Ticks Feeding on Animals Committed to Wildlife Rehabilitation Centers and Implications for Lyme Disease Ecology

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Abstract Lyme disease has become a significant infectious disease concern in the Western United States. This study investigates the ecology and transmission of the spirochete causative agent of Lyme disease, *Borrelia burgdorferi*, in an area of Northern California, USA by examining the host selection of different tick species and life stages. Ticks were collected from a wildlife rehabilitation center near the San Francisco Bay to investigate disease transmission and how it relates to tick and host animal ecology in the area. Tick species, tick life stage, and host species were compared to infer their relationship to local Lyme disease transmission. Despite lower than expected sample sizes, this study uncovered some evidence of novel host selection relationships, and provided an initial evaluation of this new parasite collection method. This research has allowed for valuable insight into the enzootic maintenance and cycling of spirochete populations and the relative importance of different ticks and vertebrate hosts to this process.

Introduction

Lyme disease, caused by the tick-borne spirochete *Borrelia burgdorferi*, is the most prevalent arthropod vectored disease in North America (Orloski et al. 2000). Infection most commonly manifests itself as the characteristic skin lesion Erythema migrans (Grubhoffer et al. 2005), but can develop into a range of more serious symptoms, such as arthritis, meningitis, and unilateral facial palsy (Piesman and Gern 2004). *B. burgdorferi* is maintained through enzootic cycles in temperate environments throughout the world (Brown and Lane, 1992). However, the ecology of its maintenance and transmission shows extreme variability between different habitats. The far western United States exhibits a much lower prevalence of Lyme disease than the eastern states due to a spirochete transmission cycle created by a unique fauna of ticks and vertebrate hosts.

Ticks are one of the most influential ectoparasites to the health of humans and domestic animals due to their extensive vectoring of pathogenic organisms (Furman and Loomis, 1984). Transmission of *B. burgdorferi* between a variety of vertebrate hosts occurs via Ixodidae ticks, commonly known as "hard ticks." Their proficiency as a vector is due to the fact that Ixodids typically remain attached to their host for several days, allowing for transmission of *B. burgdorferi* (Parola and Raoult, 2001).

The majority of Ixodid ticks exhibit a 3-part active life cycle (larva, nymph, and adult) in which they feed on a host once per stage (Sonenshine, 1991), permitting three points in a tick's life where *B. burgdorferi* can potentially be transmitted. The host selection of each tick species, at each of these life stages, is paramount in importance to the spread of Lyme disease among a community of vertebrate hosts that often includes humans.

Forty-nine tick species are present in California (Furman and Loomis, 1984). Three species, *Ixodes pacificus, Ixodes spinipalpis*, and *Ixodes jellisoni* have been identified as the primary vectors of *B. burgdorferi* (Piesman, 2002). However, several other species are also competent vectors (able to maintain and transmit *B. burgdorferi*). Thus, several ixodid species hold considerable importance to the transmission of Lyme disease in California; in contrast to the eastern United States where one tick species, *I. scapularis*, is paramount to both spirochete maintenance and transmission to humans (Lane et al., 1991). *I. pacificus* is California's primary vector to humans, but due to its lack of host specificity, which includes feeding on reservoir-incompetent vertebrate hosts, it is considered relatively unimportant in spirochete maintenance

(Burgdorfer et al., 1985). In California, *I. pacificus* has been found in 53 of 58 counties, at elevations up to 2150 meters (Furman and Loomis, 1984). This wide distribution and propensity to feed on humans constitutes a major public health concern in the state of California.

B. burgdorferi has been detected in 32 species of birds and mammals in California (Lane et al., 2005). However, significantly fewer have been identified as reservoir competent, meaning that after being infected they maintain the spirochetes long enough to infect subsequently feeding ticks. Three rodent species (the western gray squirrel, California kangaroo rat, and dusky-footed wood rat) are commonly parasitized and reservoir competent hosts (Brown and Lane, 1996; Lane et al., 2005). Some research has also identified avian species as capable of *B. burgdorferi* infection, which helps explain the homogeneity of spirochete populations across great distances; however results are inconsistent (Wright et al., 2006; Slowik and Lane, 2001).

The web of transmission is further complicated in California by *Sceloporus occidentalis*, the western fence lizard. *S. occidentalis* has the novel ability to kill *B. burgdorferi* in the ticks that feed on it (Lane and Quistad, 1998). The spirochetes activate two separate molecular pathways that lyse the invading cells (Kochi and Johnson, 1988) leaving both the tick and lizard effectively disinfected. *S. occidentalis* is also the primary host for sub adult *I. pacificus* (Furman and Loomis, 1984; Lane and Loye, 1989) and as such has a cleansing effect on the population of tick vectors that infect humans. The high frequency of ticks feeding of *S. occidentalis* also reduces the force of *B. burgdorferi* transmission by diverting ticks from feeding on reservoir-competent vectors like the western gray squirrel (Lane and Quistad, 1998). The presence of *S. occidentalis* is thus a major contributor to the lower frequency of Lyme disease found in California, relative to the Eastern United States.

The extreme variability in the prevalence of *B. burgdorferi* and its patterns of transmission at different locations highlights the importance of tick-host interactions to the enzootic cycle. This ecological relationship has been the subject of a large body of research, particularly in the western United States, which exhibits a unique system of enzootic maintenance and transmission to humans. However, the vast majority of research has focused on a few species thought to be central to spirochete transmission. The influence of many other tick hosts, such as predatory mammals, birds of prey, and marsupials, is relatively unknown.

To gain new insight into the tick-host ecology behind spirochete cycling this study employs a novel collection methodology: tick collection from wildlife rehabilitation centers. The effectiveness of this collection method is herein evaluated to determine the viability of its application in future studies and the problem of *B. burgdorferi* transmission is approached from a broad ecological perspective by examining the relationship of tick species and life stage to a wide range of host species.

Materials and Methods

Sampling Methods Tick samples were collected from the Lindsay Wildlife Museum (LWM), a wildlife rehabilitation center in the city of Walnut Creek, California. LWM is one of the oldest and largest wildlife rehabilitation centers in the United States and treats over 6000 animals a year. Injured animals are collected from all over California; however the majority of patients come from the San Francisco Bay Area, particularly Contra Costa County. The facility operates 7 days a week, 52 weeks a year, and sees over 200 different animal species. Injured animals are brought in by members of the public who happen upon them. LWM provided a tick collection mechanism that was able to gather samples over a longer period of time, and broader area than would ever have been possible with field collection.

Collection commenced on December 11, 2006 and was completed on April 16, 2007, giving a total of 18 weeks of collection. However, this represents a preliminary data set for the purpose of a pilot study and collection will be continued to the end of the 2007 calendar year to control for seasonal variation.

The rehabilitation facility searched every animal that they treated and collected any ticks that were found. Rehabilitation staff members were instructed to standardize their search efforts for feeding ticks in order to control for any bias toward certain animal host species. Bird, rodent, bat, and reptilian species were searched for ~2 minutes and marsupial and larger mammalian species were searched for ~3-4 minutes. Search efforts were thus roughly proportional to the size of the animal being searched. Within the confines of having staff members doing the collecting this was the most realistic and controlled method possible. I was also assured that the staff was always diligent as possible in searching the entirety of each animal for parasites.

Ticks were removed using forceps and placed into vials containing 95% ethanol. Rehab facility staff were instructed to pull ticks free by gripping as close to the host's skin as possible in order to maintain tick mouthparts to aid in identification. The ethanol solution was used to prevent desiccation and degradation of tick morphology and to preserve the DNA of potential *B.*

burgdorferi spirochetes for PCR identification at a later date. All ticks from an individual host were placed in a single vial of ethanol and the vial was labeled with the unique identification number assigned to that animal by the rehabilitation center.

All data fields corresponding to a particular animal, and the vial of ticks it carried, were entered into the existing computer system used by the rehabilitation centers. This included the identification number, date collected, animal species, location animal was found, and the number of ticks collected. The location where the animal was found was ascertained by rehabilitation center staff by interviewing the person who found the animal. The above information was also collected for the animals coming through the facility during the collection period carrying no ticks, to show negative results.

Due to the low number of ticks collected during the controlled sampling period, samples previously collected from LWM were also included. These samples were collected sporadically from 1997 to 2002. No controlled collection was utilized during this period so these samples cannot be thought of as representative of all of the tick carrying animals to pass through LWM during this period and are thus not appropriate for any statistical analysis. However, they are useful as sources of empirical evidence of host selection. All of the same information was recorded for these historically collected samples as described above.

Data Analysis Ethanol preserved tick samples were retrieved from the rehabilitation centers at a minimum of once every two weeks and brought back to the Robert Lane Laboratory at UC Berkeley for identification. Ticks were identified to their species and life stage using the guidelines of Furman and Loomis (1984) and under the guidance of Joyce Kleinjan, who is greatly experienced in the identification of California ticks.

Further analysis and statistical tests were deemed to be unnecessary due to the low sample volume produced during this initial collection period and the results were examined purely observationally.

Results

During the 18 weeks of collection, from December 11, 2006 to April 16, 2007, a total of 1030 animals were treated at LWM and searched for ticks. Table 1 shows the time course of collection, displayed as the number of animals seen per day. 103 different host species are represented in the sample set (Figure 1), the vast majority of which are bird species (76.7%), particularly passerine birds (49.8%).

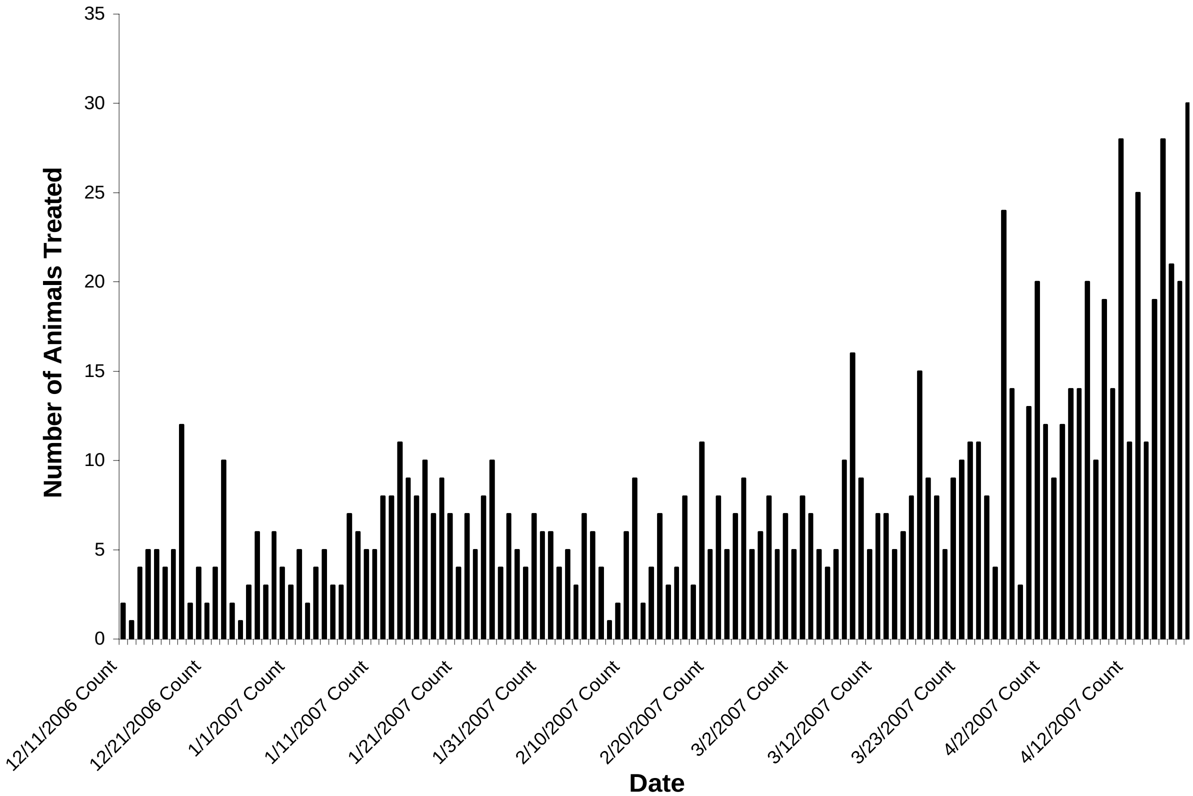

Figure 1. The number of animals treated per day at LWM from December 11, 2006 to April 12, 2007.

Table 1. Summery table of animal groupings, along with the number of individuals, the number of species, and the percent infested with ticks.

Hosts Examined	Number Collected	Number of Species	Number (%) Infested
Passerine Birds	513	41	0
Rodents	150	7	0
Raptors	83	10	0
Hummingbirds	82	1	1.22
Precocial Birds	65	10	0
Marsupials	46	1	0
Other Mammals	27	4	3.70
Sea Birds	24	12	0
Game Birds	13	3	0
Reptiles	10	6	0
Scavenging Birds	6	1	0
Amphibians	4	2	0
Wading Birds	4	3	0
Predatory Mammals	3	2	66.67
Totals	**1030**	**103**	**4**

Of the 1030 animals searched for ticks only 4 (0.4%) were found to carry them. A Black-Tailed Hare (*Lepus californicus*) was found to carry an adult *Haemaphysalis leporispalustris*. An Anna's Hummingbird (*Calypte anna*) was found to carry an adult *Ixodes brunneus*. One Gray Fox (*Urocyon cinereoargenteus*) was found to carry 8 *I. pacificus* adults and another Gray Fox was found to carry three *Ixodes rugosus* adults and three immature *I. pacificus* (2 nymphs and 1 larva).

The historically collected samples consisted of ticks taken off of 11 animals during the years of 1997 through 2002. All of the ticks collected were of two species: *Dermacentor variabilis* and *I. pacificus* (Table 2).

Table 2. Historically collected tick samples along with the date of their collection, host animal species, and the number of ticks of the two species found to be present. Letters in parentheses represent the life stage of the ticks.

Date	**Host Species**	**D. variabilis**	**I. pacificus**
5/2/1997	Western Tanager	0	2(L)
5/20/1997	Opossum	3(A)	0
6/29/1997	Coyote	4(A)	0
12/19/2000	Black tailed deer	0	3(A)
5/14/2001	Black tailed deer	80(A)	1(A)
6/15/2001	Mourning Dove	0	1(N)
9/17/2001	Brush Rabbit	7(L)	0
12/15/2001	Hermit Thrush	0	1(L)
6/1/2002	Raccoon	7(A)	0
8/5/2002	Opossum	5(A)	0
8/23/2002	Coyote	1(A)	0

Discussion

The number of ticks collected during the 18 weeks of this study was decidedly less than expected and of these results obtained some level of selection bias is likely to be inherently embedded in this collection technique. Certain types of animals are more likely to be encountered by the public and brought into rehabilitation centers. For example, small birds, often injured by domestic cats, are much more common at LWM than wood rats. Injured animals may also carry more or less ticks than healthy individuals due to ticks either preying on debilitated animals or abandoning sick ones. For these reasons the forming of any significant statistical comparisons is extremely difficult. However, this pilot study still holds considerable importance and relevance through its empirical observations, potential to guide future research, and evaluation of this new tick collection method.

The absence of large numbers of ticks collected in this study is most likely due to seasonal lulls in both the volume of animals received by the facilities and the number of questing ticks. The amount of animals to come through the rehabilitation centers is greatly influenced by the weather. During the warmer months of the year people are more likely to be outdoors and to encounter injured wildlife. This trend is illustrated by the higher animal traffic through LWM toward the end of the collection period, during the months of March and April. The seasonal variation in tick collection at wildlife rehabilitation centers is one limitation of this type of collection.

The late winter to early spring months, during which the majority of collection was conducted, also tend to be low points in questing tick activity. Eisen et al. (2002) found that *I. pacificus* nymphs don't typically become active until mid-March, and peak in density in early May. This study showed a similar increase in tick activity and collection in the later spring months.

The black-tailed hare, found to be carrying a *H. leporispalustris* tick, is a commonly identified host of this tick. Rabbits are the only known host for adult *H. leporispalustris* (Furman and Loomis, 1984) and both have been reported to carry *B. burgdorferi*. Previous research in Northern California has isolated *B. burgdorferi* spirochetes from rabbits (Peavey et al., 1997) and the *H. leporispalustris* ticks that they carry (Lane and Burgdorfer, 1988). It has been suggested that rabbits may serve as a maintenance pool of spirochetes and pass them to other vertebrates through more generalist tick species. This study is inconclusive on the matter without collection of more rabbits.

Both of the gray foxes treated during the collection period carried ticks. *I. pacificus* adults, nymphs, and larvae were found between the two foxes. This is a testament to how common the tick is in California and how wide their range of hosts is. *I. pacificus* of every life stage have been collected during every season in California and are found on 52 host species (Furman and Loomis, 1984).

I. rugosus, found on one of the foxes, are much less commonly collected in California. Their primary host is the spotted skunk (*Spilogale gracilis*) but adults have been found on the gray fox and other carnivores (Furman and Loomis, 1984). *B. burgdorferi* has never been detected in *I. rugosus* so they are unlikely to contribute to the maintenance or transmission of the spirochetes. However, this is an example of a rarely seen ecological relationship.

Anna's hummingbird is a formerly unidentified host for *I. brunneus*. This is the first record of *I. brunneus* being recovered from this particular bird species that the author is aware of. *I. brunneus* is commonly found on ground-feeding birds and is thought to be harmful to its host (Bishopp and Trembley, 1945). In the case of the hummingbird, this adverse affect may have lead to its injury and committal to the wildlife rehabilitation center. Anna's hummingbirds are also migratory species and may provide a mechanism for long distance transport of *B. burgdorferi* (Russell, 1996). However, it's not known if they're competent spirochete hosts, which requires further investigation.

The historically collected samples were more characteristic of ticks in California. *I. pacificus* and *D. variabilis* are two of the most common ticks, with some of the broadest host ranges. Both have been recorded year round, (Furman and Loomis, 1984) however in this case all but two of the collections were made during the months of May-September. This observation helps explain the low number of samples collected during the controlled period, from December to April. This also illustrates the potential of year round collection from LWM and other wildlife rehabilitation centers.

This paper has allowed valuable insight into the tick host ecology in one region of Northern California and illustrated some of the strengths and limitations of tick collection from wildlife rehabilitation centers. The collection of host species underrepresented in previous research has provided evidence that some species not traditionally considered to be contributors to spirochete cycling may have a considerable impact. It has also allowed for a better general understanding of tick ecology. However, further research is needed to draw any powerful conclusions. A broad spectrum of vertebrate species need to be collected in field studies, that are less vulnerable to host selection bias, and tested for the presence of spirochete infection. It can then be concluded whether or not they are competent hosts for *B. burgdorferi* and what their actual contribution to spirochete ecology might be.

Lyme disease constitutes a significant and emerging public health threat and further research is important to understand how spirochete populations are maintained and reach humans. With changing climate and local host ecology, traditional thinking of Lyme disease ecology may not be appropriate. As host populations change, different species may change in their relative importance to *B. burgdorferi* transmission. Further research is needed to keep up with this dynamic environment and novel collection methods such as this may become increasingly important.

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