DOWN THE WOODCHUCK HOLE: INVESTIGATING THE POTENTIAL FOR *PEROMYSCUS LEUCOPUS* TO ACT AS A MIXING VESSEL FOR THE POWASSAN VIRUS AND DEER TICK VIRUS DISEASE SYSTEMS

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Abstract. Powassan viral encephalitis is a rapidly emerging and potentially deadly disease. Although serious cases are extremely rare, incidence has been increasing in the northeastern United States for the last 10 years. The virus that causes the disease is carried and transmitted by the woodchuck tick, *Ixodes cookei*, a species almost exclusively found in burrows of medium-sized mammals. Because of its specific habitat, the woodchuck tick is unlikely to come into contact with humans. How, then, is the frequency of Powassan disease increasing in the human population? One hypothesis is that the blacklegged tick, *Ixodes scapularis*, is responsible. This species of tick, which commonly encounters the human population, has been found to carry Deer Tick Virus (DTV)–a nearly identical relative of Powassan Virus (POWV).

It is highly likely that the DTV and POWV disease systems are linked, although it is unknown how the hosts, ticks, and pathogens encounter each other. I investigated one proposed mechanism: that the white-footed mouse (*Peromyscus leucopus*), a common, widespread host species for *I.scapularis*, enters woodchuck burrows, thus encountering *I.cookei* and consequently acting as a mixing vessel for both ticks and pathogens. I tested this hypothesis by trapping mice within two meters of woodchuck burrows at seven sites at the Cary Institute of Ecosystem Studies in Millbrook, New York. Mice were implanted with unique microchip tags at the time of capture and held for three days to assess their tick burden. Scanners to detect the microchip tags in the mice were placed at the entrance to woodchuck burrows; the scanners recorded visits by mice to determine frequency of burrow use. Of the 13 tagged mice, 11 used the burrows at least once, with several making frequent visits to burrows in a single night and over multiple nights. The captured mice were found to have typical *I.scapularis* burdens, but no *I.cookei* were recovered. Interestingly, of mice that visited the burrows at least once in the scanning period, those that visited more often had a slightly higher *I.scapularis* burden than those that visited less often (R^2=.34). This suggests that if mice living in the vicinity of woodchuck burrows also host *Ixodes cookei*, they may play an important role in POWV/DTV transmission.

INTRODUCTION

Powassan encephalitis, caused by the Powassan virus, a tick-borne flavivirus, is a rare disease associated with serious long term side effects and a high rate of fatality. The virus was discovered in 1958 with its first diagnosed victim, a 5 year old boy from Powassan, Ontario, and the disease has been diagnosed in 26 additional cases between then and 1998 (Mclean and Donahue 1959; Gholam et al. 1999). Although it is extremely rare, it is of concern because its frequency is increasing. Nine cases were confirmed between 1999 and 2005, for an average of 1.29 cases per year, up drastically from 0.66 cases per year from 1958-1998. In 2009, two of the six cases were in Dutchess County, NY, the location of the Cary Institute of Ecosystem Studies (USGS 2009).

Powassan virus can be transmitted in only 15 minutes of tick attachment, unlike other tick-borne diseases, such as Lyme disease, which require 24-48 hours of tick attachment (Ebel and Kramer 2004). The seriousness of Powassan viral encephalitis coupled with its rapid transmission and increasing frequency, warrants studies that address its ecology and transmission in order to understand how the disease may spread in the future. Much is still unknown about Powassan, but this only heightens the importance of each study. Ultimately, research on the ecology of Powassan is necessary for finding ways to prevent this rapidly emerging disease.

POWV has been isolated from only a few types of ticks and mammal hosts. It has traditionally been associated with *Ixodes cookei*–the woodchuck tick–as the vector, and woodchucks (*Marmota monax*) and a few other burrowing mammals including skunks and weasels as hosts (Main et al. 1979; Artsob 1989). A study by Ko in 1971 found that 29% of woodchucks were infested with *I.cookei*. The life cycle and ecology of woodchuck ticks are not well understood, however it has been established that woodchuck ticks are found exclusively in burrows. Of hundreds of thousands of questing ticks collected during routine monitoring of tick abundances at the Cary Institute, and tens of thousands of mice inspected, no observations of *I.cookei* have been made (R.S. Ostfeld, pers. comm.).

If the main vector of POWV is the woodchuck tick, a species which only rarely comes into contact with humans, how is the frequency of the disease increasing in the human population? One hypothesis is that the blacklegged tick (*I.scapularis*)–a common, widespread species that infects dozens of hosts–is responsible. This tick is commonly encountered by humans and feeds on many of the same hosts as the woodchuck tick. Furthermore, *I.scapularis* has been found to carry Deer Tick Virus (DTV), an antigenic variant of POWV (Kuno et al. 2001). A study at the Cary Institute indicated that 1% of local *I.scapularis* are carrying the disease (Ostfeld and Kramer, unpublished). *I.scapularis* can also carry and transmit POWV (Costero and Grayson 1996). While this seems like a probable explanation for the increase in cases, much is unknown about how the hosts and vectors encounter each other and transfer the virus among them, and how this may lead to an increasing disease risk in the future.

One possibility is that *Peromyscus leucopus*, the white-footed mouse, enters woodchuck burrows during its nightly movements and ultimately acts as a host for both species of ticks. White-footed mice use the area within 2 meters of woodchuck burrows more extensively than the area 10-12 meters away, establishing the plausibility of mice entering woodchuck burrows (Swihart and Picone 1990). This notion also is supported by the observation that 3-4% of white-footed mice sampled in Massachusetts, Wisconsin, and Rhode Island tested positive for POWV (Ebel et al. 2000). Furthermore, the increase in the mouse population density as a result of a fragmenting habitat, particularly in the northeastern deciduous forests, may account for the increase in the number of cases in this area, as is known to occur with Lyme disease (Ostfeld and Keesing 2000; Allan et al. 2003). It is possible that the increase in Powassan encephalitis in the past 10 years is due to an increased mouse population density, the evolution of the virus to use different vectors and hosts, and/or some other factors.

Study Objectives

The overarching purpose of this study is to investigate the potential for the white-footed mouse to act as a host species for both blacklegged ticks and woodchuck ticks, and consequently the Powassan Virus and Deer Tick Virus disease systems. This question leads to two main study objectives: to determine if white-footed mice living in the vicinity of woodchuck burrows use or enter woodchuck burrows, and to determine if white-footed mice living in the vicinity of woodchuck burrows carry woodchuck ticks. Two distinct experiments were carried out to investigate these two questions.

METHODS

Site Selection

This study was carried out on the property of the Cary Institute of Ecosystem Studies in Millbrook, New York, in the area of Dutchess County within the Hudson valley. Sites consisted of active (currently occupied) woodchuck burrows and two meters of the surrounding area. Burrows were identified by observing the resident woodchucks during their afternoon summer feeding sessions and watching where they ran when startled. In this way, seven sites were chosen for the study. Sites varied in their proximity to manmade structures and in their environmental composition. See the detailed site descriptions below for more information on each site. All seven were included in the tick burden study to determine if mice even live near woodchuck burrows, and if these mice living in the vicinity of woodchuck burrows carry woodchuck ticks and/or blacklegged ticks.

Tick Burden

Mice were captured at seven sites to assess their tick burdens. Ten baited Sherman traps per site were used in the nighttime between June 17th, 2010 and August 5th, 2010. A combination of a smear of peanut butter and a seed and grain mix was used for bait. Traps were placed at approximately 6pm at night and were checked in the morning between 7am and 8am. Mice were brought in to the Cary Institute's Rearing Facility, an approved animal holding facility, tagged with uniquely numbered ear tags, and kept for three days, after which time they were released in the same place they were captured. Mice were kept in wire mesh cages suspended over plastic tubs edged with Vaseline. In this way the engorged ticks from the mice dropped into the pan after feeding and remained in the tub for counting the next morning. Daily care for the mice included fresh water, fresh seed and grain mix, apple slices, and plastic tub cleaning. Every day the tubs were 'picked' for ticks, and the engorged individuals were placed in humidified vials. The vials of ticks were taken to the lab and observed under a microscope to determine species and stage (larval, nymphal, or adult) and findings were recorded.

Burrow Use

To determine whether white-footed mice spend time in woodchuck burrows, captured mice were implanted with PIT tags, a type of subcutaneous microchip. Ring-shaped scanners capable of detecting and recording the uniquely numbered PIT tags were placed over the entrance to the woodchuck burrows overnight. In the morning the scanners were retrieved and the recorded data were compiled.

Analysis

Scanners were programmed to scan for mice continuously throughout the night. An initial timestamp was recorded at the first moment a mouse passed the scanner, and if the mouse stayed within the scanner's range for more than five seconds, a timestamp five seconds later was recorded. For example, a mouse staying at the entrance to a burrow for 12 seconds beginning at 12:00:00 would be recorded at 12:00:00, 12:00:05, and 12:00:10. A visit of less than five seconds would be recorded as a single timestamp. To simplify the analysis, I counted the number of timestamps recorded per mouse. I divided this number by the maximum number of days (based on the date of capture) that the mouse possibly could have been recorded to produce a calculation of visitation, a way to take into account both the number of visits and the length of each visit per night. I also calculated "average nightly visitiness" by dividing the number of timestamps per night by the number of nights the animal visited the burrow at all. I compared these calculations to the tick burden data of the mice to determine if there was a correlation between burrow visiting, *I.scapularis* burden, and *I.cookei* burden.

RESULTS

Tick Burden

Of the seven sites where trapping took place, five had white-footed mice mice living in the vicinity of woodchuck burrows. The two sites with no mice were abandoned after 90-100 trap nights (1 Sherman trap set for 1 night = 1 trap night). A total of 26 white-footed mice were captured at the five sites. No *I.cookei* were found on the mice during this study but a typical *I.scapularis* burden was observed. A range of 0-75 engorged blacklegged ticks were collected from individual mice in the three days they were kept in the Rearing Facility for an average of 4.00 ticks per mouse (156 total ticks/39 mouse captures). The median number of ticks was 0.5 ticks/mouse. Additionally, the seasonal *I.scapularis* larval peak was observed between July 26th and July 28th (see Figure 1). 72.4% of the total engorged ticks found on mice throughout the study were collected during that three day period; 108 were larvae and five were nymphs.

Burrow Use

From the initial seven sites, only three were used for the burrow use study. Two sites were unusable for this phase of the research project due to there being no mice living in the area, and two due to the burrow being unsuitable for scanner placement. The three sites used for the burrow use study included Cold Storage (CS), Smith Cottage (SC) and Raccoon Coop (RC). Ultimately, 11 of the 13 microchipped mice at these sites visited the burrows at least one night (see Figure 2), with nine returning more than one night. One mouse was recorded visiting the burrow every night during the eight-night recording period at that site (see Figure 4). See Figure 3 for a compilation of all burrow visits across three sites. Interestingly, the period of least "visitiness" was observed around the time of the full moon.

Tick Burden Relative to Burrow Use

Initially I hypothesized that the *I.cookei* burden of white-footed mice would be positively correlated with the frequency of burrow visits. However, since no *I.cookei* were found on the mice captured near woodchuck burrows, I was unable to either support or reject this hypothesis. Therefore I examined the relationship between mouse *I.scapularis* burden and burrow use frequency. I found that there is a positive correlation between a mouse's overall visitation and *I.scapularis* burden (R^2=.34, see Figure 5). In general, mice that visited more often had more blacklegged ticks. In order to account for individuals that may have not visited the burrows more than a few times because they did not live in the area anymore or had died, I considered how many times a mouse visited per night on nights they visited at all (average nightly visitation). This correlation was also positive but weaker (R^2=.11), and therefore suggests that this may not be a very significant measurement (see Figure 6). A large sample size would improve certainty about whether or not this is a useful comparison.

DISCUSSION

Tick Burden

No engorged woodchuck ticks were collected from the captured mice in this study. There are a few possible reasons for this result. First, it is possible that no woodchuck ticks were present on these mice. Another possibility is that woodchuck ticks were present, but since they feed for a longer duration of time than blacklegged ticks (about 7 days), they did not have time to fully feed and drop off during the three days the mice were kept in the Rearing Facility (Ko 1971). However, this reasoning might explain a low *I.cookei* count, but perhaps not the complete absence of *I.cookei*. One more potential reason no woodchuck ticks were collected is perhaps they do not drop off their hosts until they feel they are in a suitable environment, i.e. a burrow. Attempting this study again and manually removing all visible ticks from the white-footed mice even before they are engorged would likely give a more accurate count of both *I.cookei* and *I.scapularis*.

Burrow Use

With no *I.cookei* found on the mouse sample population, I wanted to know if white-footed mice living in the vicinity of woodchuck burrows enter the burrows as part of their nightly routine. The scanners I set up to record the PIT tags revealed exciting new information that white-footed mice cross the entrance of woodchuck burrows, and go at least a few inches down them very often. Although some mice stopped visiting the burrows after a few days, they were also not captured in the area anymore, and it is proposed that those individuals dispersed or died. One interesting case of burrow use was observed in mouse Q950. This individual was recorded at two different burrow sites about 50 yards apart from each other on the same night. It seems that this mouse's territory includes both burrow entrances. However, the burrows were not exclusive territory, as 2-3 different mice often visited each burrow nightly (See Figure 4 for the "visitiness" of three mice at one site over time).

Implications for POWV, DTV, and Powassan Disease Risk

Since no woodchuck ticks were collected from the sample mouse population of this study, no decisive conclusions can be made about the white-footed mouse's role in the increasing frequency of Powassan Disease. However, the result that mice which spend more time around woodchuck burrow entrances may have a higher blacklegged tick burden is potentially very important in considering disease risk. If white-footed mice ever do host woodchuck ticks, the mice which are most likely to be hosts-based on how much time they spend in and around burrows-also carry a higher number of blacklegged ticks, which are thought to increasingly carry the disease to humans.

Recommendations for Further Study

The discovery that mice do cross and potentially enter woodchucks' burrows during their nighttime activity suggests that further research should be conducted in this area. In a future study it would be advisable to scan the burrow at one site continuously rather than moving between sites. This would allow more complete data to be collected. The nature of the white-footed mouse's use of woodchuck burrows should also be investigated, as that was one of the principal limitations of this study. The scanners allowed us to know that mice do cross over the entrance to woodchuck burrows, often nightly and multiple times per night, but it is impossible to tell if they spend much time inside the burrows, how deep they travel, and what purpose visiting a burrow has in their nightly routine. Relatedly, why do mice that visit the burrows more often have a slightly higher blacklegged tick burden on average? Perhaps infrared video cameras or other experiments could elucidate answers to these questions.

Furthermore, the woodchucks themselves, as well as other burrowing mammals, should be considered as potentially more important host species for the DTV and POWV viruses and the blacklegged ticks and woodchuck ticks. Since very little is known about POWV and DTV, it is possible that mice do not actually play a large role in transmission. Other factors, such as a changing environment that affects the distribution and populations of large rodents and other mammals may be a factor in the evolution and increasing frequency of Powassan disease. Alternatively, perhaps an environment suffering increasing fragmentation, such as that of Dutchess County, is increasing the frequency of human and woodchuck conflicts, leading to greater disease risk due to the shear proximity or burrows to human establishments.

Lastly, more information about the ecology of woodchuck ticks would enable us to be more successful at identifying them. It is possible that *I.cookei* were present on the mice tested in this study, but that they feed longer than *I.scapularis*, or only drop off their hosts when they are in a suitable environment for molting, such as a burrow. This could possibly explain the lack of woodchuck ticks found on the sample population. More information about woodchuck ticks in general, combined with an improved method for collecting them from host species, as well as a larger sample size of mice trapped near woodchuck burrows would increase the chances of being able to come to a decisive conclusion about the potential for mice to be a mixing vessel for the POWV and DTV disease systems. Finally, an experiment to determine if *I.cookei* are present in the burrows in the first place would be helpful in supporting or rejecting the hypothesis that mice are responsible for the increase in Powassan disease cases in humans.

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APPENDIX

TABLE 1. Detailed description of woodchuck burrow sites used in this study.

Name	Trapping?	Scanning?	Description of Location
Field Lab	Yes	No, unsuitable for scanner placement	Off a dirt road in the forest on the edges of the Cary property
Weather Station	Yes	No, no mice captured	Flat wooden platform surrounded by a few meters of short lawn, adjacent to a small shed
Lee Lovelace	Yes	No, unsuitable for scanner placement	Located in a forest and lawn edge area, close to the paved road but at least 50 meters from the house itself
Smith House	Yes	No, no mice captured	Inside a 3-sided shed, surrounded on all sides by at least a meter of short lawn
Smith Cottage	Yes	Yes	Behind a house near a small strip of trees and brush, including a woodpile.
Cold Storage	Yes	Yes	On a hill overlooking the Cary rearing facility, abutting the walls of the Cold Storage building
Raccoon Coop	Yes	Yes	About 50 meters from Cold Storage site, on the edge of lawn and forest

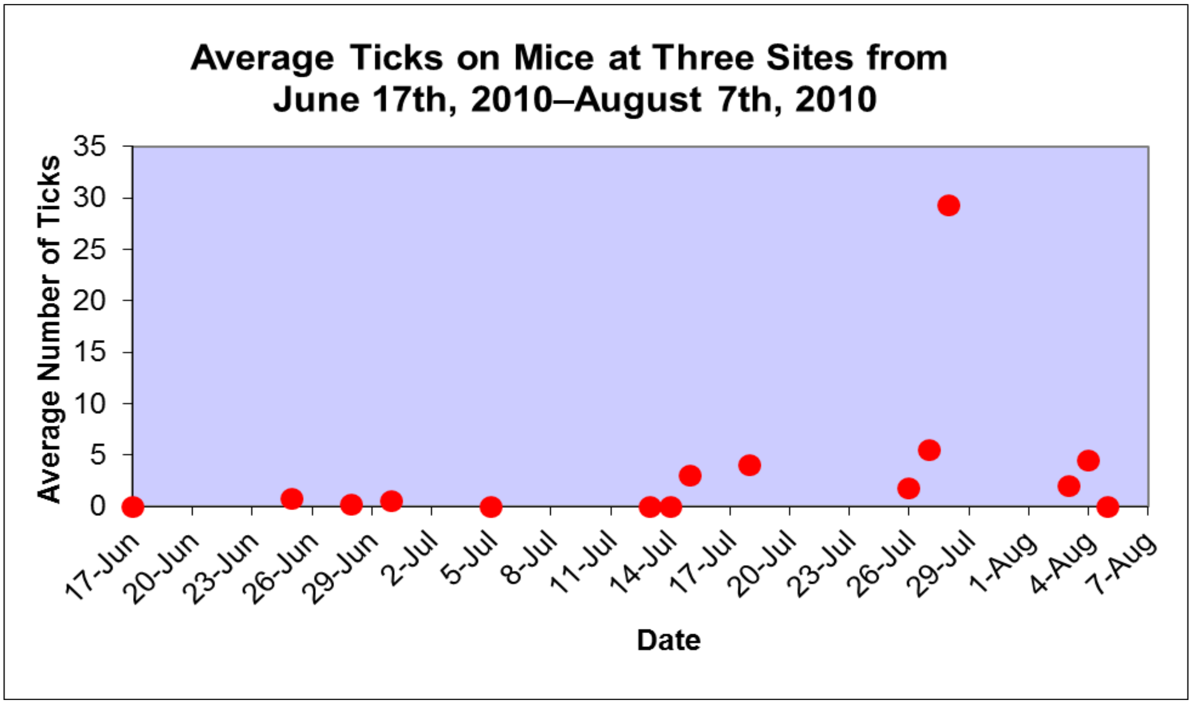

FIGURE 1. Average blacklegged ticks per mouse at three sites. The seasonal larval peak was observed around the 26th of July into early August. N = 39.

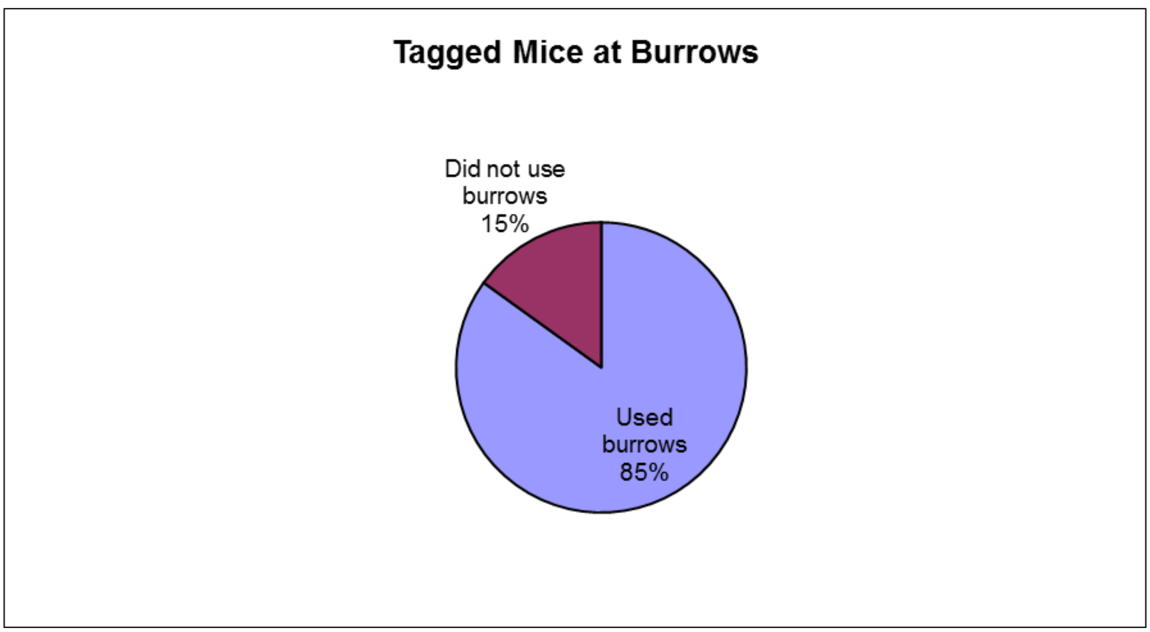

FIGURE 2. Of the 13 microchipped mice, 11 visited the burrow at least once.

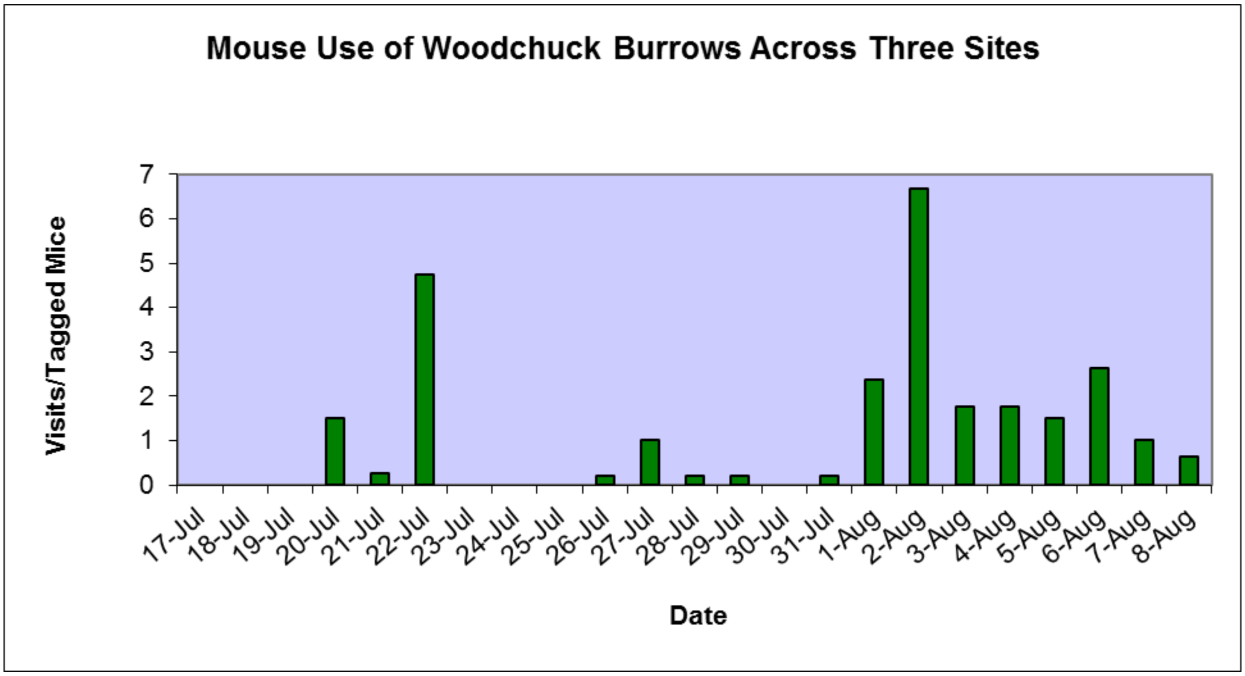

FIGURE 3. Total mouse use of woodchuck burrows across three sites over three weeks. Interestingly, the least amount of burrow use occurred around the time of the full moon (July 26th).

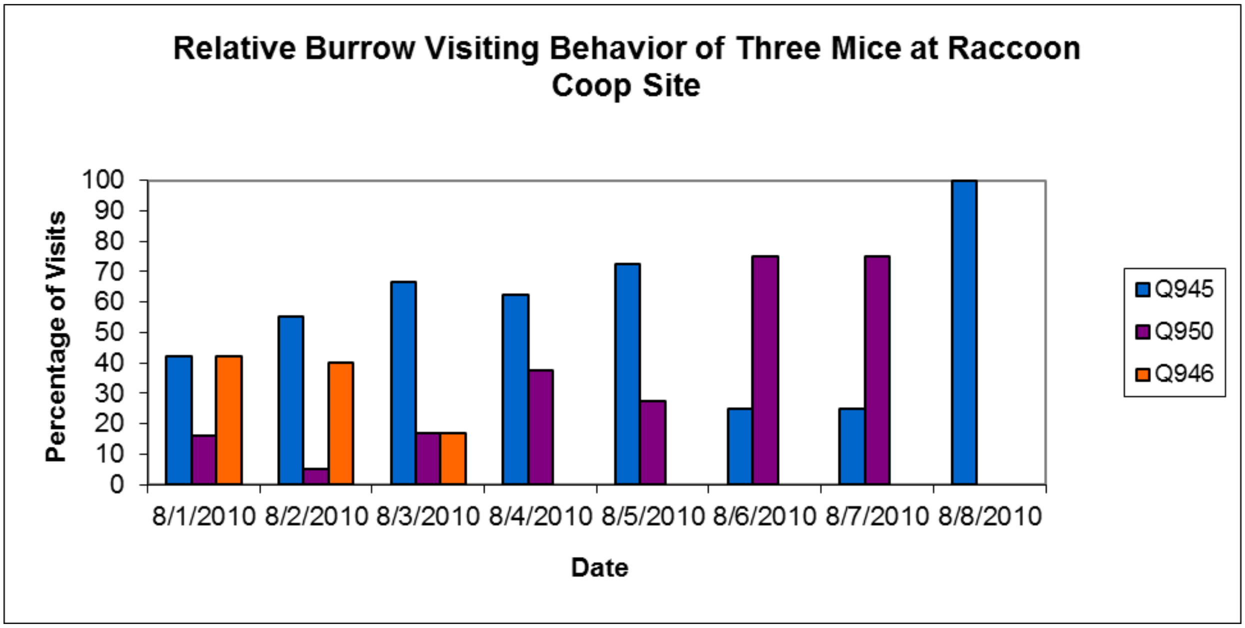

FIGURE 4. This chart shows the relative visiting behavior of three individual mice at one site.

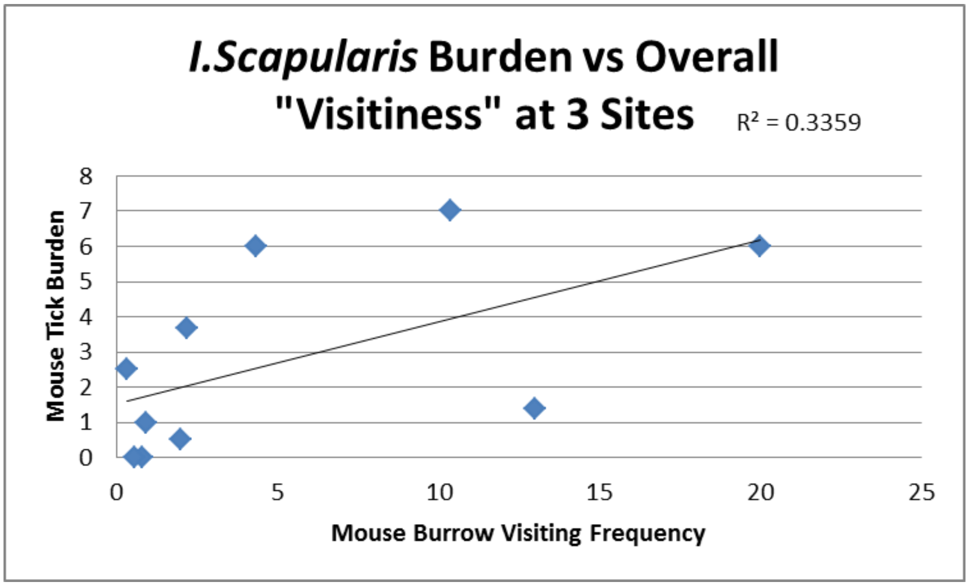

FIGURE 5. This graph shows blacklegged tick burden in relation to "visitiness" of the mice. A positive correlation was observed (R^2=.34).

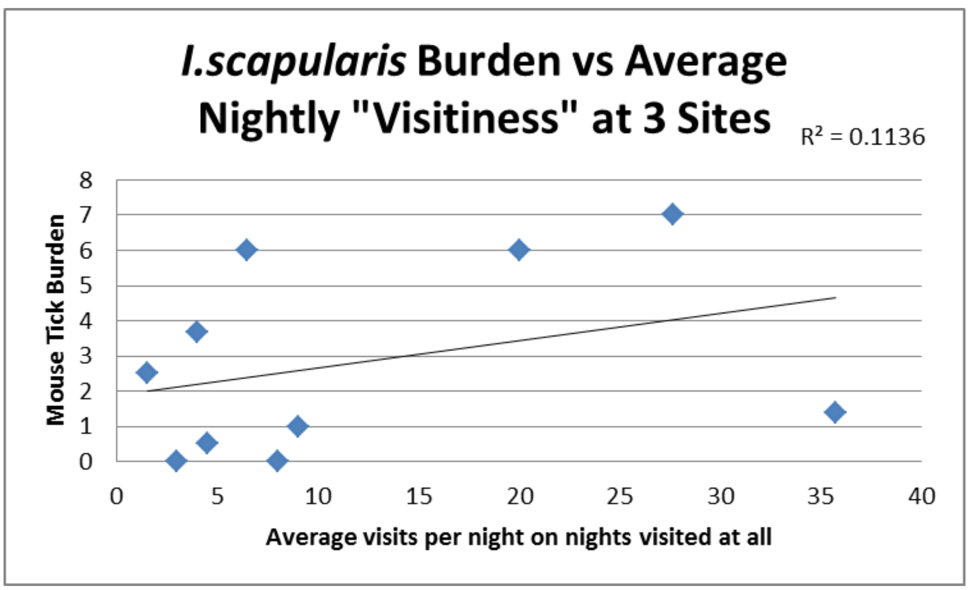

FIGURE 6. Out of the nights a mouse visited the burrows at all, how active a mouse was on those nights compared to its tick burden. R^2= .11, a value less than overall visitiness (see Figure 5).