Collection of Ticks for Surveillance of Disease Agents on a Mountain in Central Virginia

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Abstract

Tick-borne disease is found all over the world, and interest in disease surveillance for tick-borne illnesses has increased, partly because some of the illnesses are becoming more common. Tick collection is an integral and necessary part of disease surveillance, and knowledge of the ticks' habitats, life cycles, and different collection methods increases the chance of their capture. Beginning in March of 2015, ticks were collected using CO_2 traps from Candlers Mountain in central Virginia. The tapes used in the traps were experimentally tested using a force transducer to create a trap that would capture the greatest number of ticks. From March through June, 62 deer and lone star ticks were collected using this method. The lab experiments suggested that duct tape and colored lab tape would be the best choice for a CO_2 trap. The researchers believe that better education about tick species, habitats, and the potential risk of tick-borne diseases will help people in the Lynchburg area and around the world protect both themselves and their pets.

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Introduction

Suburbanization and an increase in population size have placed unsuspecting humans in a more vulnerable position for exposure to ticks (Institute of Medicine, 2011). While it is unpleasant enough to be bitten by a tick, the possibility of receiving a tick-borne disease is a much more serious and perhaps deadly consequence of a tick bite. Ticks carry agents in their bodily fluids which may cause disease when passed on to a host through the feeding process. The prevalence of tick-borne diseases such as Lyme disease, Ehrlichiosis, and Rocky Mountain spotted fever has increased over the past few decades in the United States and across the world. This is in part due to the increasing interest in outdoor recreational activities and a need because of population growth to expand cities and towns further into more rural territories where ticks are found (Institute of Medicine, 2011). This causes increased exposure of a population.

Because these diseases can cause serious health problems and even death for both humans and animals, it is important to know their presence in any geographic location. The method of determining if a certain disease is in an area is known as *disease surveillance* (Petersen et al. 2015). This study documents efforts made to determine how abundant ticks were in a certain area and whether or not any carried the diseases in question. Research of this type has been done in many places all over the world. Local tick knowledge can help prevent the spread of the diseases by increasing public awareness of the presence of the diseases in certain areas and the inherent risks one may face when in tick territory (Petersen et al. 2015). The collection of ticks directly from the outdoors, as was done in this study, is considered *active surveillance* as compared to *passive surveillance*, which is just the reported cases of the diseases in humans and animals (Petersen et al. 2015).

Testing for Disease Agents

The collection of ticks, which might be considered the first phase of the overall study to determine the presence of disease-causing agents, is the primary subject of this thesis and was accomplished during March through June of 2015. The second phase, though not yet completed, involves testing the ticks for certain diseases and will be briefly introduced to explain the ultimate goal of this study and to emphasize the importance of successful tick collection. To properly determine pathogen presence, the ticks must be genetically tested. To do this, the nucleic acids must first be extracted from the ticks in order to collect their DNA, a process which is usually done by freezing the ticks and then crushing them into very small pieces (Crowder et al. 2010). They also may be frozen with liquid nitrogen, offering a very fast and effective method of both killing and freezing the ticks (Brinkerhoff, Gilliam, & Gaines, 2014). The ticks are frozen so that they can more easily be divided into small pieces that can be tested; whereas if they were soft if would be very hard to cut them up to the desired size. However, before the ticks are crushed, they should be rinsed with 70 percent ethanol and then water in order to kill any surface pathogens on the ticks that may react with the primers used in later steps and skew the results (Sparagano et al. 1999).

PCR, or polymerase chain reaction, is then done in order to amplify the sample of tick DNA (Sparagano et al. 1999). This is crucial because if a tick was infected with only a small number of pathogens, the disease may not be detected (Sparagano et al. 1999). The amplification of the sample allows the pathogen DNA to be identified even when only present in small numbers. Specific primers are added to the sample to attach to and amplify specific parts of the pathogenic DNA that will prove the presence of the antigens in the ticks (Crowder et al. 2007; Sparagano et al. 1999). The results would then be positive or negative for the presence of disease. PCR is considered to be perhaps the most successful and accurate method of detecting the presence of and identifying the pathogens that cause these diseases (Sparagano et al. 1999).

Hypothesis

The researchers first wondered if ticks would be caught at a pre-determined site located near a popular trail on Candlers Mountain in central Virginia. If ticks were caught, their number, gender, and species would be documented. The second phase of the project would investigate whether or not the ticks were infected with the agents causing Lyme disease, Rocky Mountain spotted fever, and Ehrlichiosis. With these questions answered, the public could be informed of what pathogens may be present on Candlers Mountain, a fact which would help protect both humans and their pets. As outdoorsmen are educated about the potential disease risk that comes with ticks, their awareness may save both people and animals from sickness and possible death. Candlers Mountain is an important area to test as many people frequent the woods, whether hiking, camping, or biking, and their safety is important. The hypothesis of this study was that if an efficient method of collection was determined, then deer ticks, American dog ticks, and lone star ticks would be captured at the testing site. An introduction to the different tick species (what is called their natural history) and the diseases they potentially carry is important in order to understand the methods behind tick collection and the reasons for doing so.

Tick Species

It is important to understand the life habits and to be familiar with the different species of ticks in order to be successful in the capture of ticks. To collect ticks and accurately document their numbers, one must know when ticks are active, where they are most likely to be located, and on which animals they prefer to feed. Knowledge of a preferred habitat gave researchers a good advantage for quickly identifying the type of area in which the highest number of ticks would be found. That is, awareness of their life cycle made it more likely to catch them. The three tick species that are most frequently associated with the diseases and were of the greatest interest to the researchers were the deer tick (*Ixodes scapularis*), the lone star tick (*Amblyomma americanum*), and the American dog tick (*Dermacentor variabilis*). Each of these species has a specific disease that it is known to carry. What follows is a brief review of the important natural history of these ticks.

Ticks are actually not insects and belong to Class Arachnida and Family Ixodidae (Institute of Medicine, 2011). The ixodids are considered to be hard ticks as compared to the soft ticks in the family Argasidae, and possess highly sclerotized bodies that provide an immense amount of protection (Petersen et al. 2015). These ticks typically live anywhere from one to three years, during which they progress from an egg, to a six-legged larvae, to an eight-legged nymph, and finally to an adult (Institute of Medicine, 2011). A larva must obtain a blood meal before it can molt into a nymph, and the nymph must also feed from a host before molting into an adult (Institute of Medicine, 2011). The adults mate while actually on a host, and the female lays several thousand eggs on the ground once she has fully fed and has dropped off the host (Chan & Kaufman, 2008). The female then dies along with the males (Chan & Kaufman, 2008).

They are considered obligate blood feeders, meaning they completely rely on feeding on the blood of a host to survive, and feed once at every stage of life (Institute of Medicine, 2011). To help the feeding process, the ticks possess mouthparts which allow them to secure themselves to the host; further security is added by the secretion of a substance from the salivary glands that resembles cement (Petersen et al. 2015). This enables the ticks to remain attached for several days if they require that much time to feed. This long attachment time increases the chances for the pathogens to be transferred into the host as the tick secretes its saliva, which contains the pathogens, into the bite while feeding (Petersen et al. 2015). Even though the eggs almost always hatch into larvae free of any infection, they can become infected after they have taken their first blood meal if they happen to feed on an infected host (Institute of Medicine, 2011). This also means that they can transmit diseases at both the nymphal and adult stages, which is very dangerous because the ticks are very small at this age and are not likely to be noticed on a human host.

It seems very unlikely that a person or animal would just happen to get close enough to a tick for it to climb on to the host. However, ticks engage in a behavior called *questing*, where they climb some short distance off the ground onto some brush, grass, or tree limbs and "wave their legs as a potential host approaches" (Chan & Kaufman, 2008). This allows the tick to easily grab on to the animal or person when it brushes against the foliage where the tick is perched (Chan & Kaufman, 2008). Ticks can also recognize a potential host by sensing body heat, the carbon dioxide given off in the breath of an animal, and by feeling vibrations which can inform ticks that an animal is approaching (Center for Disease Control, 2015). Interestingly, research has shown that ticks often congregate in larger numbers alongside the edge of paths or trails, most likely because they can detect the smell of the hosts that move along that trail and identify that it is well-traveled (McNemee, Sames, & Maloney, 2003). The ticks would have a better chance of encountering a host if they quest in areas that are popularly traveled (McNemee et al. 2003). This dramatically increases human risk of being bitten by a tick, as humans mostly use these trails.

American dog tick. The American dog tick (*Dermacentor variabilis*) is the first tick of interest in this research and is located east of the Rocky Mountains, where it is most abundant in those states on the east coast (Chan & Kaufman, 2008). These ticks need a certain amount of humidity and moisture to prevent desiccation and are therefore found in deciduous forests, preferring to inhabit areas near fields and forests with second growth (McDade & Newhouse, 1986). They are usually found in forested areas with lots of low underbrush, and seem to also prefer areas with tall grass (Chan & Kaufman, 2008). The shrubbery and grass allow the tick to be able to climb to a height where it can easily attach to its host. In Virginia, this tick first becomes active during the first few weeks of April and continues to seek a blood meal until around August or September (Burg, 2001). However, there are several times during these months of activity where the ticks are at the highest level of activity and are actively seeking hosts. These peaks in activity occur from the time in April when they first become active through the month of May and once again during July (Burg, 2001). Because of this, humans run an increased risk of becoming bitten and infected with the disease-causing pathogens during these months as the ticks are moving around and more actively searching for a meal while people are enjoying the summer weather. The American dog tick commonly is found feeding on small mammals such as voles, the white-footed mouse, opossums, skunks, and raccoons, as well as larger animals such as horses and livestock (Burg, 2001; Chan & Kaufman, 2008). However, its preferred host as an adult is dogs, thus giving the tick its name (Chan & Kaufman, 2008).

The American dog tick is distinguishable from other species by its color, size, and shape. Both males and females have a brown body with light grey markings on their scutum, or the area behind the head (Chan & Kaufman, 2008). As seen in Figure 1, the gender can be determined by this lighter pattern of color, as the light color on the male is much more patterned. Also, the scutum of a male is very large and covers most of the dorsal portion of its body, whereas the female's pale scutum is much shorter and covers only a certain area directly behind the head (Chan & Kaufman, 2008). No American dog ticks were captured during this study.

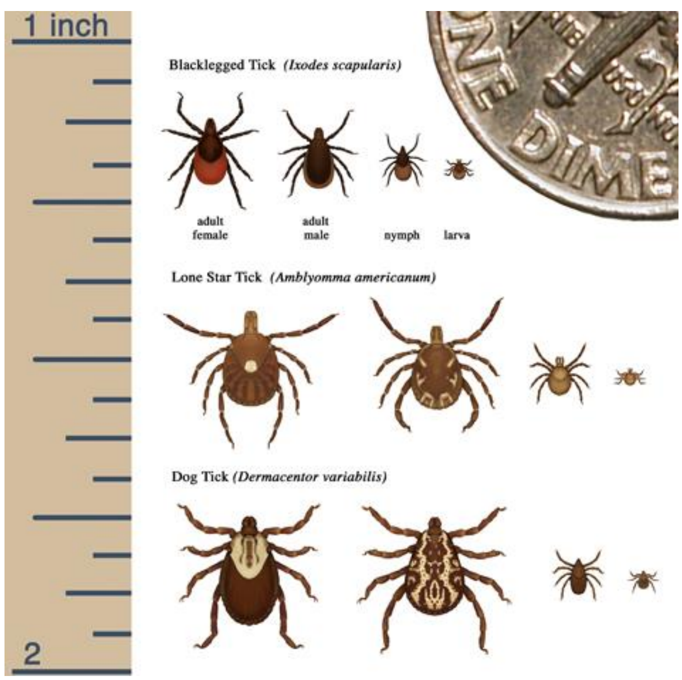

Figure 1. The sizes of the different life stages of the deer tick (blacklegged tick, top), lone star tick (middle), and American dog tick (bottom). The differences in body shape between the species and coloration between male and female can be observed. Taken from (Center for Disease Control, 2015).

Lone star tick. The Lone star tick (*Amblyomma americanum*) is the second tick of interest in this research and occupies a range extending from Texas, north to Michigan, and all across the eastern states where it is the most abundant (Pasketwitz, n.d.). These ticks usually inhabit wooded areas, especially favoring those that offer the preferred habitat and contain a large number of mammals on which for them to feed (Pasketwitz, n.d.). They are not specific in their choice of host animal and will quickly attach to any animal that gives them the opportunity to feed (Pasketwitz, n.d.). The smaller larvae and nymphs typically find meals on smaller mammals such as birds, rabbits, and squirrels, but will also feed on larger mammals and humans if given the chance (Pasketwitz, n.d.). The adults usually choose larger hosts and are commonly found on white-tailed deer, foxes, raccoons, possums, and livestock (Pasketwitz, n.d.; Kollars et al. 2000). Lone star ticks can be found year round, although their activity significantly increases during the months of May, June, and July (Kollars et al. 2000). The nymphs remain at this high state of activity through the month of August, and the larvae are very active all the way through September (Kollars et al. 2000).

These ticks are probably the easiest species to identify, with the female having a large white spot on the back of the tick (Figure 1). The more circular body shape of both male and female also aids in their identification, as American dog ticks and deer ticks both have more of a “tear-drop shape” to their bodies (Pasketwitz, n.d.). Males are typically smaller and darker brown with a white pattern decorating the circumference of their body while the females are larger and are more of a reddish-brown color with the bold white dot on their dorsum (Pasketwitz, n.d.). This was by far the most commonly captured tick at the site.

Deer tick. The deer tick (*Ixodes scapularis*) is the last tick of interest in this study. Like the other species, these ticks are found mostly on the east coast, but are also found all the way to Texas and Minnesota (Patnaude & Mather, 2000; Thevanayagam, 2012). They are found in areas inhabited by white-tailed deer, as these mammals are the main hosts for the adult ticks (Chan & Kaufman, 2008). The larvae tend to choose smaller animals as prey such as birds, small mammals, and the white-footed mouse, which is their favorite host (Thevanayagam, 2012). As the ticks grow to larger stages, they choose larger mammals until they are feeding primarily on deer (Thevanayagam, 2012). Deer ticks tend to be found in those areas that deer inhabit, such as wooded forests with an abundance of undergrowth (Chan & Kaufman, 2008). When forests are cleared and then allowed to re-grow, the result is a “second-growth forest”; a favorite habitat for deer and therefore prime territory for ticks to thrive (Chan & Kaufman, 2008). Adult ticks can be found year round but are less active during the winter when temperatures are near freezing (Chan & Kaufman, 2008). The larvae are the most active in August, which is a bit later than the peak months for the American dog tick and the Lone star tick (Chan & Kaufman, 2008). The nymphs are also active during the summer months and usually molt into their final adult stage in October (Chan & Kaufman, 2008). The adults are then active from October to the spring and early summer months, where they reach peak activity and are actively feeding and reproducing (Chan & Kaufman, 2008). The males die after mating, and the females die soon after laying several thousand eggs on the ground (Thevanayagam, 2012).

These ticks are the smallest of the species studied in this research, and because of this they are sometimes very difficult to detect when attached and feeding. They are only about three millimeters long when fully grown and the larvae and nymphs are usually no larger than a pinhead (Thevanayagam, 2012). Larvae and lymphal ticks are especially difficult to see when feeding on a host because of their very small size. Unlike the other two species that were described, these ticks have no white markings; however, the male and female do have differing appearances while sharing obvious black legs (Chan & Kaufman, 2008). While the males are a very dark brown color, the body of the female is actually a lighter reddish-brown color besides the black head and scutum (Figure 1) (Chan & Kaufman, 2008). Only a few of these ticks were captured in this study.

Common Diseases

Lyme disease. Lyme disease, along with other diseases transmitted by ticks, is considered a *zoonotic disease*, where the disease agent is transmitted through an animal vector into a human (Institute of Medicine, 2011). It is also the most common disease that is carried by a vector in North America (Rim & Eppes, 2007). In the Eastern United States, *Ixodes scapularis*, known commonly as the black-legged or deer tick, is the main transmitter of the disease (Bacon, Kugeler, & Mead, 2008). However, a bite from a deer tick does not guarantee that a person or animal will contract Lyme disease, as the tick must be infected with bacterial organisms known as *Borrelia burgdorferi* in order to pass on the disease (Bacon et al. 2008). These spirochetes are the causative agent of Lyme disease and are commonly found in the white-footed mouse (*Peromyscus leucopus*), "the major reservoir of the bacteria" (Rim & Eppes, 2007). This mouse is a preferred host for young deer ticks and therefore passes some of its supply of bacteria to each tick that feeds on an infected mouse. Lyme disease is most commonly found in the eastern states along with some states in the upper midwest area of North America (Rim & Eppes, 2007).

The first characteristic indication that a person has been infected with the spirochetes that cause Lyme disease is the presence of a bull's-eye shaped rash around the area of the tick bite (Rim & Eppes, 2007). This rash is known as *erythema migrans* and appears after the tick has been attached for approximately 24 hours (Rim & Eppes, 2007). The disease-bearing spirochetes can only penetrate the dermis of the host after this 24-hour period, so the sooner a tick is removed, the greater chance one has of not contracting Lyme disease (Rim & Eppes, 2007). After the rash, patients may then experience cardiac and neurological problems, joint and muscle pain, headache, and fatigue (Institute of Medicine, 2011). Lyme can cause an abnormally slow heart beat which if severe enough can be fatal (Rim & Eppes, 2007). It may also cause cranial neuropathy, a condition most commonly found in the form of Bell's palsy (Rim & Eppes, 2007). Also, if left untreated or if treatment is delayed, Lyme disease often causes acrodermatitis and chronic arthritis, a condition that can be debilitating to patients as the joints become swollen and difficult to move (Institute of Medicine, 2011). This arthritis is most common during winter months and usually affects the knees (Rim & Eppes, 2007).

Ehrlichiosis. Ehrlichiosis is another tick borne disease that, although less common than Lyme disease, is becoming more widespread and is often found infecting both humans and animals (Institute of Medicine, 2011). Formally known as human monocytic ehrlichiosis, it is caused by rickettsia bacteria known as *Ehrlichia chaffeensis* which attack monocytes in the blood and the macrophages that are found in body tissues (Institute of Medicine, 2011; Rim & Eppes, 2007). States located in the southeastern and south central areas of the United States have the highest numbers of reported cases of ehrlichiosis, in part because of their high populations of lone star ticks (Rim & Eppes, 2007). The lone star tick, or *Amblyomma americanum,* is the most common vector for this disease, but in some cases the disease is transmitted via the American dog tick (Rim & Eppes, 2007; Schutz, 2006). Also, the white-tailed deer is known as the main reservoir for *Ehrlichia chaffeensis* and frequently contains the bacteria (Rim & Eppes, 2007).

Clinical symptoms of ehrlichiosis usually appear between 5 to 10 days after the patient was bitten (Rim & Eppes, 2007). “Fever, chills, headache, myalgias, and malaise” are all early indicators that the infection has begun (Rim & Eppes, 2007). Later symptoms include “nausea, vomiting, anorexia, and weight loss” with a rash developing in some adults, while found more frequently in children (Rim & Eppes, 2007). This rash can be found anywhere on the trunk of the body or on the arms and legs, which helps distinguish the disease from Rocky Mountain spotted fever (Schutz, 2006). More serious neurological conditions can occur along with seizures, coma, renal failure, respiratory failure, and congestive heart failure (Rim & Eppes, 2007). Death can occur from these serious complications and the risk of death is increased in those patients with compromised immune systems (Institute of Medicine, 2011). Most cases of ehrlichiosis emerge in the spring and summer months when the ticks are the most active (Schutz, 2006). In Lynchburg, hunting dogs often show high levels of antibodies to *Ehrlichia* when brought into veterinary facilities, suggesting that ticks in the area do carry the disease agent (D. McGuirt, personal communication, August, 2015).

Rocky Mountain spotted fever. Rocky Mountain spotted fever, or RMSF, is considered to be the most common rickettsial disease in the country and is also the most serious (Lin & Decker, 2012). This potentially deadly disease is caused by *Rickettsia rickettsii,* a bacterium that attacks the “endothelial cells that line the small vessels of all major tissues and organs," leading to vasculitis, or inflammation of the blood vessels (Rim & Eppes, 2007). This infection can ultimately damage the blood vessels (Institute of Medicine, 2011). *R. rickettsii* are gram-negative coccobacilli bacteria that actually penetrate the cells and live within them, causing the disease's symptoms (Lin & Decker, 2012). They reproduce inside the epithelial cells and spread from cell to cell, an action which causes the characteristic rash of this disease (Lin & Decker, 2012). This tick-borne disease is most common in the southeastern portion of the country as well as in the more southern states, where the ticks that serve as a vector for the disease are abundantly found (Rim & Eppes, 2007). The disease initially originated from the area of the Rocky Mountains in the late 1800s, thus giving the disease its name (Lin & Decker, 2012). In the eastern and southern states, the American dog tick (*Dermacentor variabilis*) is known as the main vector and transmits the disease (Rim & Eppes, 2007). Moreover, the organism that serves as the reservoir for RMSF is actually the tick itself, whereas mammals like mice and deer are the reservoirs for Lyme disease and Ehrlichiosis (Rim & Eppes, 2007).

However, only less than 0.1% of American dog ticks actually carry the *R. rickettsii* that cause RMSF (Lin & Decker, 2012). Even if they do carry the bacteria, the ticks must be attached to a host for six to ten hours before they transmit *R. rickettsii* through their saliva (Lin & Decker, 2012). Therefore, the earlier ticks are found and removed, the less chance one has of contracting the disease. However, the tick must be carefully removed from the skin. If the tick is crushed or squeezed during removal, the pathogens may be released into the host through the body fluids even if it has not been feeding the required amount of time for transmission (Lin & Decker, 2012).

The symptoms of RMSF usually appear 2 to 14 days after receiving a tick bite (Rim & Eppes, 2007). A patient may feel general symptoms such as severe abdominal pain, “fever, myalgias, malaise, severe headache, anorexia, nausea, vomiting, and diarrhea,” which could be symptoms of any number of diseases (Rim & Eppes, 2007; Lin & Decker, 2012). The first symptom in almost all patients is usually a fever above 102°F (Lin & Decker, 2012). However, the characteristic rash that appears several days after the initial symptoms begin is the determining symptom of RMSF (Rim & Eppes, 2007). This rash begins on the outer extremities of the body, specifically the wrists and ankles, and then progresses towards the central trunk of the body (Rim & Eppes, 2007). More severe cases of RMSF occur when the disease goes untreated for some time, resulting in severe neurological issues such as seizures, deafness, focal neurologic deficits, and discomfort to light exposure (Rim & Eppes, 2007). Patients who do not receive treatment may also experience kidney failure, pulmonary edema, and gangrene of the extremities, leading to loss of limbs (Rim & Eppes, 2007; Lin & Decker, 2012). This tick-borne disease can cause death, most frequently in young children and older adults, but even if a patient survives, he may be left with permanent hearing loss, nerve problems, gangrene, and incontinence (Rim & Eppes, 2007). Chances of surviving this illness and reducing the negative and potentially permanent side effects increase when the disease is detected and treated while still in the early stages (Lin & Decker, 2012).

Common Collection Methods

To determine the presence of these diseases, ticks must first be collected to be tested. There are several different methods used to capture ticks that are popular for surveying or collecting ticks. Each method is beneficial for different cases, depending on what species of tick one is trying to collect and the terrain of the area the tick collection is taking place. Certain methods are more successful for different species of ticks, as will be further discussed.

Tick flagging. This method is perhaps the most commonly used method for the collection of ticks because of its accuracy in showing the number and actual location of the ticks in the area being tested, and also in demonstrating how a person may obtain ticks when one walks through the area. This method involves the use of a large rectangular piece of cloth, usually white fleece or flannel, stapled on one end to a wooden rod (Petersen et al. 2015). Rope is attached to the wooden rod to create a handle so that a person wanting to collect ticks can pull the cloth behind him (Petersen et al. 2015). This cloth is then dragged along the ground and pulled over vegetation to collect any ticks that are in those locations (Falco & Fish, 1992). The ground and lower vegetation are usually where ticks are found because that is where they are most likely to encounter a host. Because of their nature, ticks will grab onto anything that passes by, especially soft cloth, when they are seeking their next host. The area of collection may be divided into quadrants to collect the maximum number of ticks, or the dragging may be purely random to simulate the path a person may take (Falco & Fish, 1992). Also, the drag is checked regularly to collect any ticks that have grabbed on to the cloth and are very visible against the light background (Falco & Fish, 1992). Kollars et al. (2000) found that lone star ticks of all stages were easily captured using this method and collected several thousand of them. Flagging also was very successful in capturing both immature and adult deer ticks (Ginsberg & Ewing, 1989). This method was attempted early on in this study but was soon abandoned because of the large amount of underbrush in the study area that kept entangling the flags.

CO_2 traps. Ticks are attracted to carbon dioxide and use the gas given off from animals to locate potential hosts. This may be why CO_2 traps are so successful in attracting and capturing ticks. These traps use dry ice placed in a cooler to attract the ticks, which are caught in tape surrounding the cooler when they come to investigate. These traps have been found to capture several life stages of the deer tick (Falco & Fish, 1992) but are more likely to capture the lone star tick (Petersen et al. 2015). The lone star tick is very active and is considered to be fast moving while aggressively hunting for hosts (Ginsberg & Ewing, 1989). In contrast, the deer tick is less likely to be able to reach the trap from farther distances because the species partakes in very small amounts of horizontal movement, whereas the lone star tick and American dog tick both travel considerably further distances (Falco & Fish, 1991). This success of this method of trapping varies from species to species, as some ticks are more attracted to carbon dioxide, such as the lone star tick (Petersen et al. 2015). Ginsberg and Ewing (1989) found in an experiment that more lone star ticks than deer ticks were captured in the trap even when the deer ticks were more plentiful in the area.

The researchers chose this method and found it to work well. To make the traps as efficient as possible, the tapes used on the traps were tested to determine which would capture the highest number of ticks that crawled onto their surfaces. There was difficulty in observing the success of the tapes in the field because the traps were left out overnight and could not be observed, so the tapes were tested in the lab. These lab experiments used artificial methods to determine the strength and efficiency of the tapes.

Wildlife trapping. In some cases it is more efficient to search for the ticks' hosts rather than the ticks themselves. Some researchers harvest ticks from deer that have been killed during hunting season and then test both the deer and the ticks for any presence of disease pathogens (Petersen et al. 2015). Others choose to directly trap smaller mammals such as mice, chipmunks, voles, and ground squirrels; animals which are known for being the "primary hosts for immature stages of ticks" (Petersen et al. 2015). The animals are put under anesthesia so that they can be handled safely and thoroughly inspected for ticks (Burg, 2001). This method of trapping enables researchers to collect ticks from several different species of live animals and the hosts can be released with no harm done to them. Also, the immature stages of ticks are more likely to be found on these small animals, and testing done on the host mammal can determine what species of wildlife have acquired the pathogens from the ticks (Petersen et al. 2015).

A variation of this method occurs when ticks are collected from both animals and people when they visit veterinary hospitals or remove a tick at home (Petersen et al. 2015). Many times, pet owners do not notice ticks on their dogs or cats; instead, they are found when veterinarians do thorough investigations of the animal for a checkup. Many times, those ticks that are found are sent in for disease testing. These ticks are very helpful in determining a human's risk for disease in that area because pets regularly frequent areas where ticks may be found, such as woods and fields (Petersen et al. 2015). Any tick on a pet is brought closer to the owner and therefore increases his or her chance of also being bitten and contracting a tick-borne disease (Petersen et al. 2015). Also, veterinarians can perform tests while the dog is still in the office to determine if the dog has been infected by a tick. This test is called a four way snap test, which tests the dog's blood to determine if it is infected with heartworms, Lyme disease, ehrlichiosis, or anaplasmosis (Hookstead, 2012).

Methods and Materials

Study Location

This study was conducted on Candlers Mountain in Lynchburg, Virginia near a popular hiking trail. This area was chosen to more accurately assess the risk of human contact with diseased ticks, and also because of the previous research mentioned before that suggests ticks congregate alongside trails (McNemee et al. 2003). The traps were placed in a ravine about 10 to 20 meters from the hiking trail found in the closed-canopy deciduous forest. The ground in the area was covered with a thick layer of leaves and had a relatively dense population of small shrubs and trees among the larger deciduous trees. Traps were set out and ticks were collected starting in March of 2015 and continuing until June of 2015.

Method of Collection

Initially, the flagging method was used to attempt the capture of ticks. The short end of a 1x1 meter sheet of white flannel cloth was stapled to a wooden rod which had a three foot long rope attached to each side, forming a handle. The flag was then pulled over the leaves and underbrush in hopes that ticks would grab on to the soft fabric. However, the flag became entangled in the bushes because of the dense nature of the underbrush, rendering it very difficult to catch ticks. It was then decided that a carbon dioxide trap would be more successful in that area.

The carbon dioxide trap was created using a small Styrofoam cooler roughly 0.5 meters long, 0.3 meters wide, and 0.3 meters tall. The cooler was taped onto the center of a plastic lid of a two foot long plastic storage container, which served as the base to hold the cooler and to hold the tape that would catch the ticks. A wire cage was constructed inside the cooler to contain the dry ice and to prevent the ice from touching the walls of the cooler, which would cause the exhaust holes to become blocked. Two holes approximately one centimeter in diameter were punched through the walls near the bottom of each side wall of the cooler to enable the carbon dioxide to escape the cooler. Small wisps of smoke could be seen escaping the holes. Also, a layer of tape was placed with the sticky side up around the perimeter of the storage lid so that ticks would have to crawl across the tape to reach the attractive dry ice and in the process would become stuck to the tape and captured. Two traps were baited and set out together each time and were located approximately 3 to 6 meters from each other.

Several different tapes were used on the traps, including double-sided carpet tape, Gorilla duct tape, and clear packaging tape. Whenever the tape had lost its stickiness or had become contaminated with leaves and other insects, it was removed and replaced with new tape. All traps were put out in the morning or early afternoon, left out overnight, and checked the next day, giving the ticks around a full day of access to the traps. Usually a small amount of dry ice remained in the traps the next day. When ticks were found to be stuck in the tape, they were removed from the tape with metal forceps and were placed in sealable plastic bags that were labeled with the date of capture and number of ticks captured.

Once bagged, the ticks were brought back to the lab and each was identified as to gender, life-stage, and species using a microscope and a dichotomous key. Once identified, the ticks were placed in a freezer at a temperature of -80°F (-62°C) so that they would be preserved for further testing.

Determination of Tape Strength

Further testing in the lab was carried out to determine which tape or sticky surface would be the most efficient at capturing ticks. Since the traps were left unattended for many hours, it was not known whether or not the tape was actually capturing all of the ticks. It is entirely possible that some ticks may have been able to escape from certain tapes if they were not sticky enough. It is necessary to have a tape that will quickly capture and hold the ticks for an indefinite period of time until they could be collected because without test subjects the testing for disease could not be done. To determine which tape would be the strongest and stickiest in order to catch the largest amount of ticks possible, several different kinds of tape were tested in the lab and compared with each other. An instrument called a force transducer was used in the experiment to measure the amount of force needed to pull an object that resembled a tick leg off the tape. This instrument uses an attached force plate to measure the amount of electricity generated by the force asserted by an object, giving an indication of how strong or sticky the tape was. The force plate is a flat metal knife-like object that bends when force is applied and generates electricity. It then relays that reading electronically to the force transducer, which creates a visual of the magnitude of force in the form of a pulse wave.

The force transducer was calibrated so that the amount of force needed to remove an object from the tape was equated as grams of pressure. A 0.54 gram dissecting pin that was weighed on a Scout Pro scale was used to calibrate the force transducer, and was placed on top of the force plate so that the system was allowed to calibrate until the system read zero grams. This created a weighing scale so that the grams of pressure produced by the tape on the artificial tick leg could be determined. A thin wire was used to simulate a tick leg because after examination of the end of the wire under a dissecting microscope, it was discovered that the tip had a hook that was comparable to the hooks on a tick leg (Figure 2). This shared physical characteristic meant that the wire and the tick leg should react similarly when placed into contact with the tape. The wire was placed parallel to the dorsal surface of the force plate and was secured by placing a thin piece of lab tape perpendicularly over it. The end of the wire overhung the end of the force plate by approximately ¾ of an inch, and this overhanging piece was then bent so that it was perpendicular to the plate.

Figure 2. The tip of the piece of wire from a twist tie used in the experiment as seen under a dissecting microscope. The tip has a hook that is similar to the hooks on a tick's leg.

The force plate and wire were then slowly lowered down until the tip of the wire lightly touched the tape, and then was pulled back off the tape. Several different tapes were tested using this method, including Pic Window Fly Traps tape, strings of Pic Fly Ribbon tape, yellow lab tape, duct tape, and clear packing tape. The wire was touched to each tape 10 times to gather an accurate representation of the force needed to pull the wire off the tapes. Since the force plate bends when force is applied, the moment when the wire stuck to the tape while it was being pulled off caused the force plate to be pulled down slightly. The force transducer recorded the amount of pressure in grams needed to pull the wire off of the tape, giving an indication of the stickiness of each kind of tape.

Results and Discussion

Tick Collection

Carbon dioxide traps were set out in the designated collection spot every week or every other week during the months of March, April, May, and June of 2015. Some days the CO_2 traps did not capture any ticks. On March 13, one male lone star tick was captured in the tape. On March 23, a total of three ticks were caught. A male and a female lone star tick were captured along with a female deer tick. All ticks in this information are adults unless otherwise specified. On April 5, a total of 13 ticks were collected consisting of seven female lone star ticks, five male lone star ticks, and 1 deer tick. Next, three male lone star ticks were captured along with 1 female lone star tick on April 22. This day was interesting because when the traps were set out, the ticks could be seen immediately climbing out of the leaves surrounding the traps and crawling towards the CO_2. May 14 had the biggest capture of ticks, with 35 male and female lone star ticks captured along with 1 deer tick. Five nymphs were also collected on this day, but their species and gender were not identified.

Tape Strength

After doing 10 trials of touching the wire to the Window Fly Traps tape, the average grams of force required to pull the wire off of the tape was 4.34 grams and the average force asserted as the wire was touched to the tape was -16.97 grams (Figure 3). This number is negative because the force plate was pushed slightly upwards as the wire was touched to the tape, instead of being pulled downwards as the wire sticks to the tape.

Also, the average grams of force that is required to pull the wire off of the tape can be considered the “stickiness” of the tape.

Figure 3. The results of the grams of force for the Window Fly Trap strips as recorded by the force transducer. The flat horizontal line is the baseline of zero, where no force was recorded. The negative spike is the force asserted as the wire was pressed onto the tape, and the positive spike is the amount of force needed to pull the wire off of the tape. This peak is a measure of how sticky the tape is. This explanation of the graph is applicable to each of the following figures.

Also, after several trials, it was determined that the fly ribbon tape residue had an average stickiness or force with which it holds the wire on its surface of 3.99 grams (Figure 4). The average force used to push the wire onto the residue was -18.12 grams. For the yellow lab tape, the average stickiness was 10.85 grams, and the average force asserted when the wire touched the tape was -20.88 grams (Figure 5). The average force needed to pull the wire off of the duct tape was 8.26 grams, and the downward force of touching the tape was -21.49 grams (Figure 6). Finally, the last tape tested was the clear packing tape, which was calculated to have an average stickiness of 2.05 grams, and an average asserted force of -18.54 grams (Figure 7).

Figure 4. The results of the grams of force for the Fly strip residue as recorded by the force transducer.

Figure 5. The results of the grams of force for the yellow lab tape as recorded by the force transducer.

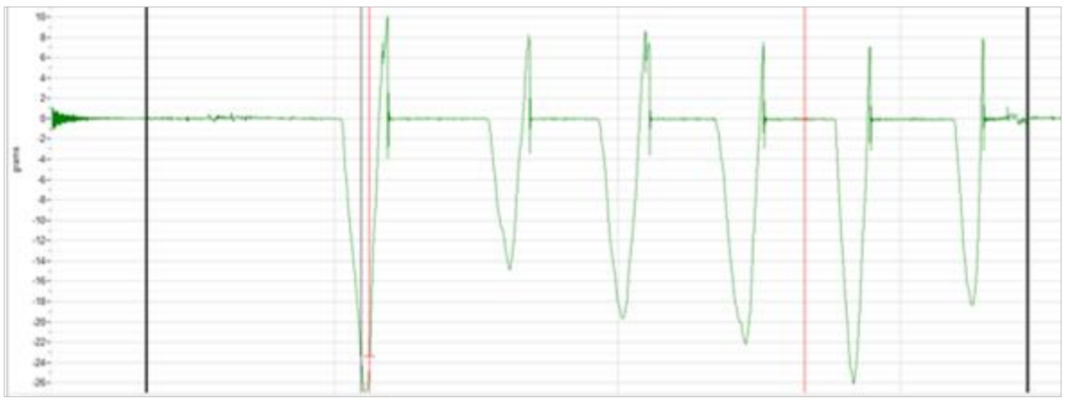

Figure 6. The results of the grams of force for the duct tape as recorded by the force transducer.

Figure 7. The results of the grams of force for the clear packing tape as recorded by the force transducer.

From these results, it can be determined that the average asserted force of the wire being pressed onto the tape is relatively similar for each of the tapes. Therefore, the average stickiness that was calculated for each tape is comparable because the wire was pushed into the tape with around the same force for each tape. From this method of testing the effectiveness of the tapes, the yellow lab tape was the stickiest and required the most force to pull the wire off the tape. Even from observations it was seen that this tape held strongly to the wire tip, because the resistance could be felt when the tip was pulled from the tape. However, the least sticky tape was the clear packing tape. The wire barely stuck at all when it was touched to the tape and some readings on the force transducer were very close to zero grams of force (Figure 8).

Conclusions

Overall, it would appear that the carbon dioxide trap is an effective method for catching lone star ticks in this area, but flagging should also be considered to collect different species of ticks such as deer ticks and American dog ticks that are not as attracted to CO_2. An area nearby to the CO_2 trap should be chosen that is not so overgrown that a flag cannot be used, therefore avoiding the difficulties with entanglement as experienced in this study. Flagging this area will hopefully collect those other species of ticks that are in the same area as the CO_2 traps, allowing the disease risk of all tick species in the area to be determined. Also, duct tape and yellow lab tape were determined to be the more efficient tapes to catch and hold ticks for an extended period of time until they can be collected, and should therefore be used to create the best design of a CO_2 trap. Obviously, this was determined using experimental testing of artificial items and therefore is not as accurate as testing live ticks, but it gives a good indication of what tapes may best catch ticks. When this knowledge of the habitats, preferred hosts, life cycles, and periods of activity of ticks is combined with the different collection methods and materials, the capture of ticks to be tested for disease will be much more successful in the United States and around the world. The next phase of this research will be to test these ticks for pathogens that have been collected.

Once there is an understanding of the disease threats in this area, students and locals in Lynchburg will be able to plan how to protect both themselves and their pets when entering areas such as Candlers Mountain where ticks and disease may be present. Also, with the information presented on the different tick-borne diseases and their symptoms, people all around the world may be able to recognize if he has been infected with a tick-borne disease and will be able to seek potentially life-saving treatment. If a disease is in fact proven to be found on this mountain, people will take the threat more seriously than if there was only a faint chance of encountering a diseased tick. It is very possible that these ticks that have been collected for this thesis may contain Lyme disease, Ehrlichiosis, or Rocky Mountain spotted fever. Lynchburg, Virginia is located within the area where each of these diseases is the most commonly found, and it is very important that the public is made aware of the potential threats that await them in the outdoors. Knowing that each tick may possibly be carrying a disease may encourage people to take preventative measures when in the outdoors such as wearing bug spray, the appropriate clothing, and checking for ticks after leaving tick habitat.

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