Not Man's Best Friend: How *Ixodes scapularis* Negatively Affects the Canine Population in Roanoke, Virginia

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INTRODUCTION

Over 850 tick species are found around the world, but only about 90 of them are present in the United States (1). Even with an abundance of species of ticks, only a few of them transmit diseases. Ticks are "blood-feeding arthropods" and are considered vectors for various disease-causing pathogens (2). In southwestern Virginia, there are five ticks that are known to inhabit the area: *Ixodes scapularis, Amblyomma americanum, Dermacentor variabilis, Rhipicephalus sanguineus,* and *Amblyomma maculatum* (3). Each of these species has the potential to spread different diseases. For instance, *D. variabilis* could transmit pathogens that cause Rocky Mountain spotted fever, ehrlichiosis, and tularemia (4). Other ticks can carry the same pathogens as well, such as *A. americanum* and *I. scapularis* carrying the causative agents of ehrlichiosis and tularemia (4). It is important to note that combined infections are possible, meaning an individual can be infected with more than one pathogen at the same time (4). Not only is tick species an important determinant of disease transmission but so is the life stage of the tick.

Three-host ticks, or ticks that attach to three different hosts throughout their lives, go through four life stages: egg, larva, nymph, and adult (5). The life cycle begins when an engorged female detaches from a host to lay thousands of eggs in soft, warm locations, like leaf litter (6,7). Soon the eggs hatch into six-legged larvae at which point they must attach to a host, typically a small host such as a chipmunk or shrew, for a blood meal (6,8). It is important to note that only females blood-feed (8). Following their attachment to a host and their blood meal, the tick will detach and molt into the nymph phase (6). Nymphs then attach to a larger host, such as a deer or dog, for their next blood meal, detach, and molt into an adult (6,8). As an adult, the tick feeds on a new host until it becomes engorged and detaches to lay the next generation of eggs, dying after doing so (6). It will often take two to three years for ticks to progress through the life stages with many never reaching adulthood because they cannot find a host for their blood meal, dying as a consequence (6,9). Hosts are essential to the survival of ticks as they provide the vector with its meal. In order to seek out a host, ticks will quest, a process in which ticks grasp leaves or grass with their third and fourth pair of legs while outstretching with their first pair (9,10). When a host brushes up against the tick's outstretched arms, the tick will attach and climb on to the host (9).

Of particular interest is three-host *I. scapularis,* more commonly known as the deer tick because of its common attachment to white-tailed deer. It is during the nymphal and adult stage that *I. scapularis* can transmit disease (8). This species of tick is primarily found in the northeastern region of the country as well as Wisconsin and Minnesota. Recently, however, the area in which *I. scapularis* inhabits has been increasing (8). As seen in Figure 1, there has been an increase in both reported and established cases from 1996 to 2015, likely due to the increase in suitable habitats (8).

The climate plays an important role in the dispersal of *I. scapularis*. This species is seasonally active, peaking during months of warmer temperatures and higher humidities (10). If the temperature falls below a certain threshold (45°F), the tick will either die or fail to quest (11). Additionally, warmer weather is correlated with both faster development and lower mortality rates (11,12). The connection between climate and tick dispersal is clear, leading to many concerns regarding climate change. This warmer temperature favors the tick life cycle and poses a problem: expansion of ticks. As the minimum temperatures rise in colder regions, ticks are beginning to infiltrate new areas. For instance, the warm temperatures are beginning to make areas of Canada suitable for ticks (11). Similarly, higher elevations, such as the Appalachian Mountains, can better sustain ticks as they continue to warm as well (12).

Another factor that is contributing to the spread of ticks is the spread of mammals, as host movement is essential in the movement of the vectors (11). Of particular interest is the white-tailed deer, the most common host for deer ticks (13,14). One study found that in areas with significant populations of both deer and ticks, reducing the deer population would subsequently reduce the tick population as well, limiting the number of cases of Lyme disease (13). This finding concurs with that of Wilson's study in 1990 that found a correlation between the distribution of white-tailed deer and ticks (15).

Of particular concern for this paper is the emergence and spread of ticks in southwestern Virginia. It was initially thought that *I. scapularis* was absent from southwestern Virginia as it appeared to be endemic to only the northeastern region of the United States (16,17). Slowly, however, northern Virginia began reporting an increase in Lyme disease cases and soon cases spread to the southern and western regions of the state (17). In 2013, Virginia had an incidence rate of 11.2 cases per 10,000 people with a 33% prevalence of Lyme disease in the southwestern corner of the state (16,18). Looking specifically at this southwestern region, the cases of Lyme disease have been on the rise. There has been a significant and steady increase in cases in Roanoke, Blacksburg, and Lynchburg since the early 2000s, as well as an increase in areas of higher elevations (18,19).

This spread of *I. scapularis* is of concern because this species has the potential to transmit the causative agent of Lyme disease, *Borrelia burgdorferi*. This pathogen is a spirochete, or a spiral shaped bacterium, that is able to live in the *I. scapularis* species (5). After *I. scapularis* has a blood meal from an infected host, the pathogen begins to reproduce in the midgut region, with a doubling time of about four hours (20). After about 48-72 hours the *B. burgdorferi* travels to the salivary glands of the tick (20). The pathogen can then transfer from the salivary glands of the tick to the host during a blood meal. In contrast to many other pathogens, *B. burgdorferi* does not transmit transovarially, or from the mother to her offspring, and instead must be transmitted via blood (21,22).

Lyme disease is the most common tick-borne disease in the United States and is commonly identified and addressed in humans. An infection of Lyme disease typically takes place in three stages, the first of which starts about a week after being bitten and causes the individual to experience flu-like symptoms, including headache, nausea, and fatigue (4). About 75% of infected individuals, report having erythema migrans, a bullseye rash that often takes form around the site of the bite (4). In the second stage, the infection may progress into higher fevers and problems with the central nervous system (4). Finally, stage three is characterized by symptoms such as chronic arthritis, keratitis, and dermatitis (4).

Interestingly, dogs do not follow the same progression of the disease. In fact, 95% of dogs are asymptomatic for the disease in the short term, compared to the only 10% in humans (23). Many of the symptomatic dogs are puppies who experience arthritic-like symptoms, but not until two to five months after the initial bite (23). Because of the lack of early symptoms associated with Lyme disease in dogs, it is difficult to diagnose and treat. Veterinarian clinics often check dogs for tick-borne diseases during their check-ups and, should a dog test positive, there are treatments to get rid of the disease. Similar to human treatments, Lyme disease in dogs can be treated with doxycycline, amoxicillin, or cefovecin (24). Despite immediate benefits, the long term effectiveness is unclear and must have further research to better understand (24). The problem with treatment, however, is actually identifying the disease. Dogs who are not tested at the veterinarian clinic might go unnoticed and untreated due to their lack of symptoms. By not identifying the disease early on, the pathogen could persist in the animal for years undetected resulting in a slow progression of adverse effects related to the disease (5). It is possible for infected dogs to ultimately exhibit joint disorders as well as fever, fatigue, and loss of appetite (25). In extreme cases, *B. burgdorferi* may play a role in renal failure (26-30). It is important, then, for veterinarians and dog owners to prevent Lyme disease in their dogs.

The intent of this study is to evaluate the abundance and prevalence of *I. scapularis* and *B. burgdorferi* in southwestern Virginia by sampling locations that pet owners might take their dogs. Lyme disease is debilitating, so understanding the prevalence of the tick species and this pathogen is essential in preventing transmission both to humans and pets. It is expected that *I. scapularis* will be the second most abundant tick, following *A. americanum*, but that it will also have an increased abundance since previous years because of the increase in suitable habitat. Similarly, because the abundance of *I. scapularis* would have increased, it is predicted that many *I. scapularis* will test positive for *B. burgdorferi*.

METHODS

Collection Sites. Ticks were collected at Carvins Cove, Mill Mountain, and the Salem Rotary Dog Park. Both Carvins Cove and Mill Mountain had multiple trails that were sampled (Table 1). Each location was sampled once a week between April 29, 2020 and June 30, 2020. Plots were set up at each location and the edges of the trails were sampled as well. Plot sizes ranged from 0.01 hectares (1358 ft^2) to 0.05 hectares (5,583 ft^2). The number of dogs at each site was also observed.

Collection Protocol. Ticks were collected using the flagging technique. Large squares of white felt were attached to a wooden dowel and weighted down with fishing weights. The white felt was swept over the vegetation or leaf litter and surveyed for ticks every 20-30 seconds. Ticks that hung to the felt were transferred to a collection tube containing ethanol to be identified later.

Tick Identification. Ticks were identified morphologically using a dissecting scope. Features that aided in identification included festoons, capitulum, and scutum (Figure 2). The dorsal sides of the ticks were essential in identifications.

DNA Extraction. DNA was extracted from all *I. scapularis* ticks using the ThermoScientific GeneJET Genomic DNA Purification Kit. The directions provided in the kit were followed.

PCR. PCRs were run on all extracted DNA to detect the *B. burgdorferi* fla gene FL6/7. The sequences of the genes are outlined here:

BorFL6F: TTC AGG GTC TCA AGC GTC TTG GAC T

BorFL7R: GCA TTT TCA ATT TTA GCA AGT GAT G

A super stock mix was created using Hot Start MasterMix, PCR grade water, and both the forward and reverse primers. 15µl of the super stock mix was added to 5µl of DNA from *I. scapularis* samples. The samples were placed in the PCR machine and the Bor FL6/7 program was run. The program began with a one minute denaturation at 95°C (31). This was followed by 40 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 55°C, and one minute extension at 72°C (31). These cycles were concluded with a single three minute extension at 72°C (31).

Gel Electrophoresis. The PCR samples were placed in a gel box containing a 1.5% gel made with 1xTAE. The gel was run at 100V for about 45 minutes. 4µl of 10,000 x sybersafe was added to the gel which sat for another 25 minutes covered by tin foil. At the end of these 25 minutes, an ultraviolet light was used to see the bands and determine if *B. burgdorferi* was present in the samples. Positive samples were at 296 bp (32).

RESULTS

A total of 288 ticks were collected across all collection sites during nine weeks in the summer. About 78% (224/288) of them were *A. americanum,* while only 19% (54/288) were *I. scapularis* (Figure 3). Of the 54 *I. scapularis*, only one of them was an adult and it was female (Table 2). The majority (46/54) of these were collected from sites at Mill Mountain with the remaining 8 found at Carvins Cove (Figure 4). No ticks were collected at Salem Rotary Dog Park. Even when accounting for plot size, Mill Mountain had the most *I. scapularis* per hectare (Figure 5 and Table 3). All collected *I. scapularis* were tested for *B. burgdorferi* but only the adult female tested positive (Figure 6 and Table 4).

The greatest volume of ticks collected appeared during the sixth week of collection (Figure 7). This pattern could be due to weather factors and linear regressions were run to determine if so. Temperature and the number of ticks collected had a statistically significant relationship ($p=0.022$) (Figure 8). Precipitation and humidity were measured as well, although they were not statistically significant ($p=0.436$, $p=0.329$) (Figures 9 and 10). Finally, the relationship between wind speeds and collected *I. scapularis* was evaluated but it was not statistically significant ($p=0.412$) (Figure 11).

DISCUSSION

The study aimed to identify the abundance and prevalence of *I. scapularis* and the causative agent of Lyme disease, *B. burgdorferi*. The expansion of tick range led to a prediction that *I. scapularis* abundance would have increased since the previous years. However, it was suspected that it would not outnumber the *A. americanum* population. Because of the proposed increase in *I. scapularis* abundance, it was hypothesized that *B. burgdorferi* positive results would be present in high numbers. This information is essential in aiding the canine population.

***Ixodes scapularis* Abundance.** The findings of this study support the hypothesis that *I. scapularis* would represent the second most abundant tick species in the Roanoke Valley, following *A. americanum* (Figure 3). The large abundance of *A. americanum* could be due to the fact that this species is known as a “hunter tick.” Attracted by host carbon dioxide odors, *A. americanum* can crawl towards their host, increasing their range and enhancing their chances of attachment (33,34). During the collection period, trails were followed out and back, suggesting that *A. americanum* sensed the carbon dioxide odors and moved towards the trail after the initial walk by. On the return trip, this species would have been waiting along the trail for its hosts to return. On the other hand, *I. scapularis* solely quest, remaining relatively stationary during their search for a potential host (9,10). Without actively seeking a host, *I. scapularis* are forced to wait for a host to come to them, likely resulting in the lower collected numbers of this species.

According to the national CDC data, the number of tick bites in 2020 that resulted in emergency room visits was nearly half of what it was in previous years (35). Although specific data regarding the number of *I. scapularis* bites are not included, it suggests that as the overall tick bites declined, so did tick bites from *I. scapularis*. This might be a result of a decreased *I. scapularis* density or reduced reporting of tick bites.

Weather Patterns. Various weather conditions were measured during the collection period but only the relationship between temperature and the number of collected ticks was statistically significant (Figure 8). *I. scapularis* prefer warmer habitats as it facilitates faster reproduction and increases biting rates (11,12). These warmer temperatures are also what allows the southern Appalachian Mountains, including Roanoke, Virginia, to be habitable by this species of tick (12). If temperatures are below their threshold (45°F) ticks will die out, highlighting how warmer temperatures elicit lower mortality rates (11). It is likely that as the temperature in the Roanoke Valley became warmer, *I. scapularis* were not limited to staying under leaf litter to maintain warmth. They were able to leave the leaf litter to quest in the warmer weather, explaining the increase in collected ticks during the warmer periods.

In addition to temperature, humidity has been noted to play a role in tick abundance and their behaviors. It has been suggested that in less humid areas *I. scapularis* mortality increases, however, not all studies have concluded this (36). Humidity still appears to be important as *I. scapularis* retreat to ground cover which provides humidity and allows them to avoid desiccation, or drying out (33). Extremely sensitive to desiccation, humidity might still be an important factor in the abundance of *I. scapularis*, although this study did not find a statistically significant relationship (Figure 10). Humidity is simply the amount of water vapor in the air, so this factor could be connected to precipitation. Similarly, precipitation did not produce a significant relationship with the abundance of ticks, concurring with other studies (Figure 9)(37).

Wind patterns did not appear to play a role in tick abundance (Figure 11). It is likely that the wind speeds were low enough (below 10 mph) to not blow questing ticks off of their site. Had speeds been higher, it is speculated that *I. scapularis* abundance would be lower due to the inability to hold on while questing.

Ticks have such a short life cycle and are subject to adverse weather patterns during their lives. *I. scapularis* also rely heavily on host life and patterns in order to develop into their next life stage and various environmental factors can impact the lives of potential hosts as well. It might just be that monitoring weather is too simple to predict tick patterns. It is more likely that a more complex interaction, including various abiotic and biotic factors, influences *I. scapularis* populations. Additionally, Schulze et al. found that over the years the average temperature and precipitation each month did not alter significantly, suggesting that weather changes might not be great enough to significantly influence a tick population.

Vegetation. Of the locations where the ticks were collected, there was a stark contrast in the surrounding vegetation. The Salem Rotary Dog Park was a well-kept grassy area that housed few trees. The trails at Mill Mountain were primarily surrounded by a deciduous forest with leaf litter covering the floor. The locations at Carvins Cove had a less dense deciduous forest and less leaf litter on the ground. The majority of *I. scapularis* ticks were collected at the sites on Mill Mountain, where the deciduous trees were most prominent. Even when accounting for plot sizes, *I. scapularis* density was consistently greater at Mill Mountain locations (Figure 5 and Table 3). Commonly found in brushy, deciduous forests, *I. scapularis* find an ideal habitat to survive in (33,36). The leaf litter on the ground provides a place of warmth and high humidity levels that are sufficient enough to prevent them from desiccating (33). The Salem Rotary Dog Park completely lacked this environment and locations at Carvins Cove were more open with less leaf litter, suggesting that the lack of ticks found at these sites could be due to the lack of sufficient surrounding vegetation. Similarly, the vast deciduous landscape at Mill Mountain might better maintain *I. scapularis* populations, yielding greater collection numbers.

Also interesting to note is how vegetation impacts the deer population. For deer, deciduous forests act as a food source, place to rest, and an area to raise their young (38). Dense and variable deciduous forests, such as those found at Mill Mountain, are ideal locations for deer to inhabit. The low lying green vegetation that is found at Mill Mountain can act as a food source for these herbivores (39). Additionally, deer can find refuge among deciduous trees and shrubs, acting as protection from potential dangers, such as humans, other animals, and even extreme weather (38). Mill Mountain had a greater diversity of vegetation than Carvins Cove and Salem Rotary Dog Park, suggesting that deer were more likely to inhabit locations near Mill Mountain. Because the abundance of deer is correlated to the abundance of *I. scapularis* and Mill Mountain sites housed the majority of ticks, it is likely that this region is commonly inhabited by deer as well (13-15). It appears, then, that the habitat at Mill Mountain was most sufficient to harbor both deer and tick populations, likely explaining the great abundance of collected *I. scapularis* at this location.

***Borrelia burgdorferi* Abundance.** The CDC reports a growing trend in the number of Lyme disease cases amongst humans since 2009. The reported number of yearly cases waivers around 900 to 1,000 cases throughout the entire state of Virginia (40). In 2018, the incidence rate of Lyme disease in the state was 8.7 cases per 100,000 persons, a significant decrease from 2017 which reported an incidence of 12.3 cases per 100,000 persons (40). The data from the current study reported only one tick as positive for the pathogen (<2%), suggesting a potential decline in the prevalence of *B. burgdorferi*. However, it is challenging to tell whether this was a coincidence or a pattern we might see in the future.

The few positive *B. burgdorferi* results could be due to the fact that *Borrelia* sp. are not transmitted transovarially in *Ixodes* sp. (21,22). The offspring are unable to acquire the pathogen from their mother's ovaries and must rather come in contact with it via a blood meal from an infected host. The collection dates took place at the end of spring and beginning of summer, a time when either tick eggs are being laid or when nymphs that have molted over the winter are looking for their next host (7). It is in this early summer stage that there is an abundance of nymphs questing for a new host but few adult *I. scapularis* doing the same. Because of this, it is no surprise that only one adult female was collected and the remainder were nymphs. Similarly, because mothers cannot pass *B. burgdorferi* to their offspring, the absence of this pathogen in all of the nymphs is not surprising. At some point before they were collected, these nymphs needed to have a blood meal from an infected host in order to have any chance of harboring the pathogen. While there may not have been many positive results for the collected ticks, various mammals still act as reservoirs for the pathogen, allowing the ticks to acquire it later in life and in the summer weeks following this study period. It is hard to say how the prevalence of *B. burgdorferi* changed over the course of a few years because data from the end of the summer was not obtained. Due to the tick life cycle and their tendency to feed on hosts, it is expected that more positive samples would have been collected in the subsequent weeks, increasing the prevalence of the pathogen in *I. scapularis*.

Dog Sightings. Although a strict tally of dog sightings was not kept, all sites were examined for the presence and abundance of dogs. Each of the sites were frequented by pet owners and their dogs on at least one occasion, if not more. In general, the Salem Rotary Dog Park had more sightings than any of the other sites. However, no ticks were collected during the collection period, making this the lowest risk site. Mill Mountain and Carvins Cove were frequented less consistently than the dog park but pet owners would still walk their dogs along trails at these two sites. Dogs were often off leash or would wander off the path into the leaf litter and vegetation, likely increasing their chances of tick attachment than if they remained on the cleared path. Because of the higher density of ticks at Mill Mountain, it is deemed the highest risk of the three sites.

IMPLICATIONS

Renal Failure Associated with *B. burgdorferi*. The presence of *B. burgdorferi* in dogs has been associated with long-lasting effects if left untreated, despite the fact that dogs typically remain asymptomatic when they are first infected. Various studies have examined the relationship between *B. burgdorferi* and the onset of renal failure in canines (26-30). Certain breeds, most commonly Labrador and Golden retrievers, appear to have a predilection to succumbing to Lyme disease and developing renal failure (26,27). It is suggested that this predisposition comes from host immune systems, which tends to be weaker in these breeds (27). In one study, researchers found that all (18/18) dogs that had renal failure also tested positive for the presence of *B. burgdorferi* (27). The spirochaete can essentially “hide” from the immune system, down-regulating its outer surface proteins to avoid detection by the immune system and allow it to persist in the body (30). This could account for its persistence of the pathogen in the dog’s body if left untreated or undetected. Interestingly, however, in another study the pathogen was not identified in any of the kidney samples (28). They neglected to test blood samples, a common place the pathogen is found, but the overall study suggested that direct invasion of the kidney by the pathogen did not happen in these cases (28). It is suggested that perhaps there is a co-infecting agent that might work alongside *B. burgdorferi* in causing renal failure among canines (28).

While this relationship is not completely understood and is still being studied, some sort of connection appears to exist. Labrador and Golden retrievers are still more likely than other breeds to develop Lyme disease and are 6.4 and 4.9 times more likely to develop a renal lesion, respectively (27). Renal failure among dogs has a poor prognosis, with over half of the affected patients passing or being euthanized within just a few weeks or months after the diagnosis (26,29). With a possible connection between Lyme disease and renal failure, it is important to understand the risks associated with *I. scapularis* and the damage the tick can do to dogs.

Other Tick-Borne Diseases. Although Lyme disease is the most well-known tick-borne disease in the United States, it is not the only one that poses a threat to canines. *A. americanum*, the most abundant tick found in this study, has the ability to transmit pathogens that cause ehrlichiosis, tularemia, STARI, Heartland virus disease, and Bourbon virus disease (4,41). Additionally, *I. scapularis* can carry other disease causing agents such as those inflicting anaplasmosis, babesiosis, and Powassan virus (41). All of the aforementioned diseases can negatively affect both humans and dogs. Coinfection is also a possibility, so dogs may suffer from multiple tick-borne diseases concurrently. For example, there is a 23% chance that a dog with Lyme disease is also infected with the causative agent of babesiosis, both caused by an infection from *I. scapularis* (4). Needless to say, Lyme disease is only one of the potential diseases transmitted by ticks.

PREVENTION STRATEGIES

Maintenance. Because of the plethora of diseases that can be spread by ticks to canines, and the increasing tick populations in southwestern Virginia, it is important to take proper preventative measures (17,18). Simple precautions such as clearing leaf litter and maintaining tall grass areas in individual yards are effective enough to control tick populations and limit contact with canines (23,42). One study suggests utilizing pesticides and acaricides to kill ticks, however, resistance within the tick population might develop, reducing the effectiveness of this measure (43). Preventative measures such as these eliminate habitats that ticks prefer, increasing the barrier between ticks and civilization. It is equally important to check a dog for ticks after it has been outside, especially after coming into contact with wooded areas, such as after a hike.

Medication. Medication is also highly recommended by veterinarians, especially for those that are in areas where ticks are endemic (2,44). Many treatments are topical and reduce the likelihood of tick bites on dogs. In lab settings, using a collar, fipronil, or permethrin was effective in preventing transmission (5). This suggests that medications are effective in preventing tick-borne diseases in canines. It is equally important that pet owners adhere to the veterinarian's prescription instructions. By deviating from these instructions, pet owners are putting their dogs at risk for disease progression, antibiotic resistance, and/or decreased quality of life (44). Pet owners are often unaware of the negative affects their dogs face without the proper medication, underestimating the need for tick prevention. When asked if pet owners adhered to the recommendations made by the veterinarian, 62% said they did, 14% underestimated the recommendations, and 25% had no recollection of instructions at all (44). Despite the majority of individuals stating they followed directions, only 13% of them purchased enough of what the veterinarian recommended (44). Without proper application of tick medicine, it is not as effective and transmission is more likely. It is important for pet owners, especially those in endemic areas, to understand the risks of tick-borne diseases and the importance of properly administering their dogs' medicines.

Lyme Vaccine. Lyme vaccines are available for dogs, although veterinarians often do not recommend this preventative measure in non-endemic states. One study determined that immunity to Lyme disease was high in the year following the vaccine (25). After a year, immunity declined, suggesting the need for booster shots (23). Overall, dogs with this vaccine and the proper booster shots appear to have a decreased chance of Lyme disease infection (45). Because Lyme disease is not widespread, it is recommended that pet owners consult their local veterinarians to determine if their pets should receive the vaccine.

CONCLUSION

Lyme disease is a debilitating tick-borne illness that can negatively impact the canine community. Without proper protection, canines are extremely susceptible to Lyme disease, among other tick-borne illnesses. Perhaps of more concern is the progression of the disease into more fatal illnesses, such as renal failure. This study suggests that the risk of Lyme disease is relatively low in southwestern Virginia. However, it is important to consider that the data only includes the early summer months. In general, the CDC denotes Virginia as a high incidence state, indicative of an average incidence of at least 10 cases per 100,00 persons within the last three years (40). Because Lyme disease is present in southwestern Virginia, it is essential that pet owners talk to their veterinarians in order to devise an effective plan to keep their pets safe from ticks.

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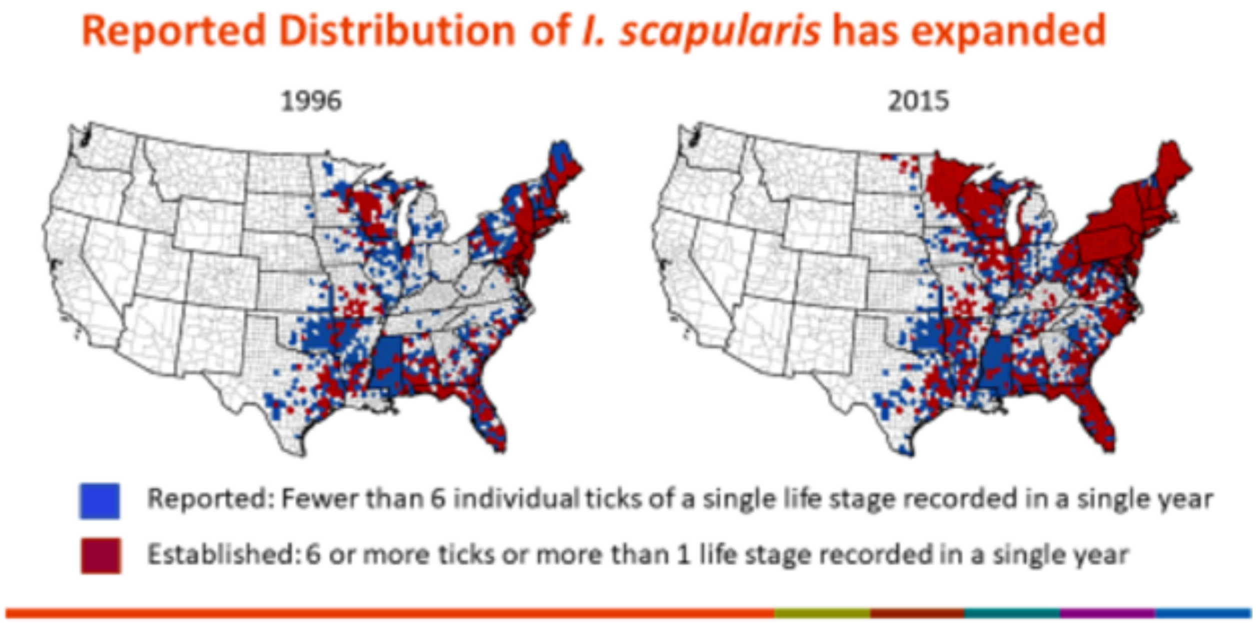

Figure 1. The distribution of *I. scapularis* in 1996 compared to 2015.

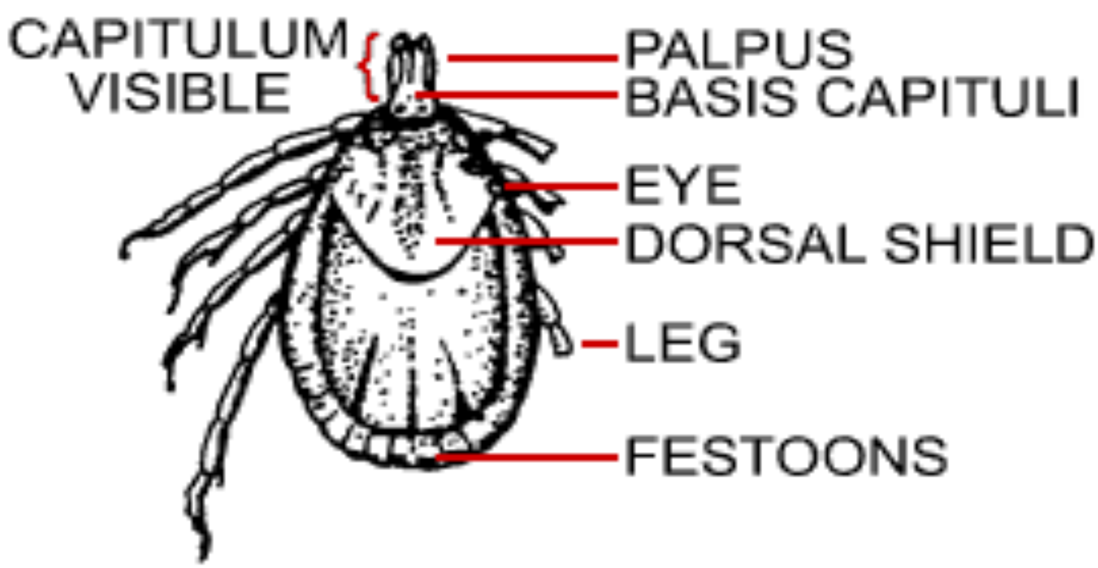

Figure 2. Body parts utilized in identifications were the festoons, capitulum, and scutum, labeled here as dorsal shield.

Table 1. Sites sampled with elevation, average temperature, average rainfall, and vegetation status noted.

Site Location		Elevation (ft)	Average Temperature (°F)	Average Rainfall (mm)	Vegetation
Carvins Cove	**Horsepen**	1,410	64	0.315	Leaf litter; little green vegetation; large trees provide shade
	Hotel	1,441	64	0.315	Some leaf litter; lots of overgrown and tall green vegetation
	Bennett Springs Loop	1,180	64	0.315	Leaf litter; little green vegetation; large trees provide shade
Mill Mountain	**Woodthrush**	1,161	63	0.315	Leaf litter; little green vegetation; large trees provide shade
	Lower Star	1,209	63	0.315	High green vegetation; large trees provide shade
Salem Rotary Dog Park	**Large Dog Pen**	1,075	65	0.315	Grass cut short; no other vegetation

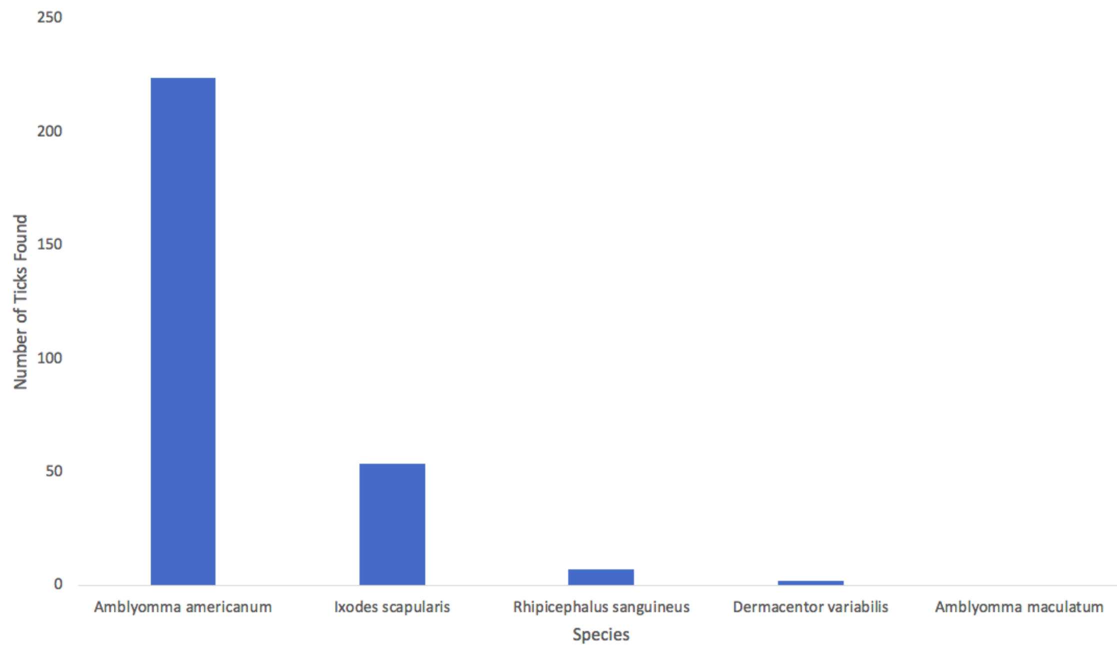

Figure 3. The number of each species found during the nine weeks of collection. No *Amblyomma maculatum* samples were collected.

Table 2. The number of adult females and nymphs collected. No adult males were identified.

	Life Stage	
Week	Females	Nymphs
Week 1 (5/3-5/9)	1	0
Week 2 (5/10-5/16)	0	1
Week 3 (5/17-5/23)	0	0
Week 4 (5/24-5/30)	0	2
Week 5 (5/31-6/6)	0	8
Week 6 (6/7-6/13)	0	20
Week 7 (6/14-6/20)	0	6
Week 8 (6/21-6/27)	0	8
Week 9 (6/28-7/4)	0	8
Total	1	53

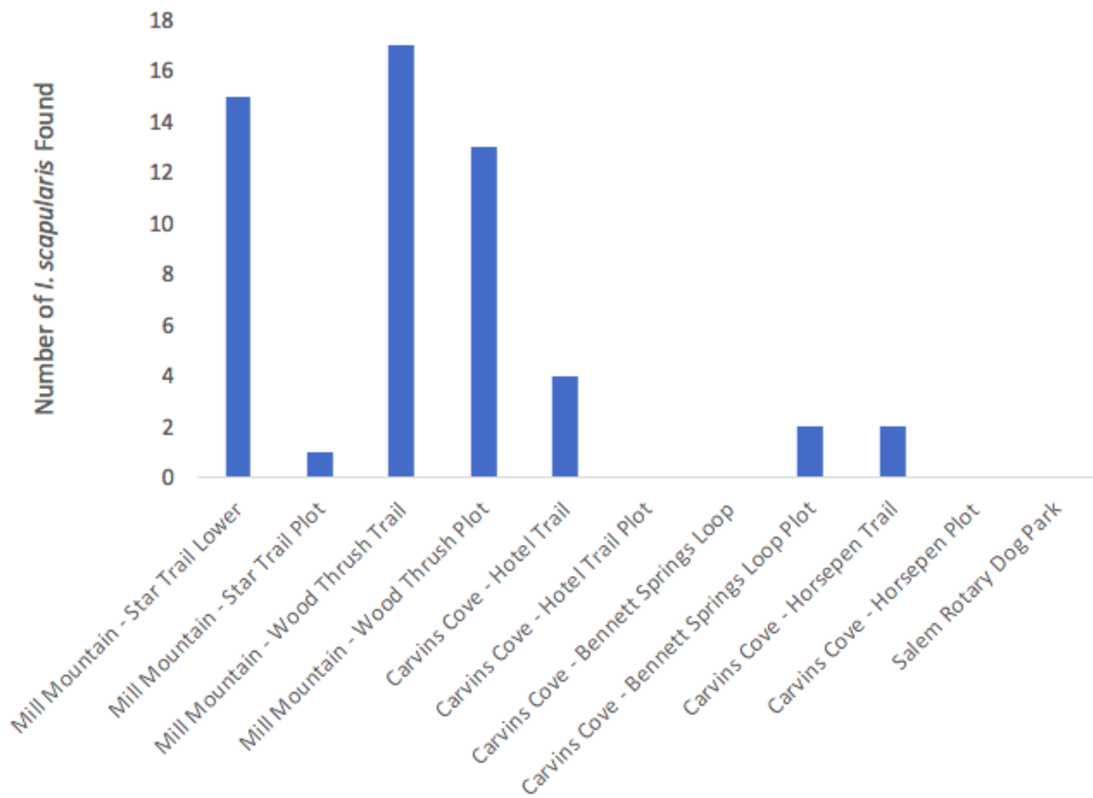

Figure 4. The number of *I. scapularis* found at each site. Minimal samples were collected at Carvins Cove.

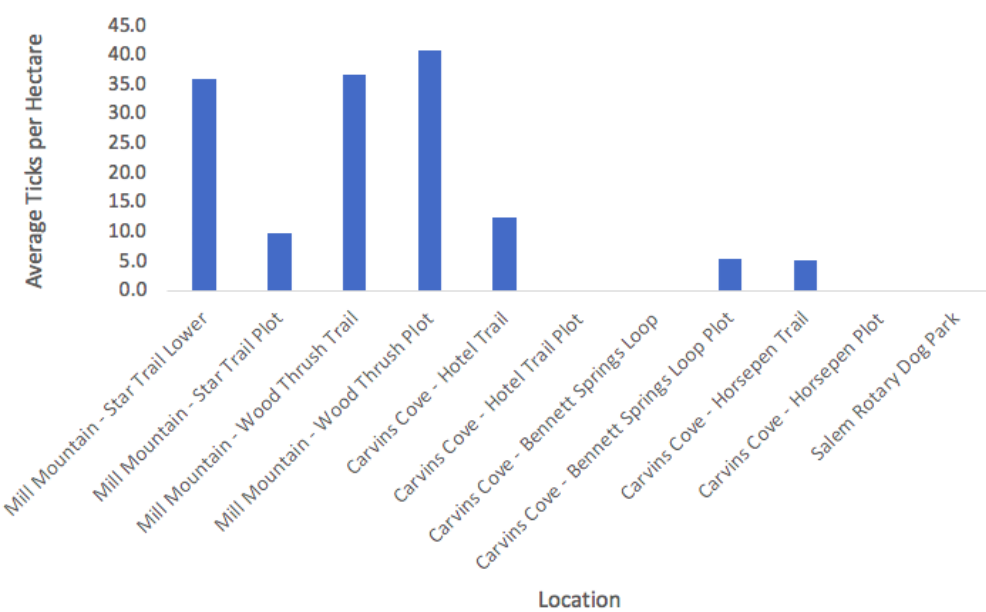

Figure 5. The average number of ticks per hectare at each collection site. The Wood Thrush trail and plots at Mill Mountain harbored the most ticks per hectare.

Table 3. Tick density per hectare was greatest at Mill Mountain sites.

I. scapularis per hectare	
Week 1	
MM - Star Trail	19.3
Week 2	
CC - Horspen Trail	21.6
Week 3	
Week 4	
CC - Hotel Trail	25.2
CC - Bennett Springs Loop Plot	22.0
Week 5	
MM - Wood Thrush Plot	122.7
MM - Star Trail	38.6
Week 6	
MM - Wood Thrush Trail	168.6
MM - Star Trail	134.9
MM - Wood Thrush Plot	20.4
CC - Hotel Trail	50.3
CC - Bennett Springs Loop Plot	22.0
CC - Horspen Trail	21.6
Week 7	
MM - Wood Thrush Plot	102.2
MM - Star Trail	19.3
Week 8	
MM - Wood Thrush Trail	63.2
MM - Star Trail	57.8
MM - Star Trail Plot	79.3
CC - Hotel Trail	25.2
Week 9	
MM - Wood Thrush Trail	63.2
MM - Wood Thrush Plot	81.8
MM - Star Trail	19.3

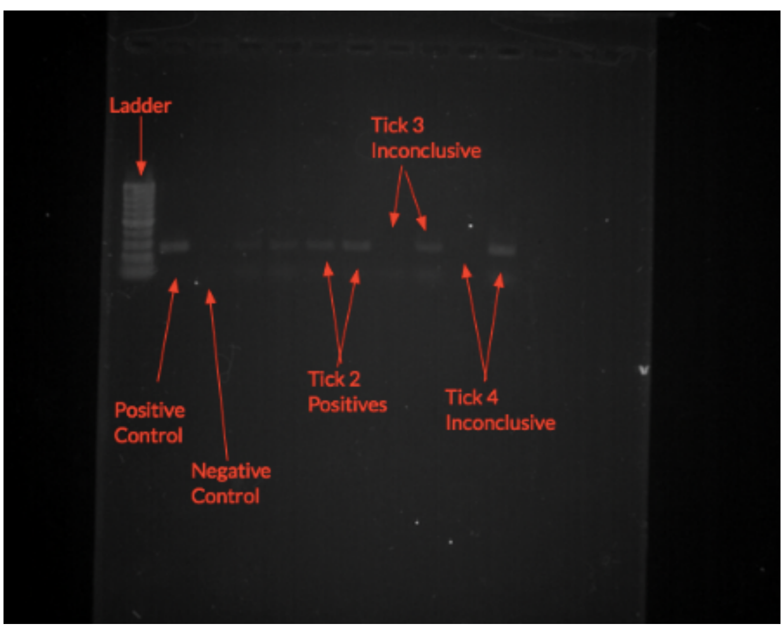

Figure 6. Gel run to determine the presence of *B. burgdorferi.* One sample was positive and the other two inconclusive.

Table 4. The number of positive results for *B. burgdorferi* of each *I. scapularis* found at the collection sites.

Location	Life Stage	Positive IDs
Mill Mountain - Star Trail Lower	female	1 (1)
Mill Mountain - Star Trail Lower	nymph	14 (0)
Mill Mountain - Star Trail Plot	nymph	1 (0)
Mill Mountain - Wood Thrush	nymph	17 (0)
Mill Mountain - Wood Thrush Plot	nymph	13 (0)
Carvins Cove - Hotel Trail	nymph	4 (0)
Carvins Cove - Bennett Springs Loop Plot	nymph	2 (0)
Carvins Cove - Horsepen Trail	nymph	2 (0)

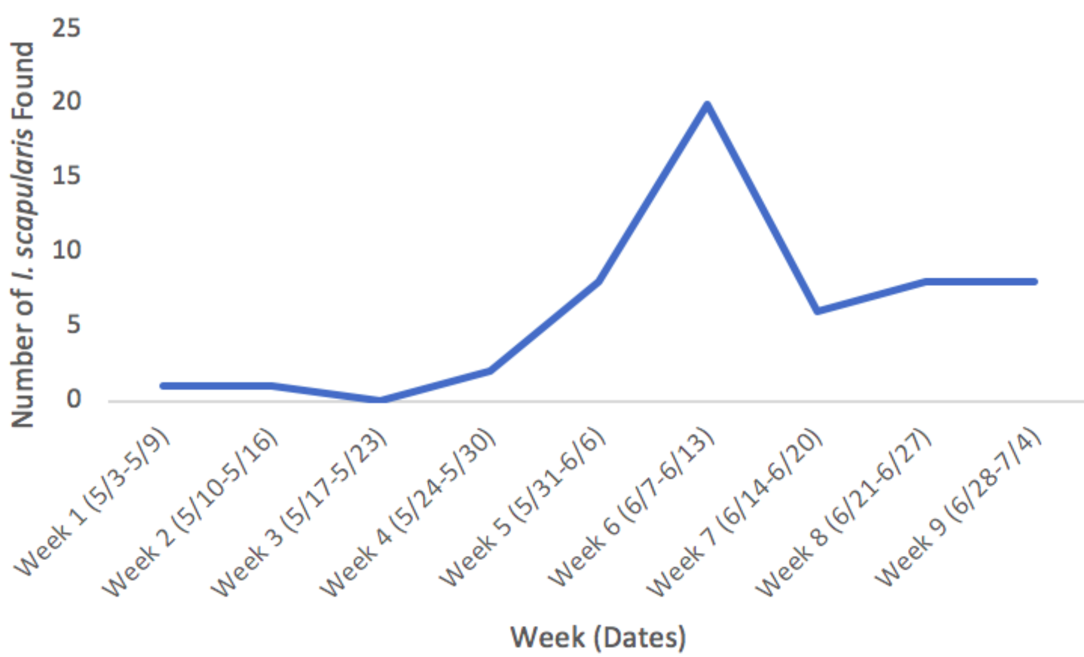

Figure 7. The majority of *I. scapularis* collected was in late May and early June.

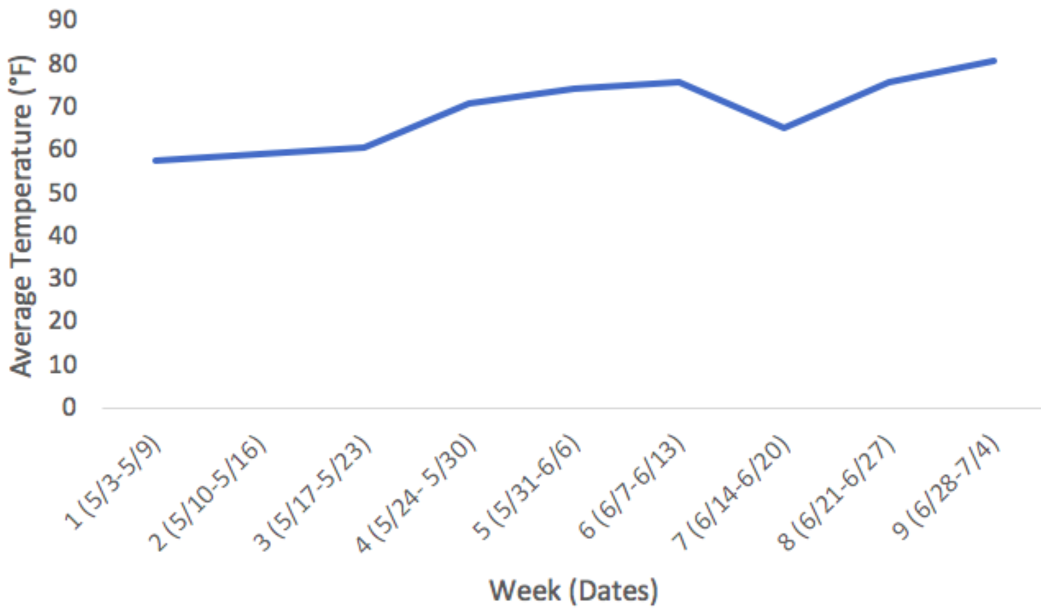

Figure 8. The temperature increases steadily over the nine weeks, peaking during the last week.

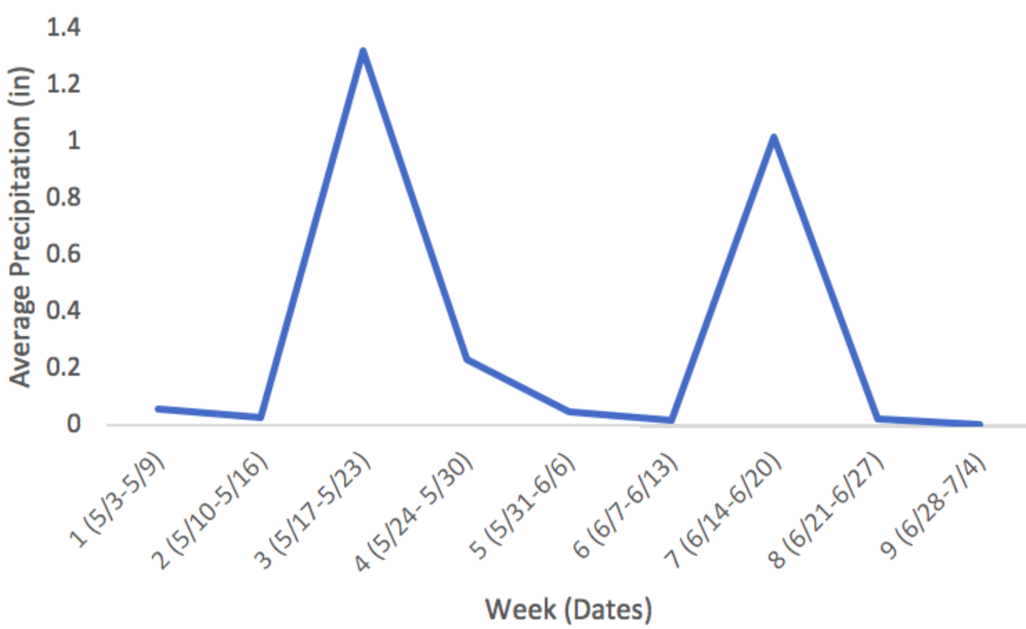

Figure 9. There were two peaks in precipitation, one at week 3 and the other at week 7, both reaching about 1 inch.

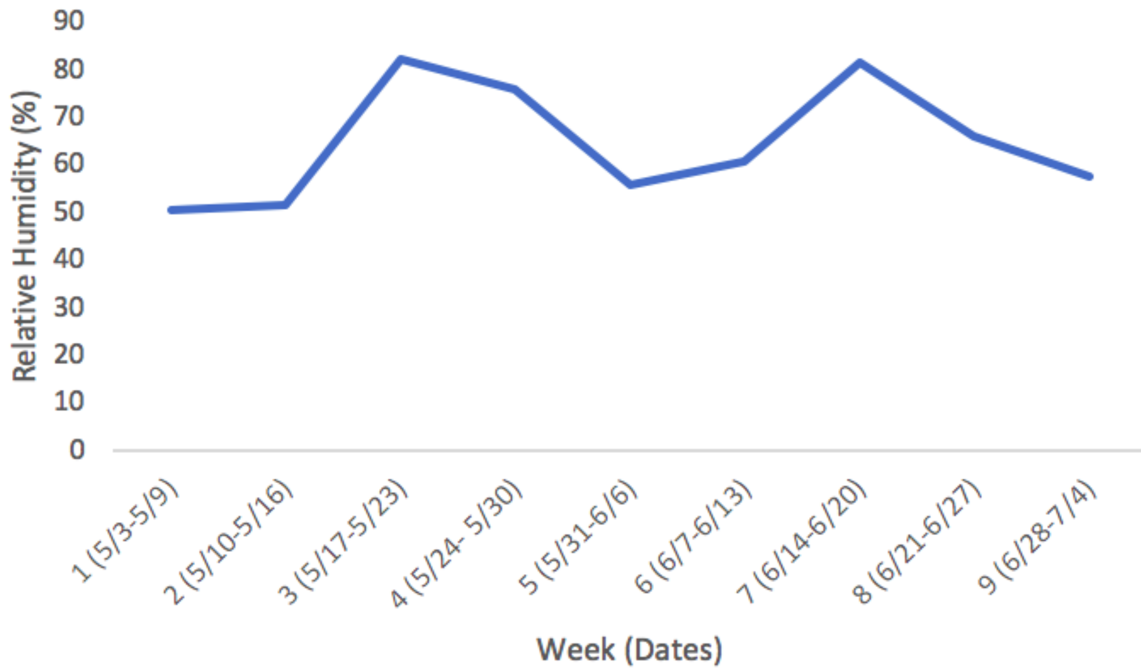

Figure 10. The relative humidity peaked at weeks 3 and 7, reaching about 80%. In the other weeks, relative humidity stayed between 50-70%.

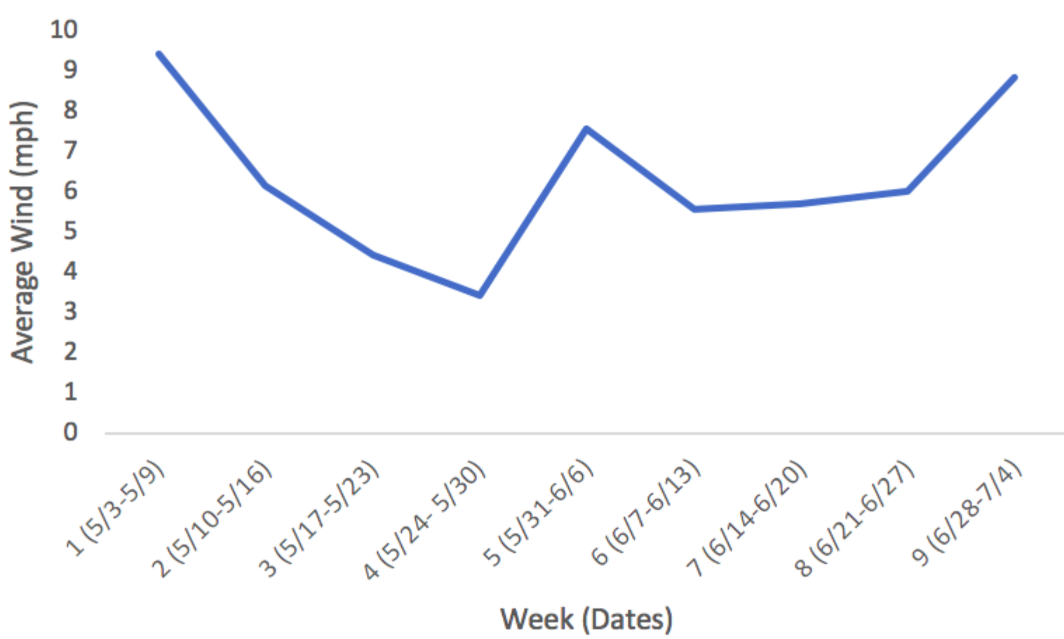

Figure 11. Wind speeds remained under 10 mph, although it peaked in the first week nearing 10 mph. There was a significant drop in speed between the first and fourth weeks. Wind speed rose after the fourth week.

Table 5. Number of *I. scapularis* collected in various life stages at different sites over the nine weeks. No ticks were found during the third week.

Life Stage	Location						
	CC - Horspen Trail	CC - Hotel Trail	CC - Bennett Springs Loop Plot	MM - Wood Thrush Plot	MM - Wood Thrush Trail	MM - Star Trail Plot	MM - Star Trail
				Week 1			
Female	0	0	0	0	0	0	1
				Week 2			
Nymph	1	0	0	0	0	0	0
				Week 3			
N/A	0	0	0	0	0	0	0
				Week 4			
Nymph	0	1	1	0	0	0	0
				Week 5			
Nymph	0	0	0	6	0	0	2
				Week 6			
Nymph	1	2	1	1	8	0	7
				Week 7			
Nymph	0	0	0	5	0	0	1
				Week 8			
Nymph	0	1	0	0	3	1	3
				Week 9			
Nymph	0	0	0	4	3	0	1