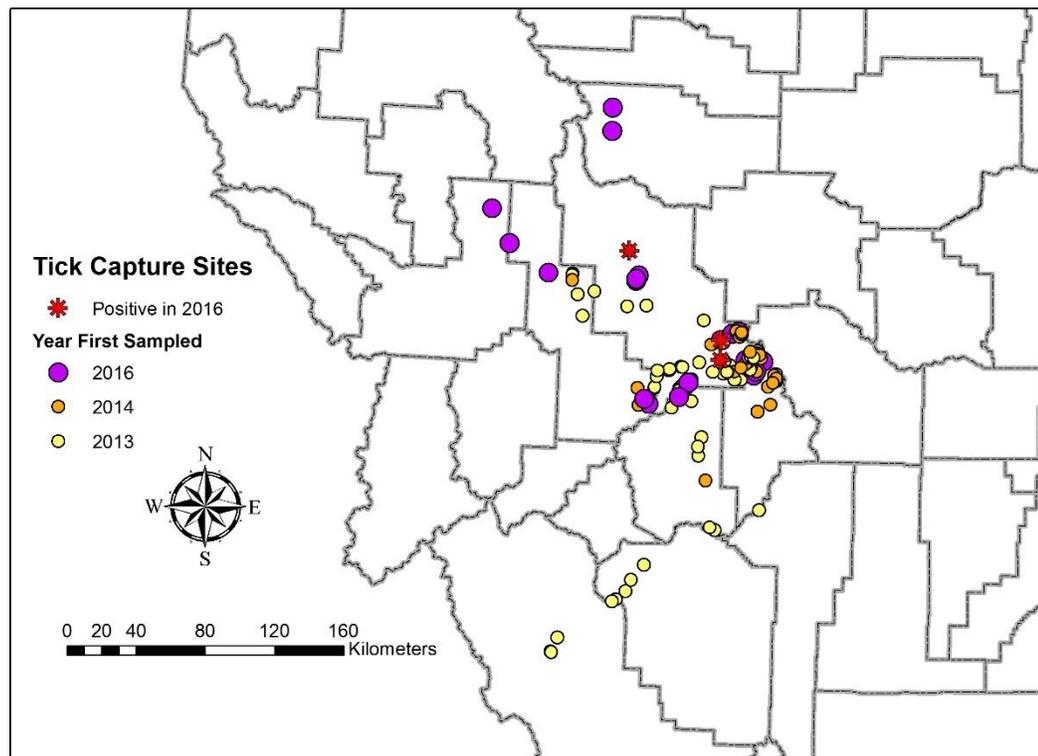

Figure 5. Map of CTF positive tick sites sampled in Montana's Rocky Mountain Region.

Discussion:

      This study found three climatic factors that may influence *D. andersoni* abundance in Montana's Rocky Mountain Region. Because there was no obvious correlation between high moisture availability and a low relative tick abundance, I rejected my hypothesis that increasing moisture content will decrease the relative abundance of *D. andersoni* ticks.

Soil Temperature

      This study found that high soil temperature indicated high relative tick abundance. Because *D. andersoni* ticks hibernate underground through winter, on-site spring and early summer data are not sufficient to determine soil temperature effect on tick abundance. Average annual soil temperature data would be necessary to further strengthen the correlation between high soil temperature and high tick abundance.

Precipitation

      This study found that elevated precipitation indicated high relative tick abundance, which contradicts the overhydration hypothesis. Although *D. andersoni* is considered a xerophilic species, desiccation is the primary cause of mortality in ticks as they are prone to water loss due to their high surface area to volume ratio (Yoder et al. 2007). Thus, locations with high precipitation could decrease the probability of desiccation. Because the results of this study were only significant within the range of values recorded, precipitation levels above and below the observed values would be needed to further understand the moisture tolerance of *D. andersoni*.

Air Temperature

This study found that lower average annual air temperature indicated increased relative tick abundance. On-site air temperature data most likely did not show significance due to a high diurnal temperature variance in Montana. These results correlate with the findings of Yoder et al. (2007), who found that the ability for the tick to retain water is more important that its ability to obtain water. Thus, high rates of transpiration due to high air temperature could limit the distribution of *D. andersoni.*

Infection Rate

This study found a CTF infection rate of sampled *D. andersoni* populations in Montana's Rocky Mountain region of 1.05%. Because this infection rate is much lower than values observed in other studies (Dotson 2015; Eads and Smith 1983), I rejected my hypothesis that the infection rate would fall within the range of 10-15%. Further research into the CTF infection rate of various Montana sites would be useful in determining areas endemic to the virus.

Summary

Although certain climatic factors influenced the relative abundance of *D. andersoni* ticks throughout Western Montana in this study, further research would be necessary to strengthen the correlation and increase generalizability. Year round data from sampled sites would be useful in determining whether winter climate has an effect on tick distribution in addition to spring and summer climate. Also, sampling in climates outside of the range of values recorded in this study could better indicate factors limiting the tick distribution.

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