SHREWS (*BLARINA BREVICAUDA*) AND THEIR EFFECT ON LYME DISEASE RISK

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INTRODUCTION

According to the CDC, reports of Lyme disease have been on the rise since 1991, with 19,804 reported cases in 2004 (CDC, 2005). Lyme disease is the most common vector born disease in the United States (Ostfeld and Keesing, 2000) and a serious threat to human health. Although beginning symptoms may be mild, the bacterium that causes the disease, *Borrelia burgdorferi*, may spread to the joints and nervous system, producing much more devastating effects (CDC, 2005). It is therefore critical to understand the factors affecting the distribution and transmission in an effort to minimize future human infections.

B. burgdorferi is maintained in animal hosts, and transmitted between hosts, including humans, by the black-legged tick (Ostfeld 1997). The black-legged tick (*Ixodes scapularis*) has three life stages, each requiring a single blood meal (Van Buskirk and Ostfeld 1995). *B. burgdorferi* is not transmitted from parents to offspring of the black-legged tick; therefore larvae emerge from eggs uninfected with *B. burgdorferi*. Their subsequent infection in the nymphal and adult stage depends on whether the animals they feed on are infected (Patrican 1997). Once a black-legged tick becomes infected, it remains infected throughout the rest of its life stages (Ostfeld 1997), allowing infected nymphs and adults to transmit the bacteria to other animals, including humans. Because the adult stage of the black-legged tick prefers the white-tailed deer, it is the nymphal ticks that commonly infect humans and other animals. The animals that these nymphs infect help to maintain a group of infected hosts able to infect the next generation of tick vectors.

In this cycle of infection between animal hosts and tick vectors, certain animal hosts are better reservoirs for *B. burgdorferi* than others (Schmidt and Ostfeld 2001). A competent reservoir is able both to sustain infection with *B. burgdorferi* and to transmit the bacteria to a vector effectively. Research has shown that the white-footed mouse (*Peromyscus leucopus*) is the most competent reservoir for this bacterium (Schmidt and Ostfeld 2001). Because white-footed mice are competent reservoirs and are bitten by many larval ticks, they are able to infect many ticks.

Although the white-footed mouse is quite common, its populations experience large fluctuations due largely to the masting of acorns, an important food source for these mice (Ostfeld et al. 1996). Because these mice are such competent reservoirs for *B. burgdorferi*, they are crucial in the maintenance of large numbers of infected ticks. The question is: what happens when mouse populations are very low? Does infection prevalence in ticks drop drastically? To answer these questions it is important to understand the role of other host species for *B. burgdorferi* when trying to understand Lyme disease risk.

According to a model created by LoGiudice et al. (2003), even in instances of zero mouse density, the nymphal infection prevalence (NIP) remains relatively high at 34%. LoGiudice et al. (2003) claim that this is due to the effects of species such as the northern short-tailed shrew (*Blarina brevicauda*), which they assume function as "rescue hosts" for Lyme disease risk. This assumption is based on data showing that shrews have high larval burdens, are competent reservoirs, and, being insectivores, do not have population fluctuations that necessarily coincide with those of mice. No one, however, has studied the nymphal burden on shrews, which may be a

critical factor determining their impacts on maintaining high rates of Lyme disease transmission even during times of low mouse density.

Because infected nymphs are responsible for infecting small mammal hosts with *B. burgdorferi* and, therefore, are an important link in the chain of events that maintain Lyme disease risk, if shrews are not fed on by nymphs, they will not become infected with *B. burgdorferi*. However, shrews are known to be regularly infected w/ *B. burgdorferi*. What, then, is the use of determining specific nymphal burdens on shrews?

Recent research by Brisson and Dykhuizen (2004) indicates that different polymorphisms of the outer surface protein C (ospC) of *B. burgdorferi* provides selective advantage in different environments. The environments, in this case, are different host animals. The research indicates that different animals allow establishment of infection of only certain strains with specific forms of the polymorphism. Importantly, humans are only susceptible to four of the fifteen common strains of *B. burgdorferi* (Seinost et al. 1999)

Taking this into consideration; in order to maintain Lyme disease risk to humans, it is not only important for hosts, such as shrews, to be exposed to *B. burgdorferi*, but for them to be exposed to humanly infections strains of *B. burgdorferi*. Shrews are susceptible to two of the four humanly infectious strains, making them a possibly dangerous reservoir (Brisson and Dykhuizen 2004).

Black-legged tick larvae have the possibility of feeding on a host with or without humanly infectious strains and thereby may molt into a nymph with or without humanly infectious strains. When this nymph encounters its next host, possibly a shrew, it has the possibility of transmitting only the strains of *B. burgdorferi* that it received from its first host. Brisson and Dykhuizen (2004) collected a total of fifteen shrews and found that only six of the fifteen strains of *B. burgdorferi* routinely infected *B. brevicauda*.

Whether or not a shrew is infected by any given strain is determined by two things: the nymphal burden on the shrew and the frequency of each strain of bacteria within the host seeking nymphs. There are two distinct possible scenarios. In one case, if shrews are bitten by few nymphs, probability may determine which strains infect shrews. In other words, if shrews have low nymphal burdens, there is a distinct possibility that the strains that Brisson and Dykhuizen (2004) found to infect *B. brevicauda* (including two humanly infectious strains) are simply the result of chance. On the other hand, shrews may be bitten by many nymphs, meaning that they are exposed to all strains, even the less frequent ones, and their bodies are only susceptible to the strains highlighted by Brisson and Dykhuizen (2004). In this scenario shrews are frequently exposed to all strains of *B. burgdorferi* and are susceptible to two humanly infectious strains. Because Brisson and Dykhuizen (2004) had a relatively small sample size, it remains unclear whether chance led to infection with these six strains, or whether *B. brevicauda* are regularly bitten by enough nymphs for them to exhibit such an infection pattern.

If *B. brevicauda* are exposed to all strains of *B. burgdorferi*, and are susceptible to two humanly infectious strains, the result is many *B. brevicauda* carrying humanly infectious strains of *B. burgdorferi*. Because *B. brevicauda* have high larval burdens, they would have the potential of infecting many ticks with humanly infectious forms of the *B. burgdorferi*.

To determine the causes of infection in *B. brevicauda*, I investigated the effects of the northern short-tailed shrew on Lyme disease risk at study sites owned by the Institute of Ecosystem Studies (IES), which is located in the town of Millbrook in Dutchess County, New York. IES has been a hub of Lyme disease research for some time (Allan et al. 2001, Schmidt and Ostfeld 2001, Brisson and Dykhuizen 2003, LoGiudice et al. 2003, Ostfeld and LoGiudice 2003). This is in part due to the fact that Dutchess reported the most cases of Lyme disease from 1992-2000 (Chow et al., 2003). In addition, IES is situated on 1,924 acres of relatively undisturbed land, making it an ideal place to study the complex enzootic cycle of Lyme disease transmission.

METHODS

In order to determine nymphal burdens on shrews, I trapped shrews between June 19th and June 25th and between July 3rd and July 23rd 2005 on the property of the Institute of Ecosystem Studies (IES) in Millbrook, New York.

In order to capture as many shrews (*Blarina brevicauda*) as possible, I trapped animals using both dry pitfall traps and Sherman live traps. Trapping was irregular and often correlated with weather conditions, with particular trapping efforts on cool, wet days and at dusk and dawn (R.S. Ostfeld, personal communication). This method of trapping proved ineffective and I eventually turned all trapping efforts to 11 X 11 permanent grids of Sherman traps. With the help of a field crew, I trapped at three sites, alternating sites each week, on IES property. The sites contained two grids each and I trapped for a total of 1815 trapnights.

When a shrew was caught, I immediately fed it as many meal worms as it could eat. After it had eaten, I transported the shrew, still inside a Sherman trap, to the animal rearing facility on IES property. I housed each shrew in an individual wire mesh cage, which I suspended above a tub lined with double sided tape and filled with ¼ inch of water. In the cage, I placed a small plastic shelter (i.e. Empty yogurt container), a small moist sponge, and a Petri dish to hold food. I placed the entire setup, including tub and cage, on a set of shelves with timed lighting. The light was time regulated, on for twelve hours a day (6 a.m. to 6 p.m.). I partially draped the cages with black plastic to provide relief from the lighting. Approximately half of each cage was sheltered by the plastic.

Shrews were normally fed every 3-4 hours with large (4-5 cm long) meal worms. At times when I could not feed the shrews as frequently, I impaled 10-12 meal worms with a thin wire and placed the wire in the cages. This prevented the meal worms from falling through the mesh of the cage, providing a more long-term food source for the shrews.

I checked and changed the water in the tub twice a day. Each time the water was checked, I recorded the number of larva and nymphs present in the water and whether they were engorged or not. The double-sided tape lining the tubs prevented any ticks from escaping. I removed the ticks from the water with a fine paint brush and placed them, according to their life-stage, into vials. The vials contained plaster, which I moistened with deionized water to provide a constant source of humidity before depositing any ticks into the vials. I placed all vials containing ticks into an incubator at 25°C and allowed the ticks to molt into their subsequent life stages.

I kept shrews for 72 + hours to ensure that all ticks had fallen off into the water and been collected. After this time, I transported each shrew in its wire mesh cage back to the site of capture and released it.

DATA ANALYSIS

For each week that I collected shrews I calculated the average number of nymphs on a shrew at time of capture. Two out of the four weeks that I captured shrews, I recorded no nymphs on shrews. For these weeks I assumed my lowest measured nymphal burden on shrews, 0.25 nymphs per shrew.

Because I captured shrews for just a few weeks of the summer, my data collection represented only a fraction of the nymphal season. In order to produce a seasonal average nymphal burden for shrews, I extrapolated nymphal burdens on shrews for weeks I had not sampled using data on the nymphal burdens on chipmunks during this time. For each week that I had not captured shrews, I compared the nymphal burden on chipmunks for this week to the nymphal burden on chipmunks during each of the 4 weeks that I had sampled. Therefore, for each week that I had not sampled, I produced four separate correction factors based on the four weeks that I had sampled. I multiplied these correction factors by the corresponding nymphal burden on *B. brevicauda* during the four weeks I did sample shrews. This produced four different projections for a given week that I had not sampled. I averaged

these four projections to obtain my projected average nymphal burden on shrews during the weeks I had not sampled.

I combined my extrapolations with my collected data to produce a seasonal average nymphal burden on shrews. I built Poisson distribution from this seasonal average from which I obtained the probability that, at any given time, a shrew would have 0-3 nymphs on it (the probability of having more than 3 nymphs on a shrew was miniscule, in fact I never captured a shrew with more than 1 nymph on it).

To predict the seasonal nymphal burden on *B. brevicauda*, I first broke the nymphal season into 26 discrete 3-day time steps because nymphs normally feed for approximately three days. Each three day time-step carried with it the probability of having 1, 2, or 3 nymphs on a shrew during that time period. I created an algorithm, using Visual Basic in Microsoft Excel that calculated the likelihood of having a given number of nymphs on a shrew in an entire nymphal season (code in appendix). For each seasonal nymphal burden, 0 – 50, the program evaluated every possible way of attaining that nymphal burden over 26 separate time periods. For each possible combination of nymphal burdens during the 26 time periods that led to the same seasonal nymphal burden, the program calculated the probability of this event. Finally it summed the probabilities of each combination of nymphal burdens leading to the same seasonal nymphal burden. This produced a distribution of probabilities over all possible seasonal nymphal burdens.

Using the frequency of each strain of *B. burgdorferi* in the host seeking nymph population (Brisson and Dykhuizen, 2004) I calculated the minimum number of nymphs needed to verify or reject the hypothesis that the 6 strains of *B. burgdorferi* found by Brisson and Dykhuizen (2004) to infect northern short-tailed shrews were not due to chance alone. To do this, I predicted, based on the probability of each of these six strains, how many shrews would be infected if a given number of nymphs fed on them. I varied the number of nymphs until the predicted number of infected shrews did not differ significantly from the actual number of shrews infected.

ANALYSIS

During my four weeks of trapping I collected a total of 11 northern short-tailed shrews, which were host to a total of three nymphs. Using this year's data collected on nymphal burdens on chipmunks (Figure 3) on IES property I extrapolated what nymphal burdens would have been during weeks that I did not sample. In weeks where chipmunks had relatively high nymphal burdens, my extrapolated nymphal burdens on *B. brevicauda* were likewise high (Figure 4). The average number of nymphs to be found on an individual through at any point throughout the nymphal season was 0.61 +/- 0.40 (two standard errors).

To predict the probability that a northern short-tailed shrew would have a given number of nymphs on it over the course of an entire nymphal season I drew from a Poisson distribution based on an average nymphal burden of 0.61. The probabilities of having 0, 1, 2, or 3 nymphs were 0.543, 0.331, 0.101, and 0.021 respectively. Therefore, at any point in time, shrews are most likely (probability of 0.543) to have zero nymphs feeding on them. Using these individual probabilities, I predicted the probability that a shrew would have seasonal nymphal burden of 0-30.

Brisson and Dykhuizen (2004) found that shrews are only susceptible to 6 of the 15 strains of *B. burgdorferi*, two of which are humanly infectious. In order to assure that these data are not due to chance, I calculated that **twelve** or more nymphs must bite each shrew throughout the course of the nymphal season. The probability that *B. brevicauda* are bitten by more than twelve nymphs in a season is 0.625.

DISCUSSION

Brisson and Dykhuizen indicate that *B. brevicauda* allows the establishment of infection of only certain strains of the Lyme disease bacterium. The establishment of infection with a strain of *B. burgdorferi* is related to the

specific ospC phenotype of the bacteria. There are fifteen variations in this protein common to the northeast. Four of these variations correspond to humanly infectious strains of Lyme disease bacterium. If *B. brevicauda* is frequently bitten twelve or more nymphs, we can assume that the findings of Brisson and Dykhuizen (2004) are, in fact, due to the widespread exposure of *B. brevicauda* to all strains of Lyme disease bacteria. This implies that *B. brevicauda* is regularly exposed to and infected by humanly infectious strains of Lyme disease bacteria. This implies that *B. brevicauda* may be considered a reservoir for the maintenance of Lyme disease infection.

Shrews infected with humanly infectious strains of *B. burgdorferi* represent a dangerous group of hosts. These shrews are bitten by many larvae (LoGiudice et al. 2003); therefore they have the potential of infecting many larvae with humanly infectious strains of *B. burgdorferi*. These larvae will molt into nymphs carrying humanly infectious strains of *B. burgdorferi* able to infect humans and other animal hosts, thereby proliferating humanly infectious strains of the bacteria (Figure 5)

Although the white footed mouse has received much attention for being the most competent reservoir for *B. burgdorferi*, the northern short-tailed shrew appears to be an important component in the maintenance of Lyme disease. Being insectivorous, the northern short-tailed shrew is unlikely to experience drastic population variation related to the masting of acorns. This means that *B. brevicauda* may, in fact, be a "rescue species," (LoGiudice et al., 2003) able to maintain high levels of Lyme disease risk even in instances of low white-footed mouse density.

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APPENDIX

	A	**B**	D	E	F	G	H	**I**	J	**K**	L	M	N	T	U
Blarina brevicauda	■		■	■	■					■				■	

FIGURE 1. Strains found by Brisson and Dykhuizen (2004) to infect *B. brevicauda*. Black boxes indicate infection in *B. brevicauda*. Large bold letters indicate strains infectious to humans. Shrews appear to be infected by strains A and K, both of which are infectious to humans.

FIGURE 2. The setup used to house shrews.

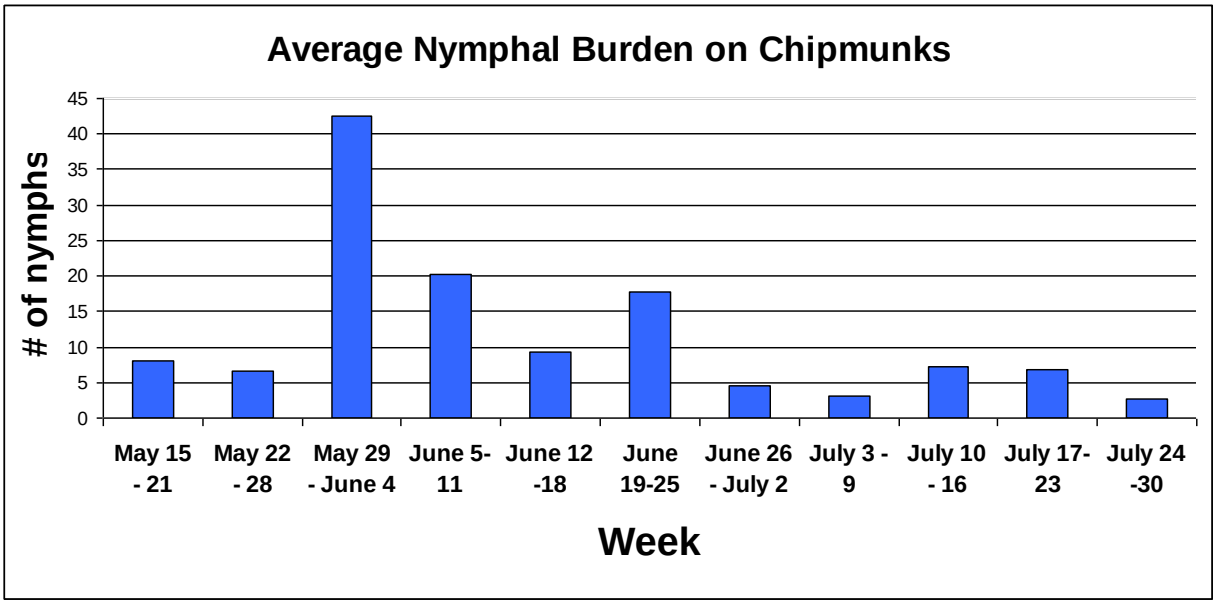

FIGURE 3. Average nymphal burden on chipmunks

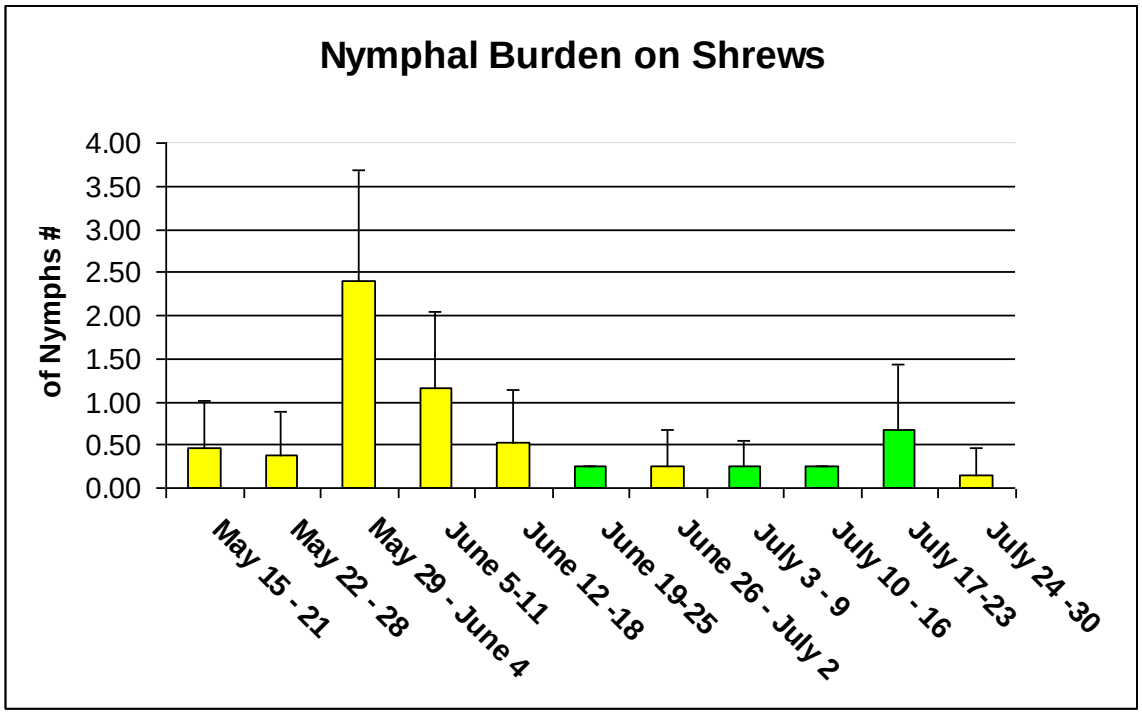

FIGURE 4. Average nymphal burdens on *B. brevicauda*. Green bars indicate weeks in which I trapped *B. brevicauda*, yellow bars indicate data calculated from the nymphal burden on chipmunks.

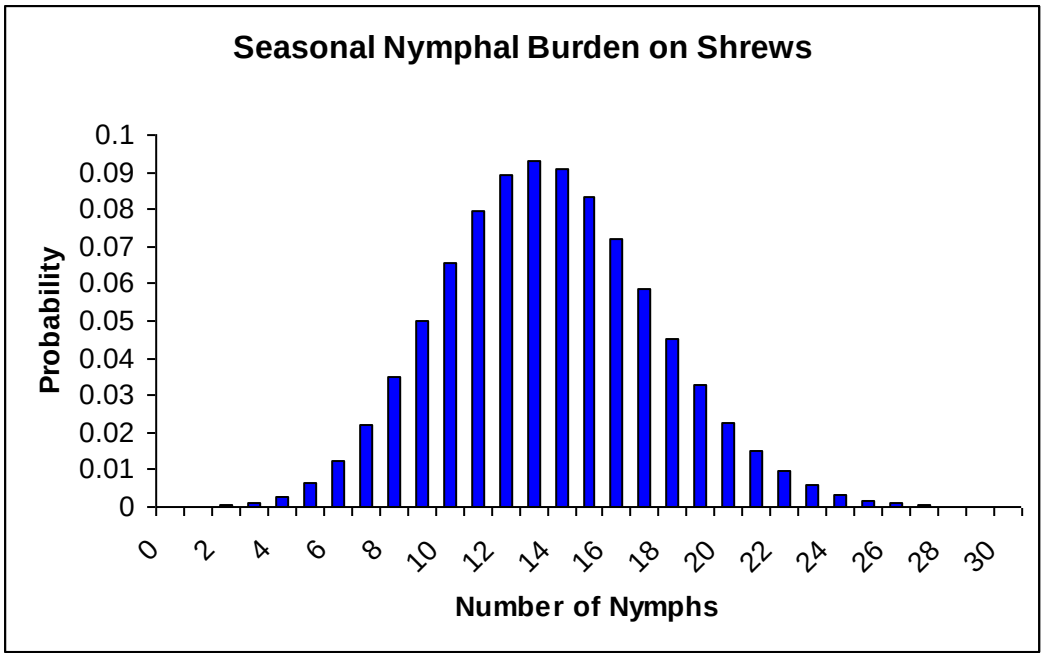

FIGURE 5. The number of nymphs is displayed along horizontal axis and the probability of these nymphal burdens is displayed along the vertical axis. The most probable nymphal burden is 13 nymphs per season.

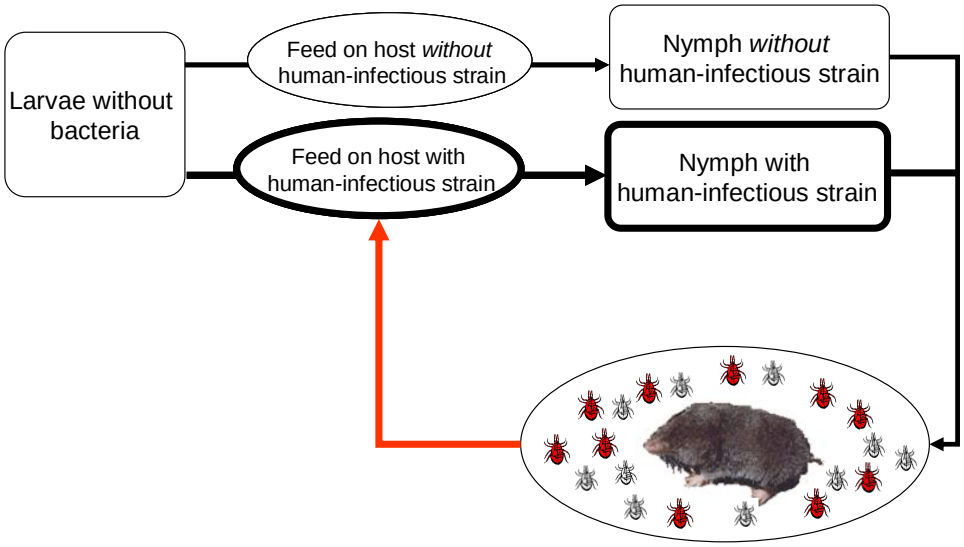

FIGURE 6. Red ticks represent those carrying humanly infectious bacteria, grey represent those that are not humanly infectious. *B. brevicauda* is frequently bitten by enough nymphs to expose it to humanly infectious strains of *B. burgdorferi*. Once infected with these strains, *B. brevicauda* may pass the strains on to uninfected ticks, increasing the number of nymphs able to infect humans with Lyme disease bacteria.