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The Carrier Rate of Colorado Tick Fever in the Rocky Mountain Wood Tick, Dermacentor andersoni

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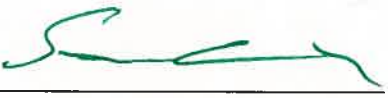

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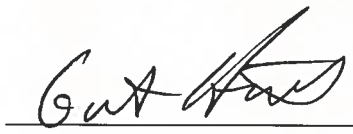

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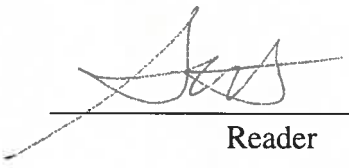

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Honors Thesis

Carroll College Department Life and Environmental Sciences

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Abstract

The adult Rocky Mountain Wood Tick, *Dermacentor andersoni*, is capable of transmitting several diseases to humans, including Colorado Tick Fever (CTF). The abundance and distribution of the virus that causes this disease is largely unknown. Ticks were collected at multiple sites in Western Montana using drag sampling methods in order to test my hypothesis that the carrier rate of CTF was within the range of previous studies. Ticks were tested for CTF by extracting their viral RNA, converting this RNA to DNA and amplifying it using Reverse Transcriptase PCR (RT-PCR). The RT-PCR product was then run on an agarose gel to visualize the DNA. Eighteen and a half percent of ticks tested were carriers of the virus. The virus was heterogeneously distributed with certain sites having virus carrier rates as high as 56% and others as low as zero percent. Although the total percentage of ticks containing the virus is within the ranges of past studies, it is not known what additional factors might account for the uneven distribution observed. Additional data should be collected in the future to determine factors influencing the virus' distribution. Data from this study and future studies can be used to generate a risk assessment map in order to decrease the risk of humans being bitten by a virus-carrying tick, and to better diagnose those who may be infected.

Introduction

Tick saliva contains many components that allow for unnoticed feeding and the transmission of pathogens (Heinze et al, 2014). Ixodid (hard-bodied) ticks like *D.*

andersoni are significant vectors of diseases around the world potentially causing major impacts on public health (Heinze et al, 2014). Because of this ticks and the distribution of their diseases should be an important area of future study.

Dermacentor andersoni, the Rocky Mountain Wood Tick has the ability to transmit several pathogens that can impact humans, including Tullaremia, Rocky Mountain Spotted Fever, Colorado Tick Fever, and Tick Paralysis. Tullaremia is a bacterial disease that generally causes flu-like symptoms including fever, chills, headache, aches, and an ulcer, which generally forms at the site of a tick's bite (Ellis et al, 2002). The most common form of Tullaremia has a mortality rate of 3% but there are other less common forms that can be more serious including an acute form which has a 30-60% mortality rate (Ellis et al, 2002). Tick paralysis is caused by a neurotoxin secreted by gravid female ticks and causes ascending paralysis, which continues until the tick is removed (Diaz, 2010). Rocky Mountain Spotted Fever is another tick born disease with a fatality rate of 1.1 to 4.0% and causes a fever, and a large rash to develop on the limbs and trunk (Lacz et al, 2005). Lastly, Colorado Tick Fever is the viral, tick borne, disease that I have focused on. It presents itself as a benign fibril disease that affects all but the very young, likely because they are rarely exposed to the ticks (Hall et al, 1968). After the virus has been injected into the blood from an infected tick bite, humans generally exhibit symptoms after an incubation period of 1-14 days (Brackney et al, 2010).

D. andersoni is the primary vector of Colorado Tick Fever (CTF; Brackney et al, 2010). In human cases the virus presents itself as an acute disease with common FLU-like symptoms, resulting in the belief that it is an under-diagnosed condition (Johnson et

al, 1997). Although CTF is usually expressed as a benign fibril disease, it can rarely lead to severe complications including death in young children (Goodpasture et al, 1978). Although the acute phase of the disease usually passes within one week of the onset of symptoms, many (48%) require greater than three weeks to completely recover from the disease (Goodpasture et al, 1978). The disease is thought to be under-diagnosed because of its nonspecific symptoms and an inability to test for it or similar diseases such as Rocky Mountain Spotted Fever (Johnson et al, 1997). The disease has seen a decline in diagnoses in recent years, despite increasing human populations and use of public lands in the endemic region (Brackney et al, 2010).

D. andersoni progresses through several life stages: eggs, larvae, nymphs, and adults. The virus is carried through each stage, and is eventually transmitted transovarially from the eggs to the offspring (Florio et al, 1950). As the tick grows, it feed on progressively larger hosts. The Golden Mantled Ground Squirrel, *Callospermophilus lateralis*, one of the host species for the larval and nymphal stages of the ticks, is thought to be the reservoir species for Colorado Tick Fever as the disease is found at a higher rate in ticks where this squirrel is found (Burgdorfer and Eklund, 1958).

D. andersoni are generally found at elevations ranging from 2,200 to 2,400 meters, with peak numbers at the middle of this range (Eisen et al, 2007). Big Sagebrush, *Artemisia tridentate*, serves as an indicator of tick presence as there is a shared climatic preference between the two species (Eisen et al, 2008). The greatest abundance of ticks within this climate has occurred on south/west facing slopes (Eisen et al, 2007). The majority of ticks have been found in grassy habitats away from rocks

and other refugia, most commonly in areas of low elk abundance, as increased grazing by ungulates decreases the preferred grassy habitat (Eisen et al, 2008). It is possible that areas with high human abundance drive away elk normally grazing on grass, potentially increasing risk of human exposure to tick-borne diseases. A lack of elk would increase the amount of gassy habitat available, subsequently increasing tick abundance and risk of human exposure.

The carrier rate of CTF in *D. andersoni* ranges from 0-40% in the Bitterroot Mountains of Montana (Burgdorfer and Eklund, 1958, 1960), to 25% in ticks collected in north central Colorado (Eads and Smith, 1983). These studies collected ticks from either a single site or several sites within the same drainage gulch.

Little is known about how virus-carrying ticks are geographically distributed (Eklund et al, 1955). It is likely that the distribution is not homogenous, as different studies have determined significantly different carrier rates. In addition, no published studies regarding the carrier rate of CTF in *D. andersoni* have been performed in the last three decades.

In this study, adult *D. andersoni* were collected in the field and tested for the virus using the method described by Johnson et al. (1997). This involves extraction of RNA from the ticks, conversion of RNA to DNA using RT-PCR, amplification of this DNA using PCR, and then detection of the viral DNA sequence with agarose gel electrophoresis. Using this method, the carrier rate of ticks collected in the Big Belt Mountains and along the North Fork of the Blackfoot River in Montana was determined.

The finding presented in this study aim to: 1) increase our knowledge of the abundance of CTF and our concern for possible infection in Montana, 2) provide the public with the knowledge to avoid areas with high tick concentrations, 3) provide preliminary data for the creation of a risk assessment map that predicts where *D. andersoni* that are carriers of CTF are located, and 4) provide the public and healthcare professionals with information to recognize CTF and optimize the management of this disease.

For my study, I hypothesized that the carrier rates of CTF in the ticks collected along the North Fork of the Blackfoot and in the Big Belt Mountains would be within the range of those ticks collected in previous studies, namely 0-25%.

Materials and Methods

D. andersoni were collected from sites in the Big Belt Mountains and on the North Fork of the Blackfoot River (Figure 1). Ticks were collected from May through July by dragging a one-meter by one-meter white flannel sheet over grasses and other vegetation at each of the sites

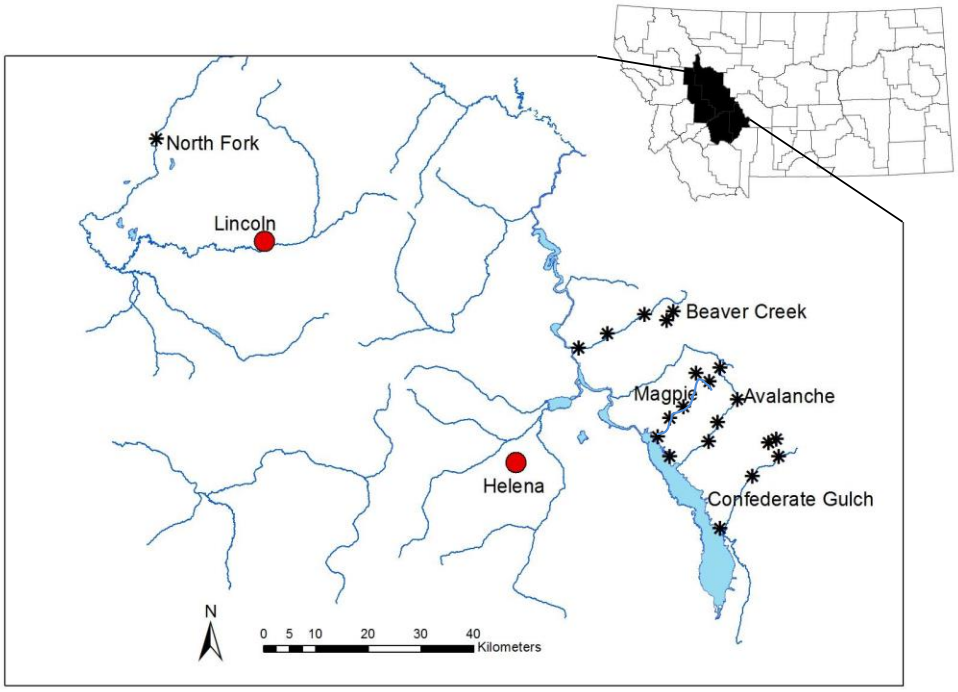

Figure 1: Map of the sites where ticks were collected.

for a search time of sixty person minutes. Once ticks were spotted, they were immediately placed into a vial of 200 proof ethanol, and upon returning to the lab one to four hours later, they were transferred into a -80 C° freezer. The collected ticks were then visualized to confirm that they were *D. andersoni* and not a tick of a different species.

D. andersoni were first homogenized individually in fast prep lysing tubes containing a quarter inch bead at 5.5 oscillations per second for 45 seconds. The RNA in each tick was extracted using a Qiagen RNeasy fibrous tissue kit (according to the product protocol, Table 1).

Table 1: RNA extraction reagents.

Reagent	Amount
RNA later	**600 ul**
Buffer RLT	**300 ul**
Buffer RPE	**350 ul**
Buffer RW1	**350 ul**
70% ethanol	**400 ul**
RNase free water	**40 ul**

Ticks were tested for CTF using a slightly modified Reverse Transcriptase PCR (Johnson et al, 1997). The virus was denatured by mixing 4 ul of RNA with 2ul of 1.45 % formamide and heating the mixture to 95 C° for five minutes. The PCR master mix in each sample contained 8.5 ul H_2O, 4 ul 5X buffer, 0.4 ul dNTPs, 2 ul each of forward and reverse primers (Johnson et al, 1997), 0.8 ul $MgSO_4$, 0.4 ul reverse transcriptase, 0.4 ul of RNA polymerase, and 1.5 ul denatured RNA. PCR was then performed with the modification to the 68 C° step changing two minutes to three minutes. The PCR product was then run on a 1% agarose gel containing ethidium bromide (Figure 2). If a band appeared around 528 base pairs then that tick was identified as a carrier of CTF.

Fisher's exact test was performed to test if the site location affects the frequency of negative versus positive results. This type of analysis is not biased by small sample sizes. All sites were first all included in the analysis and further analyzed only if the total count for the gulch was above ten, excluding Confederate and Avalanche Gulches.

Results-

Twenty-seven of 121 ticks were carriers of Colorado Tick Fever. The North Fork Bridge site had the highest percentage of carriers at 52.9% while the North Fork of the Blackfoot River had a carrier rate of 9.8%. Beaver Creek had a carrier rate of 40.7%. Avalanche Gulch had a carrier rate of 25%. Magpie and Confederate Creeks had no positive results of the fifteen and three ticks tested at each site respectively.

Statistical analysis examining if the site affects the frequency of positive versus negative results yielded a p-value of 0.0001 if all Gulches were included and a p-value of <0.0001 if Confederate and Avalanche Gulches were excluded.

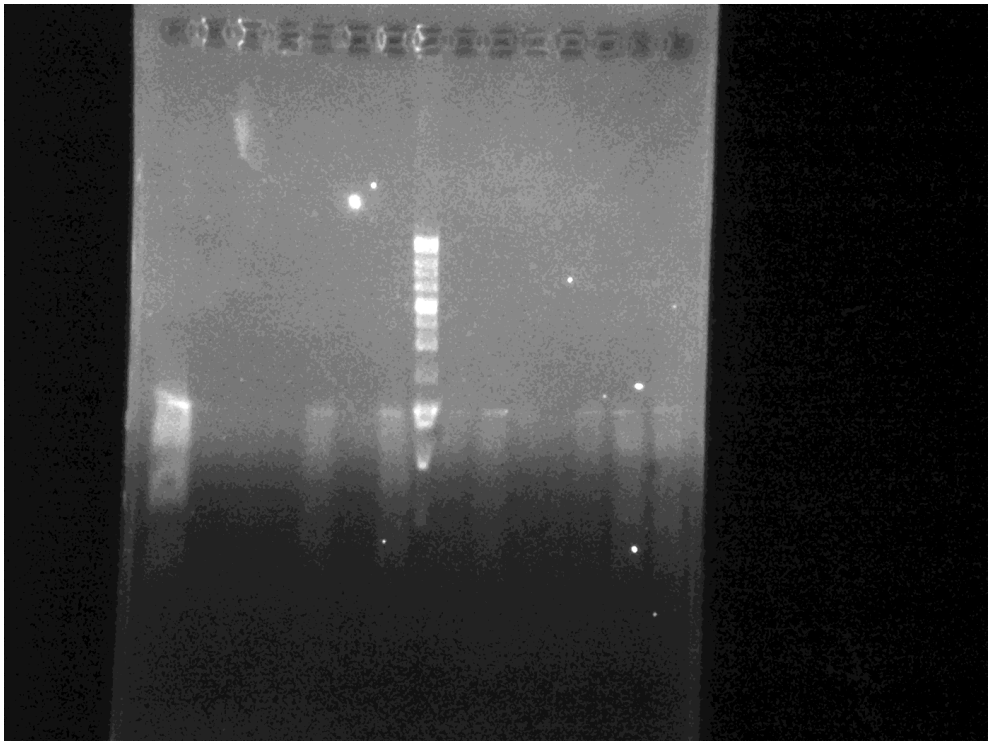

Figure 2 Gel containing both positive and negative results: lane one, on the left side of the gel, is a positive control of Colorado Tick Fever, lane eight contains a 1 Kb ladder, and lanes five, seven, ten, 13,14, and 15 are positive samples.

Discussion-

My results indicate that the average carrier rate for Colorado Tick Fever in *D. andersoni* in Montana, 22.3%, is within the range of previous studies, 0-25%. These data are in support of my hypothesis. Unexpectedly virus carrying ticks were not distributed homogeneously across the landscape but instead concentrated at specific locations. Specific locations such as Beaver Creek and the North Fork Bridge had carrier rates much higher than my initial predicted high of 25%. Of the twenty-seven positive results, nine were from the North Fork Bridge and eleven were from Beaver Creek. It is likely that other factors influence the distribution of this virus. Some influences could potentially be the presence of the golden-mantled ground squirrel, *Callospermophilus lateralis*, which is a reservoir host for the virus (Burgdorfer and Eklund, 1958). However because the virus is passed from the adult female to her offspring, once a virus carrying tick is present, I would expect these carriers to increase in abundance regardless of the presence of *C. lateralis*. Another possible explanation of this difference in carrier rate are geographic barriers to the spread of the virus such as mountains between gulches or the rivers such as the North Fork of the Blackfoot (9.8%), which was the only major feature between the main North Fork site and the North Fork Bridge site (52.9%).

The number of ticks that I tested for CTF is relatively small, particularly within the Big Belt Mountains. Increasing the sample size of these sites and comparing the abundance of the virus at sites that are geographically close to each other can better evaluate the heterogeneity of the virus' distribution. Possible uses for data such as these could be isolation or improved warning signs for those areas with high incidences of CTF

or areas with factors present that promote CTF. Increasing awareness could be particularly helpful in areas such as the North Fork of the Blackfoot River which have both a high concentration of virus carrying ticks and high levels of recreational activity. A risk assessment map could also potentially be created. All of these actions could lead to a reduction in the number of humans infected by the virus.

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Appendix 1: Tested sites, locations, presence of CTF, and number of ticks tested

Site Name	Coordinates	CTF found	# Ticks tested
North Fork of the Blackfoot River	47.12651 N, 112.96428 W	Yes	51
Beaver Creek C	46.85057 N, 111.73164 W	Yes	21
Beaver Creek D	46.86270 N, 111.67890 W	Yes	6
Avalanche A	46.61154 N, 111.65466 W	Yes	2
Avalanche riparian	46.68375 N, 111.52473 W	No	1
Avalanche random #2	46.70802 N, 111.49424 W	Yes	5
Magpie A	46.65310 N, 111.70490 W	No	1
Magpie B	46.67607 N, 111.65569 W	No	9
Magpie C	46.69738 N, 111.62336 W	No	5
Confederate C	46.61713 N, 111.38452 W	No	3
North Fork Bridge	47.10188 N, 112.96236 W	Yes	17