Detection of Colorado Tick Fever virus in Archived Whole Blood Samples from Montana Deer Mice

2017 SUMMER UNDERGRADUATE RESEARCH FELLOWSHIP PROGRAM

SUBMITTED BY

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Background

This SURF proposal is a continuation of a RAMP project that was initiated in spring 2017. The RAMP project was jointly mentored by Dr. Joel Graff and Dr. Amy Kuenzi. It involved 4 undergraduate students who had no previous research experience. One of the specific aims of this RAMP project was to have the students optimize a reverse transcriptase-polymerase chain reaction (RT-PCR) assay to detect limited quantities of Colorado Tick Fever Virus (CTFV) dsRNA genomes in small mammal blood samples. By the end of the project in April 2017, the students will have generated positive controls for their assay, determined the sensitivity of the assay, and finalized a standardized operating procedure for the assay. In addition the students will have extracted RNA from previously collected blood samples (collected from 2 study sites in 2016) and screened a small percentage of these RNA extractions for CTFV using their developed assay. We are asking for SURF funds to pay 1 full-time students to continue and expand on this project. During the SURF project, the students will: run RT-PCR on their remaining RNA extractions; extract RNA and screen blood samples from any remaining blood samples from 2016; begin extracting RNA and screening blood samples collected at 2 sites in 2017; collect ticks from the 2 study sites; extract RNA from collected ticks, and screen ticks for CTFV.

Introduction

Colorado Tick Fever (CTF) is a rare, but serious, human disease caused by the Colorado Tick Fever Virus (CTFV), a double-stranded RNA (dsRNA) virus carried primarily by *Dermacentor andersoni* (Rocky Mountain wood ticks) (Geissler et al., 2014). Humans become infected with CTFV when they are bitten by an infected adult tick during its feeding period, which is usually in the spring or early summer. Adult ticks that have fed, mate and the female lays eggs which hatch into larvae after approximately 40 days. The larval form of *D. andersoni* feeds on a variety of small mammals such as rodents, rabbits, and hares. Larvae that have fed, molt into nymphs which generally remain inactive until the following spring when they emerge to take a blood meal on a variety of small mammals and occasionally also on humans. After feeding nymphs molt into adults. Adults feed on large mammals such as cattle, horses, elk, deer, and man (Emmons, 1988).

In ticks, the virus is transmitted by transtadial (larval stage to adult), but not tranovarial (parent to offspring) means. Small mammals are important in maintaining the virus in nature. Infected nymphs carry the virus through the winter, infecting small mammals in the spring. Larval ticks feeding on these small mammals become infected, molt into nymphs and over winter, maintaining the virus until spring. As adults, the tick can pass the infection on to large animals (sheep, deer, elk, humans, others) (Burgdorfer, 1977).

For decades, occasional surveys of CTFV prevalence in small mammals have been conducted in Colorado and Montana in order to gather data on the distribution of the virus in nature. These studies have found that the relative importance of various rodent species as CTFV hosts varies with study area. In Colorado, field studies of CTFV in small mammals in Rocky Mountain National Park in the mid-1970s found that golden mantled ground squirrels (*Callospermophilus lateralis*) and least chipmunks (*Tamias minimus*) were the most important hosts of the virus, while deer mice (*Peromyscus maniculatus*) and Richardson's ground squirrels (*Urocitellus richardsonii)* were secondary hosts (Bowen et al., 1981). Burgdorfer and Eklund (1959) focused on identifying animals which serve as hosts for immature stages of hosts, and incidence of adult ticks infected with CTFV in the Bitterroot Mountains, Ravalli County, Montana (Burgdorfer, W, 1959). They found the golden mantled ground squirrels to be the preferred host for immature *D. andersoni* and the main carrier of CTFV but they did isolate the virus from other mammals including deer mice, yellow pine chipmunks (*Tamias amoenus*), and red squirrels (*Tamiasciurus hudsonicus*). Another group, also working in Ravalli County, Montana in the 1960s, found that the deer mice were the most abundant small mammal species present in the area and the most commonly CTFV-infected species (5% of deer mice were viremic) (Clark et al., 1970).

Understanding how viruses are maintained in host populations requires information on how viral prevalence in hosts varies temporally and spatially and how this correlates with environmental factors. We could find no published studies of CTFV prevalence in small mammal populations in Montana since

1966 and no studies that were conducted in counties other than Ravalli County. In addition, we could find no published studies on CTFV anywhere that examine temporal variation in host prevalence.

In mammals, CTFV is thought to preferentially infect immature hematopoietic cells that later differentiate into red blood cells (RBCs) and white blood cells (WBCs). Since mature red blood cells can circulate in blood for months, CTFV can potentially be transmitted by blood transfusion. Dr. Kuenzi has collected and archived whole blood samples from deer mice sampled at two sites in Montana for many years. Spatially and temporally diverse samples are represented in Dr. Kuenzi's frozen deer mouse blood bank. We hypothesize that deer mice (*Peromyscus maniculatus*) are reservoirs of CTFV at these study sites and that the prevalence of CFTV in this reservoir will show spatial and temporal variations.

Specific Aims

We propose to screen deer mice blood samples that have already been collected from several areas in Montana for an unrelated research project conducted by Dr. Amy Kuenzi. It will be of interest to determine where and when deer mice are most commonly infected with CTFV. This information will advise follow-up field and molecular biology collaborative studies between the Kuenzi and Graff laboratories at Montana Tech. We also propose to

Specific Aim 1: Continued screening of *Peromyscus maniculatis* samples collected in Montana for presence of CTFV. The SURF student will use the RT-PCR assay developed during the previously mentioned RAMP project to continue screening a vast collection of hantavirus-negative deer mice blood samples for viral presence. Correlations will be sought between viral prevalence in relation to seasonality and location.

Specific Aim 2. Develop a real time PCR protocol to quantify amount of virus in any screened samples that tested positive for Colorado Tick Fever virus.

Specific Aim 3. Collect Rocky Mountain wood ticks (*Dermacentor andersoni*) at the two study sites, extract RNA from the collected ticks and screen these samples for the presence of CTFV using the RT-PCR protocol developed during initial RAMP project.

Research plan

Specific Aim 1 and 2 : Continued screening of *Peromyscus maniculatis* samples collected in Montana for presence of CTFV and develop a real time PCR protocol to quantify amount of virus in any screened samples that tested positive for Colorado Tick Fever virus. CTFV has a segmented, dsRNA genome and is the representative virus of the coltiviruses (derived from "Colorado tick"), an order of viruses found within the family *Reoviridae*. Similar to other reoviruses, such as rotavirus (the focus of Dr. Graff's doctoral dissertation), the dsRNA genome of coltiviruses is extremely stable and is expected to be intact in the archived samples.

Students will continue to extract RNA from hantavirus antibody negative blood samples collected in 2016, 2017, and if time permits earlier years, from two study sites using previous developed protocols from the Kuenzi lab. They will set up RT-PCR reactions using protocols developed during the previously funded RAMP project to look for presence of viral RNA. They will examine the results of this reactions using gel electrophoresis.

Samples that contain CTFV RNA will be examined further using a real-time PCR protocol (Lambert 2007). This will allow us to determine the actual amount of virus present in these positive blood samples. The second goal of these aims is to use the data from these screenings to look for correlations in virus detection and/or viral load in the archived blood samples compared to spatial and seasonal variation.

Specific Aim 3: Collect and screen Collect Rocky Mountain wood ticks (*Dermacentor andersoni*) for the presence of CTFV. Ticks will be collected at each study site once in May and once in June Tick will be collected using flagging ((Dantas-Torres et al. 2013), which involves waiving cotton flannel flags (90 X 65 cm) over and through vegetation. Collected ticks will be frozen in plastic vials until RNA can be extracted. Ticks will be grouped by site and collection date. RNA will be extracted by methods described by Crowder et al. (2010). RT-PCR reactions will be used to look for the presence of viral RNA,

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DESCRIPTION OF STUDENT ACTIVITIES

The SURF student will be involved in all aspects of the study. The student will work 5 days a week for 6-8 hours a day for 9-10 weeks with a total time spend on the project not to exceed 400 hours. During each week the student will be working on RNA extractions, RT-PCR, gel electrophoresis, and real time PCR in the lab. The student will also sample ticks in the field in May and June and assist with sampling mice in the field. The mentors will assist the student with optimizing lab protocols, statistical analysis, and writing up results

SHORT BIOGRAPHICAL SKETCHES OF STUDENTS AND MENTOR

We are requesting funds for one full time student Zachary Hart. Zach worked on the initial RAMP project this past spring. This SURF proposal will allow Zach to continue to use the lab skills he learned during the RAMP project as well as learn several new techniques (Real time PCR, invertebrate sampling, RNA extraction from tissue). Both Dr. Graff and Dr. Kuenzi will train Zach on these new techniques, assist Zach with tick collection at the study sites, and be available for guidance over the summer.

Dr. Amy Kuenzi: Dr. Amy Kuenzi has 25 years of field and laboratory research experience working with small mammals. She is an expert in hantavirus dynamics and host ecology in the deer mouse/hantavirus system. She has participated in Montana Tech's Undergraduate research program since arriving in Butte in 1999 and has mentored or co-mentored over 22 student URP projects. Her research has resulted in numerous publications, 7 of which include undergraduate students as first or co-authors (4 of these publications involved URP or SURF projects). Dr. Kuenzi will lead the efforts in aim 2 and help oversee the completion of aim 1.

Dr. Joel Graff: Dr. Joel Graff has been conducting molecular biology research since 1999. His first nine years of basic biological research focused on the human pathogen, rotavirus. Similar to CTFV, rotavirus is a member of the viral family, *Reoviridae*, which contains a segmented, dsRNA genome. Building on his experience, Dr. Graff will lead the efforts in aim 1 and help oversee the completion of aim 2. Dr. Graff has extensive experience as a mentor of undergraduate researchers and is currently leading a group of four Montana Tech students in projects unrelated to the current proposal. Dr. Graff's strong publication record can be viewed in the accompanying CV.

Zach Hart: Zach is currently a sophomore majoring in Biology. Zach is interested in attending medical school in the future. He, and 3 of his classmates, worked with Dr. Kuenzi and Dr. Graff this spring on the initial RAMP project.

BUDGET

One full time undergraduate researcher	**$4000**
Lab supplies	**$ 500**
Faculty mentor stipend	**$1000** (willing to waive if funds are limited)
Total	**$5500** ($4500, if no mentor stipend)

Justification
We are requesting funds for 1 full time student researcher.

We are requesting the mentor stipend of $1000 but are willing to waive this stipend if funds are limited.

We are requesting $500 to help cover the cost of lab supplies for this project. These supplies include primers, reagents for RNA extraction, reagents for PCR reactions, supplies for gel electrophoresis, and disposables. Molecular biology reagents are notoriously expensive. Additional funds for lab supplies will be provided by the biology department via their lab supplies account and the department's indirect account.