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Survey of Colorado Tick Fever Virus Presence in Montana Deer Mice and Wood Ticks

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Introduction

Colorado Tick Fever virus (CTFV) is carried by Rocky Mountain wood ticks (*Demacentori andersoni*). Its double-stranded RNA genome is comprised of twelve segments. In humans, it causes a variety of flu-like symptoms, including fever, headache, sensitivity to light, and muscle soreness. Because the symptoms mimic the flu and other common diseases, it is often overlooked during clinical diagnosis.

Deer mice (*Peromyscus manisculatus*) are considered to be a reservoir for the virus. This study aimed to determine the prevalence of CTFV nucleic acid in mouse blood. The whole blood samples were screened from Polson and Gregson, Montana. These samples were collected both prior to the study for a separate Hantavirus study as well as during the study. Only Hantavirus negative samples were screened.

In addition, ninety ticks were collected. While these have not been tested, they provide another sample set to screen for the presence of CTFV.

Project Goals

- Optimize a protocol to detect the virus using a positive control.
- Determine the prevalence of CTFV in deer mouse and tick samples.
- Hypothesis→ The virus will be detected in both the mice and the ticks, but in a much higher percentage in the ticks. Also, the virus will be much more prevalent in the mice during the spring and early summer months than other times of the year, as ticks are most active at this time.

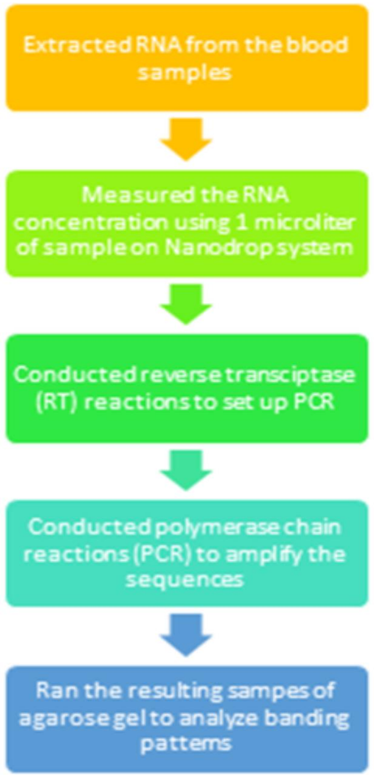
RT-PCR Optimization

The first goal of the project was accomplished. A PCR protocol was developed that show expected banding for the positive control Florio strain of the virus.

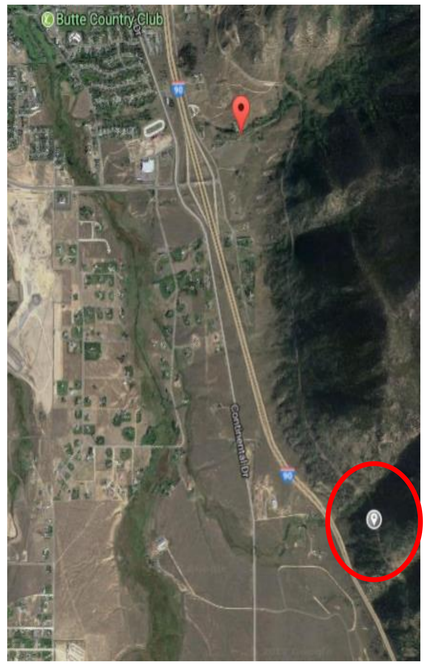
A reference strain of CTFV was used to infect Vero cells. Cell lysates were collected at the indicated time points. RT-PCR was performed with two sets of CTFV PCR primers as well as a primer set for the housekeeping gene beta-actin (ActB).

These results show that PCR with either CTFV primer set was capable of detecting CTFV nucleic acid at the 16- and 24-hour time points.

Methods



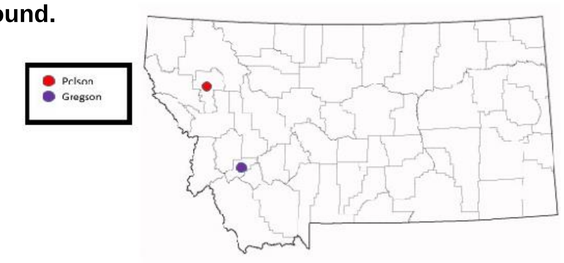
Tick Collection Site



This map displays where most ticks were collected (denoted by a red circle). The pin indicates Maud S. Canyon.

Mouse Sample Collection and Preparation

Locations where blood samples were collected from deer mice. Samples from Lake county (Polson) were taken during the months May – October and the Silver Bow county (Gregson) samples were collected year round.



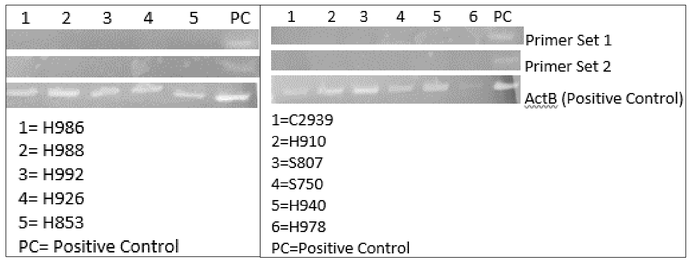
The table summarizes the progress that has been made on screening mouse blood samples from 2016. An X indicates that all samples from that month have been completed. A blank box indicates that samples from the given month have yet to be analyzed.

Month Samples Collected	Location	RNA Extraction	RT	PCR	Gel Electrophoresis
January 2016	Gregson	X	U		
February 2016	Gregson	X			
March 2016	Gregson	X	U		
April 2016	Gregson	X	U	U	U
May 2016	Gregson	X	U	U	U
June 2016	Polson	U	U	U	U
July 2016	Polson	X	U	U	U
October 2016	Polson	U	U	U	U
December 2016	Gregson	U	U		

X= all samples complete U= samples started, but not complete

Survey of Mouse Samples

CTFV was not detected in the deer mouse blood samples screened to date.



These are results from banding patters of isolated cDNA on agarose gel. The positive control (ActB) primers are producing bands in the expected area, suggesting that the PCR was conducted properly. As for the primer sets designed for CTFV, they are producing bands only for the positive control, suggesting that each of the mouse blood samples were negative.

Conclusions

This study did not detect CTFV in the deer mouse blood samples. This observation could be explained by a lack of the virus in the mice. Alternatively, the viral nucleic acid could be lower than the current level of detection of the RT-PCR assay.

Moving forward, there are still more samples to be analyzed. Also, the Florio strain of the virus could be used to better understand the basic biology of the virus through laboratory testing. The ninety ticks collected also provide opportunity for further study. They have been digested, but not evaluated with RT-PCR. Because the ticks are thought to have a higher percentage of their population carrying the virus. Testing them may be a better way to learn more about CTFV's prevalence in nature.

References

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Acknowledgements

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