

**Comparison of Capture Methods and Infection Rates for the Tick, *Dermacentor andersoni*,  
in Montana**

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**Abstract**

Montana is home to the tick *Dermacentor andersoni* which serves as a vector for Colorado tick fever. It is important to study ticks in the field to improve capture rates for the purpose of testing infection rates. To determine the most effective way to capture ticks, four separate capturing methods were assembled in two tick prevalent locations. These methods include AAAP pheromones, CO<sub>2</sub>, drag netting, and AAAP in combination with CO<sub>2</sub>. I hypothesized that the combination of AAAP and CO<sub>2</sub> would yield the best capture results. I implemented a mark-recapture study using fluorescent powder and hypothesized that CO<sub>2</sub> and pheromones combined would provide the best population estimates. My findings indicate that drag net sampling and CO<sub>2</sub> alone were the best tick attractants which could be due to pheromone amounts high enough to repel ticks rather than attract them. CO<sub>2</sub> and drag netting had similar efficacy, whereas AAAP attracted no ticks throughout all trials. I developed an RT-PCR protocol to detect the presence of Colorado tick fever in *D. andersoni* and hypothesized that infection rates would be within the range of 10-25%. At Woodlake Campground and Dearborn the infection rate of Colorado tick fever was 12%. The infection rate at Beaver Creek drainage was 10%. My results suggest that drag netting, perhaps combined with CO<sub>2</sub>, lead to higher capture rates and the most effective means of estimating population density. Also, my results show that the infection rate of ticks was within 10-12% at three sites in Lewis and Clark County.

## Introduction

Under the infectious disease ecology research project at Carroll College, an RT-PCR protocol is used to detect the presence of West Nile Virus in the mosquito, *Culex tarsalis*. This protocol is being modified to test for Colorado tick fever in the tick *Dermacentor andersoni*, also known as the Rocky Mountain Wood Tick (Johnson, 2010). Symptoms of Colorado tick fever manifest three to six days after the tick bite and include sudden fever, weakness, and muscle aches (U.S. National Library of Medicine, 2015). In addition to Colorado tick fever, *D. andersoni* is a vector of Rocky Mountain spotted fever, tularemia, tick paralysis, and anaplasmosis (Johnson, 2010). Livestock, wildlife, and humans are afflicted by over 20 tick-borne diseases worldwide (ESA, 2015). More information about the ecology and disease transmission of ticks is needed to implement effective prevention measures.

The Rocky Mountain Wood Tick, *D. andersoni*, is the known vector of Colorado tick fever and is commonly found in Montana (Johnson, 2010). *D. andersoni* is classified in the phylum Arthropoda and class, Arachnida (Zhang, 2013). Ticks are categorized into four families which include *Nuttalliellidae* and *Laelaptidae* (each comprising one species), *Ixodidae* (hard ticks) and *Argasidae* (soft ticks) (Narasimhan and Fikrig, 2015). *D. andersoni* is a hard bodied tick, due to a hard plate on its dorsal body surface, and has four life stages (eggs, larvae, nymphs, and adults) and requires one blood meal from a vertebrate host at the three latter stages (Johnson, 2010, Narasimhan and Fikrig, 2015). The feeding takes three to fourteen days to complete and the blood meal is digested apart from the host (Allan, 2010). *D. andersoni* climb vegetation and quest, which is the spreading of their forelegs, in response to stimuli such as odors, heat, vibrations, or shadows, in order to cling to the host (Allan, 2010). The complete life cycle typically takes about three years, but can vary depending on the availability of hosts (Johnson,

2010). Adults bury themselves in the soil during winter and begin to seek hosts in March or early April (Johnson, 2010).

In order to implement better tick control, the Entomological Society of America, ESA, recommends increased ecological surveillance of ticks (ESA, 2015). Studying ticks in the field is important to improve capture rates for the purpose of testing infection rates and/or infection rate variability. In the present study and to determine the most effective way to capture ticks, four separate capturing methods were assembled in two tick prevalent locations. These methods included attraction-aggregation-attachment-pheromones (AAAP), CO<sub>2</sub> (in the form of dry ice), drag netting, and AAAP in combination with CO<sub>2</sub>.

To measure the effectiveness of each method, I implemented a mark-recapture study using fluorescent powder to measure population density at sample sites. Ticks are attracted to AAAP due to its emission by feeding males and are attracted to carbon dioxide due to host exhalation (Nchu *et al.* 2009). AAAP is synthesized by mixing ortho-nitrophenol, methyl salicylate, and nonanoic acid in a 2:1:8 ratio (Nchu *et al.* 2009). I hypothesized that the combination of AAAP and CO<sub>2</sub> would yield the best capture results because up to 90% of the hard ticks, *Amblyomma variegatum*, released from the center of circular field plots were attracted to the combination of pheromones and CO<sub>2</sub> within three hours (Maranga *et al.* 2003). I hypothesized that AAAP and CO<sub>2</sub> would give a better estimate of population density via mark-recapture than any of the other methods. In addition to testing the efficacy of capture methods, I developed an RT-PCR protocol to detect the presence of Colorado tick fever in *D. andersoni* and to improve the efficiency of detection. I hypothesized that infection rates would be within the range of 10-25% since an average infection rate of 22.3% in Western Montana was found by Jordan (2015).

## Materials and Methods

### *Capture Methods*

Four separate capture methods of AAAP, CO<sub>2</sub>, CO<sub>2</sub> and AAAP, and drag sampling were assembled in two tick prevalent locations, namely North Fork of the Blackfoot River (47.125905° N 112.963738° W) near Lincoln, Montana and Beaver Creek drainage (46.854738° N 111.711139° W) in the Big Belt

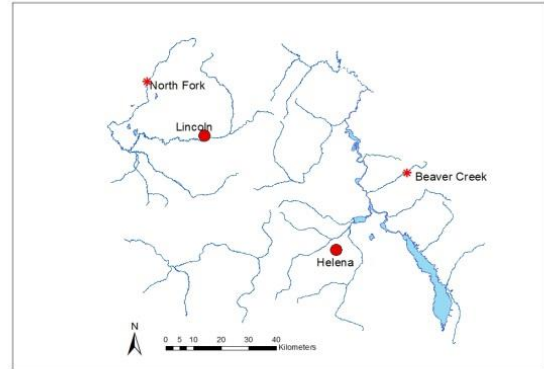


Figure 1. Map of two tick prevalent locations where capture methods were implemented: North Fork of the Blackfoot River and Beaver Creek drainage.

Mountains (Figure 1). This study was performed over a period of two days at the North Fork and three days at Beaver Creek. Three replicates of each method or twelve total sampling plots were assembled at the North Fork location, while two replicates of each method or eight total sampling plots were assembled at the Beaver Creek site. The order in which the methods were assembled was randomly assigned. Each sampling plot was measured approximately to a ten meter by ten meter area. Five replicates of the trap were set up at each sampling plot and placed approximately two meters apart (Nchu *et al.* 2009). AAAP was synthesized by mixing ortho-nitrophenol, methyl salicylate, and nonanoic acid in a 2:1:8 ratio (Nchu *et al.* 2009). Cotton-tipped applicators were used to absorb the pheromones and were then placed into the ground, placed over a one meter by one meter sheet of lab bench paper used to make ticks more visible. CO<sub>2</sub> in the form of dry ice was split into small bricks and placed in half of a Styrofoam container, atop the one meter by one meter sheet of bench paper. Drag sampling was performed using a one meter by one meter white fabric to sample vegetation for questing ticks for approximately ten minutes over the allotted sampling plot. AAAP and CO<sub>2</sub> plots were left for at least one hour in

order for the attracted ticks to move toward the sheets. Ticks found at each sampling plot were placed in a film canister full of fluorescent powder and shaken gently. Ticks were then placed in the center of the plot to implement the mark-recapture study. In all subsequent trials ticks were first checked with a UV flashlight for the presence of fluorescent powder before placing them in the film canister.

### *Statistical Analysis*

Due to the non-parametric nature of the data, a Kruskal-Wallis test was performed to test for significant differences in efficacy of the four capture methods. A Schnabel estimator was used to determine population densities obtained from the mark-recapture study (Daniels *et al.* 2000).

### *Collection of ticks for Colorado tick fever detection*

Ticks were collected using the drag net sampling technique previously described. Ticks were collected during the months of May and June at various sites across Western Montana (Figure 2).

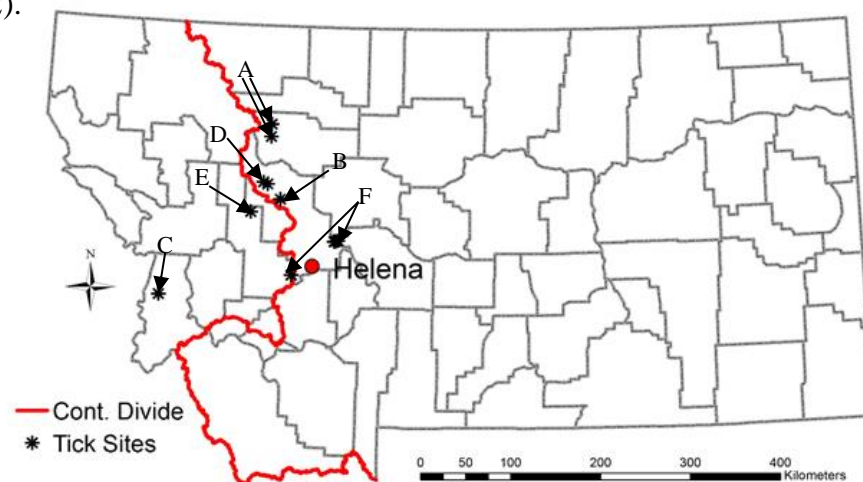


Figure 2. Sampling sites where ticks were collected across Western Montana. The sites are as follows: Blackleaf (A), Dearborn (B), Blodgett (C), Woodlake (D), North Fork (E), Helena Local including Beaver Creek (F).

### *RNA Extraction*

A QIAGEN DNeasy Blood and Tissue Kit was used to homogenize ticks. The bench protocol for that of animal tissues followed that of Garringer (2014) with modifications including an incubation time of one and a half hours and the samples being ground up using FastPrep Smart Solutions LTD and vortexed in a bead beater for 35 seconds at a speed of 30 oscillations/minute due to *D. andersoni*'s sclerotized shell (Dotson 2015). RNA was extracted from 300  $\mu$ L of homogenate in a QIAcube following the protocol of Lanicotti (2000, QIAGEN, Fitzpatrick 2015). Tick RNA was stored in a  $-80^{\circ}$  C freezer until RT-PCR was performed.

### *RT-PCR*

Tick RNA was amplified and tested for Colorado tick fever following Lanicotti's (2000) protocol for RT-PCR West Nile Virus detection. Each well of the PCR plate contained 5  $\mu$ L of sample and 20  $\mu$ L of Master Mix (Table 1). The PCR plate was transferred to a BioRad IQ500 thermocycler and samples were incubated under the following conditions: Stage 1- Reverse transcription at  $50^{\circ}$  C for 5 minutes, Stage 2- Reverse transcriptase inactivation and initial denaturation at  $95^{\circ}$  C for 20 seconds, and Stage 3- Amplification for 40 cycles of  $95^{\circ}$  C for 30 seconds followed by  $60^{\circ}$  C for 30 seconds.

Table 1. Master Mix for RT-PCR.	
Reagents	Amount ( $\mu$ L per well)
RNase-free water	9.5
Sequence 1 Forward Primer	1
Sequence 1 Reverse Primer	1
Sequence 1 Probe	1
4X TaqMan	5

## Results

Six replicates of each capture method were completed to obtain tick data (Table 2).

Table 2. Number of ticks per capture method for six replicates at Beaver Creek

AAAP	CO <sub>2</sub>	CO <sub>2</sub> & AAAP	Drag Net
0	1	0	4
0	2	1	7
0	6	0	1
0	2	1	0
0	1	0	2
0	1	0	2

A Kruskal-Wallis test was performed to compare the four capture methods and a p-value of less than 0.001 indicates there was a significant difference between capture methods (Figure 3). CO<sub>2</sub> and drag netting are of statistically similar efficacy, while pheromones did not attract ticks.

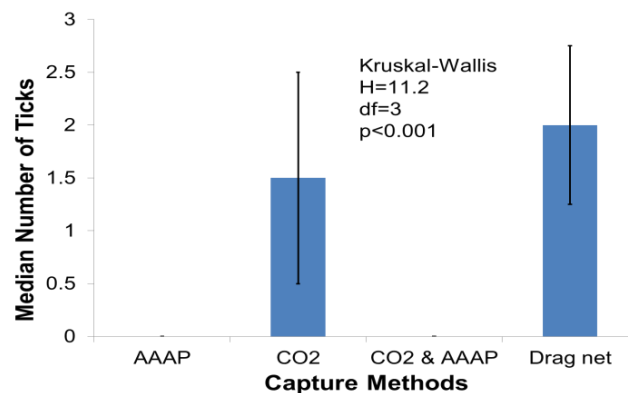


Figure 3. Median number of ticks per capture method at Beaver Creek site. Bars indicate interquartile ranges.

A Schnabel estimator was used to determine population estimates of the CO<sub>2</sub> and drag netting techniques from the mark-recapture data (Table 3). Both capture methods yielded similar population estimates indicating similar efficacy.

Table 3. Schnabel estimate of population size for mark-recapture study comparing population density of drag net and CO<sub>2</sub> methods at Beaver Creek site. Population estimate  $\pm$  standard error.

Drag Net		CO <sub>2</sub>	
Mark	Recapture	Mark	Recapture
14	2	9	4
Population Estimate		Population Estimate	
13 $\pm$ 11.467		12.6 $\pm$ 7.22	

Infection rates of Colorado tick fever were calculated for three independent sites in Lewis and Clark County (Table 4, Figure 4). Woodlake Campground was found to have an infection rate of 12%. Similarly, Dearborn's infection rate was 12%. Beaver Creek drainage had an infection rate of 10%.

Table 4. Colorado tick fever infection rates.

Site	Positive	Negative	Infection Rate
Woodlake	2	14	12%
Dearborn	2	14	12%
Beaver Creek	2	19	10%

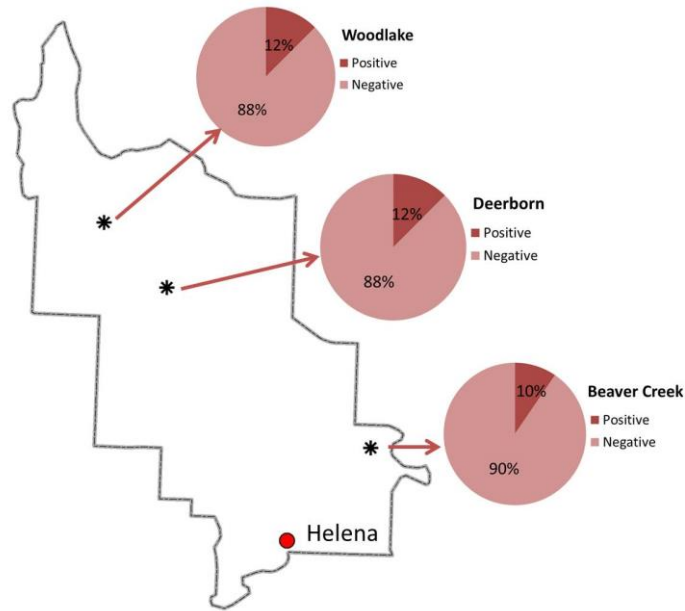


Figure 4. Colorado tick fever infection rates at three sites in Lewis and Clark County.

## Discussion

Comparison of the four methods indicates that CO<sub>2</sub> and drag net sampling are of statistically similar efficacy, while pheromones as well as the combination of pheromones and CO<sub>2</sub> did not attract ticks. However, drag netting is a more efficient means of capture due to CO<sub>2</sub> taking at least one hour of sampling in addition to thirty minutes of set-up while drag netting was completed in under ten minutes.

According to the Schnabel estimates of population, drag net sampling and CO<sub>2</sub> are both viable options for estimating population density. Due to the fact that CO<sub>2</sub> and drag net sampling were of higher efficacy in capturing ticks and estimating population density than the combination of pheromones and CO<sub>2</sub>, I reject my hypotheses that CO<sub>2</sub> and pheromones would attract more ticks and be a better estimate of population density.

Maranga *et al.* (2003) found that 90% of *A. variegatum* ticks dispensed from the center of circular field plots were attracted to the combination of pheromones and CO<sub>2</sub> within three hours.

Maranga *et al.* (2003) also found that ticks were attracted to the pheromones in the absence of CO<sub>2</sub>, but ticks were slower to aggregate and lower in proportion, suggesting variation in response to inter- and intra- specific signals. Nchu *et al.* (2009) found that 1-octen-3-ol, AAAP, and CO<sub>2</sub> attracted up to  $94 \pm 6\%$  of adult ticks from a distance of 6 m, and  $24 \pm 5.1\%$  from 8 m. These results conflict with my findings of CO<sub>2</sub> alone and drag net sampling being more effective methods than AAAP in combination with CO<sub>2</sub>. A possible reason for this discrepancy is that the compounds found in AAAP were identified in feeding males of *Amblyomma variegatum* and *A. hebraeum* but have not been identified in *D. andersoni* (Maranga *et al.* 2003). Nana *et al.* 2010 used AAAP as an effective attractant in studies performed on *Rhipicephalus appendiculatus* and *R. pulchellus* which are hard-bodied ticks like *D. andersoni*. It is unknown whether compounds of AAAP have been identified specifically in feeding *D. andersoni* males and, until this study, no known studies have been performed using AAAP to attract *D. andersoni*. It is possible that *D. andersoni* feeding males do not emit the same compounds found in AAAP, although this is unlikely due to the knowledge that other hard-bodied ticks are, in fact, attracted.

Another possible explanation for the pheromones not attracting ticks in this study is the concentration of AAAP used. “*A. variegatum* and optimum attraction was recorded at 0.022 mg of AAAP on 2 cm<sup>2</sup> odour source filter paper with an air flow rate of 5 ml/s. Higher doses of AAAP appeared to be avoided by the tick” (Nchu *et al.* 2009). Further research could use accurately measured amounts of pheromones in order to determine if higher doses were in fact repelling ticks rather than attracting them.

Colorado tick fever infection rates of 10-12% in Lewis and Clark County confirm my hypothesis. These results fall within a carrier rate of 0-40% found by Rocky Mountain Laboratories in the Bitterroot Mountains of Montana (Burgdorfer and Eklund, 1958, 1960). A

study completed one year prior in Western Montana found an average carrier rate of 22.3% which is comparable to my findings (Jordan 2015). However, Jordan's (2015) results showed high variability ranging between 0 and 52.9% between the different sites. A possible cause of the discrepancy in variation is that the instability of RNA leads to rapid degradation if not kept frozen or if proper handling is not employed. The development of RT-PCR for Colorado tick fever testing greatly improves efficiency compared to the previous method of gel electrophoresis. Utilization of this method will allow for more efficient and accurate testing of Colorado tick fever in more locations.

Understanding and broadening the knowledge of the most effective capture methods and testing mechanisms will allow for increased disease surveillance of *D. andersoni*. My results indicate that drag netting is the most effective means to capture *D. andersoni* in Montana. Colorado tick fever testing is made more efficient through the use of RT-PCR. Infection rates of 10-12% were found in three sites in Lewis and Clark County. Further testing may improve the public's knowledge of tick-borne diseases and may contribute to a risk assessment map of Colorado tick fever.

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