

USE OF A SENTINEL HOST SYSTEM TO STUDY THE QUESTING BEHAVIOR OF *IXODES SPINIPALPIS* AND ITS ROLE IN THE TRANSMISSION OF *BORRELIA BISSETTII*, HUMAN GRANULOCYTIC EHRLICHIOSIS, AND *BABESIA MICROTI*

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Abstract. *Ixodes spinipalpis* maintains *Borrelia bissettii* spirochetes in Colorado in a cycle involving wood rats and deer mice. This tick has been described as nidicolous, remaining either attached to its rodent hosts or in the rodent nest. Nidicolous ticks pose little risk of pathogen transmission to humans if they do not actively quest for hosts. To investigate the questing potential of *I. spinipalpis*, sentinel mice were placed in an area where *I. spinipalpis* had been commonly found on wood rats and deer mice. Concurrently, wild rodent populations were trapped and analyzed for Lyme disease spirochetes, the agent of human granulocytic ehrlichiosis (aoHGE), and *Babesia microti*. A total of 122 *I. spinipalpis* larvae and 10 nymphs were found on 19% of 244 sentinel mice. In addition, 4 sentinel mice became infested with *Malaraeus telchinus* or *Orchopeas neotomae* fleas. Questing *I. spinipalpis* were positively associated with woody shrubs and negatively associated with sunny and grassy areas. Four sentinel mice became infected with aoHGE after having been fed upon only by *I. spinipalpis* larvae. One sentinel mouse became infected with *B. bissettii* after having an *I. spinipalpis* nymph feed on it, and one sentinel mouse became coinfecting with aoHGE and *B. bissettii* after it was fed upon by a single *I. spinipalpis* nymph. These sentinel mouse conversions suggest the possibility that the aoHGE is transovarially transmitted by *I. spinipalpis*, and that *I. spinipalpis* is capable of simultaneously transmitting *B. bissettii* and the aoHGE. The findings that *I. spinipalpis* quest away from rodent nests and will attach to and infect sentinel mice may be of public health importance. It suggests the potential transmission of the agents of human granulocytic ehrlichiosis and Lyme disease to other hosts by *I. spinipalpis*, in regions of the western United States where *Ixodes pacificus* is not found.

INTRODUCTION

Lyme disease is the most common vector-borne disease in North America.¹ The disease has generated great interest and debate from the medical and scientific communities, including several issues regarding the epidemiology and vector-host reservoirs for *Borrelia burgdorferi* transmission. One concern is the possibility that geographic areas exist with unrecognized enzootic cycles of *B. burgdorferi sensu lato* that may be infective to humans, resulting in occasional cases.

Despite the debate on the taxonomy of the tick vector along the East Coast of the United States (*Ixodes scapularis* or *I. dammini*),² the epidemiology and ecology of the disease on the East Coast and upper Midwest has been the best studied, with *I. scapularis* being widely accepted as the vector transmitting *B. burgdorferi* among both human cases and the major reservoirs, *Peromyscus leucopus* and *Tamias striatus*.³

In the southeastern states, an anomalous situation exists where *Ixodes scapularis* is widespread but is rarely found infected with *B. burgdorferi* or attaching to humans.⁴ Although *B. burgdorferi* isolations from humans are made,⁵ such isolations are uncommon in this region, although a clinically similar disease, termed Southern Tick-Associated Rash Illness (STARI), is not unusual and is associated with the bites of *Amblyomma americanum* ticks.⁶

Lyme disease in the western United States is both less common and ecologically more complex than described on the East Coast. In California, *I. pacificus* is the vector to humans.⁷ A second tick species, *Ixodes spinipalpis*, is important in maintaining the spirochete among the principal reservoir hosts, wood rats (*Neotoma* spp.) and mice (*Peromyscus* spp.), in California,^{8,9} Oregon,¹⁰ and Colorado.¹¹ *Ix-*

odes spinipalpis, however, has been described as a nidicolous tick, being closely associated with its principal rodent hosts. Thus, *I. spinipalpis* is not believed to pose a significant threat of transmission of *B. burgdorferi* to humans.

Related to these foci is the question of whether Lyme disease is over- or underreported. A comparison of distribution maps of the recognized tick vectors to humans, *I. scapularis* and *I. pacificus*, with the areas for human cases meeting the U.S. Centers for Disease Control and Prevention (CDC) case definition of Lyme disease,¹² reveals areas where human cases are reported, but where neither of the major vectors are established.¹³ Because nationally reportable diseases are recorded by state of residence rather than state of exposure, undoubtedly some people are infected in states other than the one in which they reside. Anomalous cases may also result from misdiagnosis with clinically similar diseases (e.g., STARI in the southeastern states), or possible exposure to *B. burgdorferi sensu lato* strains that normally occur only in cryptic rodent-tick cycles.

In Colorado, *I. spinipalpis* maintains an endemic cycle of *B. bissettii*¹⁴ in the absence of either *I. pacificus* or *I. scapularis*.¹³ *Borrelia bissettii* is a member of the *B. burgdorferi sensu lato* group of spirochetes. Although not yet associated with human disease in North America, it has been detected in Europeans with clinical signs of Lyme disease.¹⁵ Recently in Oregon, *I. spinipalpis* was hypothesized to be potentially more important in the maintenance of *B. burgdorferi sensu lato* than *I. pacificus* because of the much higher *B. burgdorferi sensu lato* infection rate found in this tick.¹⁰ Even more recently, *I. spinipalpis* was discovered to transmit 2 additional human pathogens among rodents: the agent of human granulocytic ehrlichiosis (aoHGE)¹⁶ and *B. microti*.¹⁷

Although *I. spinipalpis* has been classified as a nidicolous

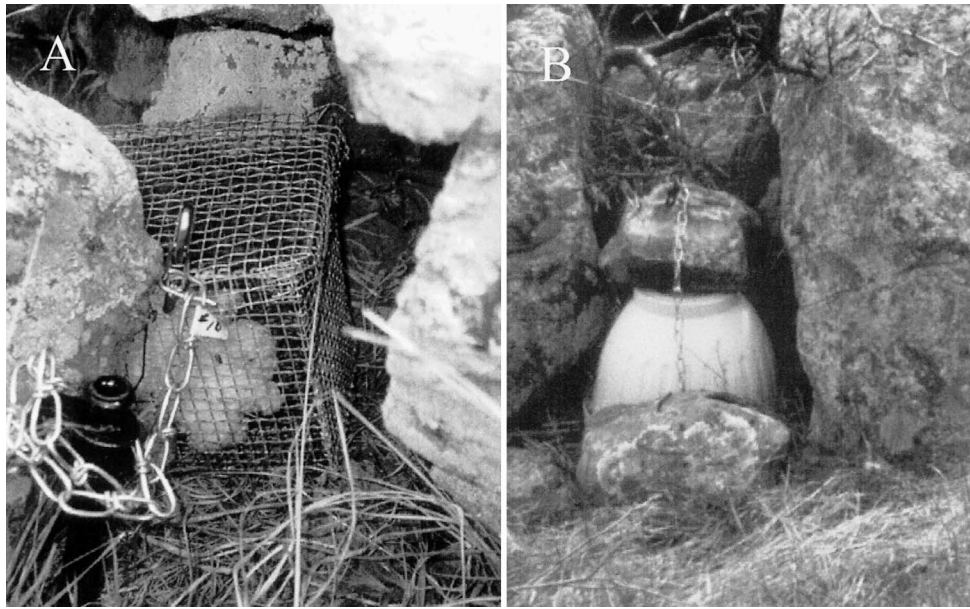


FIGURE 1. (A) Sentinel specific-pathogen-free mouse housed in a wire mesh cage with bedding material, carrot, and mouse chow. (B) In case of inclement weather, the sentinel mouse was protected in unsheltered areas by an inverted plastic flowerpot held in place with a rock.

tick, it has occasionally been collected on humans in Oregon,¹⁸ New Mexico,¹¹ and Canada¹⁹ as well as by dragging,¹⁹ and on rabbits and birds.¹⁹ The possibility exists that this tick may quest for hosts, either as a normal part of its behavior or under specific ecological conditions. Questing behavior, if it exists in *I. spinipalpis*, could not only significantly increase the potential of this tick to maintain *B. bissettii*, the aoHGE, and *B. microti* among rodents, but could potentially bring this tick in contact with humans, with the possibility of transmitting diseases associated with these pathogens.

A study was therefore conducted to define the distribution of *I. spinipalpis* in the foothills of the Rocky Mountains; an area where *I. spinipalpis* maintains endemic cycles of *B. bissettii*, the aoHGE, and *B. microti*. Because previous attempts to collect *I. spinipalpis* in this region by dragging were unsuccessful, a sentinel host system was employed. Sentinel birds are commonly used to monitor arbovirus activity,²⁰ whereas sentinel mammals have been employed to study Scrub typhus rickettsia^{21,22} and *Leishmania*²² activity, as well as to collect vector mites.^{21,22} Our results suggest that employing this method of studying the questing behavior of *I. spinipalpis* ticks was successful in detailing specific ecological niches of activity, as well as directly demonstrating this tick's ability to transmit multiple human pathogens.

MATERIALS AND METHODS

Field studies. From March through October 1999, the questing behavior of *I. spinipalpis* was studied by a sentinel host-trapping approach as described below. Concurrently, rodent populations at the study site were captured with Sherman and Tomahawk traps with rolled oats as bait. Both the study site on the Foothills Campus at Colorado State University and the trapping methods have been previously described.¹¹ Briefly, the site, at an elevation of 1,560 m, con-

sisted of rock outcroppings on an east-facing slope. Vegetation consisted primarily of skunkbrush (*Rhus trilobata*), mountain mahogany (*Cercocarpus montanus*), and various grasses.

A sentinel host system used specific-pathogen-free (SPF) white mice from the CDC colony as bait for questing ticks. Individual SPF mice were housed in wire mesh cages with bedding material, commercial mouse feed, and a carrot (Figure 1A). Each SPF mouse was placed at a defined site in the study area for 24–48 hr, weather conditions permitting (e.g., temperatures > 5°C). Inverted plastic flowerpots were placed over the wire mesh cage to provide protection from precipitation unless the sentinel site was located underneath an overhanging rock (Figure 1B). Both the mesh cage and flowerpots were secured to the site. Sites where sentinel mice were placed were characterized with respect to sun or shade, proximity to wood rat nests, presence of shrubs, rocks, and grass, and which slope the site faced (north, south, or east, or on top of a small hill).

Sentinel mice and Sherman traps (H. B. Sherman Traps, Tallahassee, FL) and Tomahawk traps (Tomahawk Live Trap, Tomahawk, WI) were checked each morning. Captured wild rodents were processed as described below. Rodents were trapped and removed from the study area to determine the prevalence of potential human pathogens in reservoir animals, as well as to enhance the possibility of questing ticks encountering a sentinel animal. Sentinel mice were returned to the laboratory and held for 6 days to allow the ectoparasites attracted to these mice to feed to repletion before identification. Ticks and fleas were identified by use of standard taxonomic keys.^{24–26} Larvae were mounted in polyvinyl alcohol mounting medium (BioQuip) before identification.

Statistical analysis. Observations on numbers of questing ticks per infested sentinel mouse were grouped for analyses. Counts of ticks from sentinel collection dates in March formed the first group. Sentinel mouse collections in April–

TABLE 1
Analyses of wild-caught animals for the presence of *Borrelia bissetii*, *Babesia microti*, and aoHGE*

Species	Common name	No. collected	<i>B. bissetii</i>	aoHGE	<i>B. microti</i>
			Positive†	Positive‡	Positive§
			% (no. positive/ no. tested)	% (no. positive/ no. tested)	% (no. positive/ no. tested)
<i>Microtus orchogaster</i>	Prairie vole	17	82 (14/17)	7 (1/17)	87 (13/15)
<i>Peromyscus maniculatus</i>	Deer mouse	80	54 (43/80)	21 (23/63)	0 (0/62)
<i>P. difficilis</i>	Rock mouse	8	12 (1/8)	0 (0/5)	0 (0/5)
<i>Neotoma mexicana</i>	Mexican wood rat	41	73 (30/41)	39 (14/36)	0 (0/36)
<i>Sylvilagus nuttallii</i>	Mountain cottontail	3	33 (1/3)	NA	0 (0/3)
<i>Reithrodontomys megalotis</i>	Western harvest mouse	3	0 (0/3)	NA	0 (0/3)

* aoHGE = agent of human granulocytic ehrlichiosis; NA = not applicable; PCR = polymerase chain reaction.

† Established by Barbour-Stoenner-Kelly-H-positive ear biopsy.

‡ Positive by PCR for the *p44* and 16S rRNA genes.

§ Positive by PCR for the 16S rRNA gene with sequence identical to the small-subunit rRNA gene (GenBank accession number U09833²⁴).

May formed the second group. Sentinel mouse observations in June–July formed the third grouping, and observations in August–September–October comprised the fourth group. Chi-square analyses of proportions of mice infested by questing *I. spinipalpis* was performed.

Laboratory studies. Captured wild rodents, as well as sentinel SPF mice, were killed with ketamine hydrochlorine (Vedco, St. Joseph, MO) before ectoparasite removal. Animals were identified according to Fitzgerald and others.²⁷ To aid identification, animals were weighed, and total body, tail, ear, and rear foot length were measured before harvesting blood by cardiac puncture and obtaining ear biopsies. In addition, spleens were removed and splenic impression smears were made before extracting DNA from the remainder of the spleen.

After blood samples were taken, thick and thin films were made and serum was separated by centrifugation. Serum samples and blood pellets were stored at -20°C if not analyzed immediately. Thick blood films were allowed to dry before removing hemoglobin by immersing in distilled water. Splenic impressions and thin films were fixed before staining with a Wright-Giemsa stain (Protocol Hema 3, Biochemical Sciences, Swedesboro, NJ). Blood films and splenic impression smears were then examined at $\times 1,000$ magnification for the presence of *Babesia* parasites.

Ear biopsies were surface-decontaminated by washing in Wescodyne (Amsco, Apex, NC) for 5 min, then two 5-min washes in 70% ethanol. Ear biopsy samples were minced before being placed in individual 4-mL snap-cap tubes with 3 mL of Barbour-Stoenner-Kelly-H culture medium supplemented with 6% rabbit serum, antibiotics, and agarose.^{28,29} Ear biopsy cultures were examined by dark-field microscopy at $\times 500$ magnification at 1, 2, and 4 weeks after culture for the presence of viable spirochetes.

We extracted DNA from both blood pellets and spleens and analyzed it by polymerase chain reaction (PCR) for the aoHGE¹⁶ and *B. microti*¹⁷ as described previously, by use of the methods of Pancholi and others,³⁰ Massung (Massung RF, unpublished data), and Persing and others,³¹ respectively. Briefly, individual spleens were teased apart between the frosted ends of 2 microscope slides and washed in phosphate-buffered saline before DNA extraction with a QIAmp DNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The DNA in the blood pellet was

extracted with a QIAmp DNA blood mini kit (Qiagen) according to the manufacturer's specifications.

The DNA was analyzed by PCR for *B. microti* with primers BAB1 and BAB4.³² Positive identification was provided by sequencing the small-subunit rRNA gene amplified by the CRYPTOFL and BAB4 primers.³¹ The CRYPTOFL and BAB4 primer set targets the first 296 bases of the *B. microti* small ribosomal RNA gene and does not cross-react with vertebrate DNA. The amplified fragments were sequenced on both strands with the ABI 377 DNA sequencer and analyzed with the SeqMan II computer program (DNASar, Madison, WI). Positive control DNA was extracted from the MN1 strain of *B. microti*³¹ and run in parallel with experimental field samples.

The DNA was analyzed by PCR for the aoHGE with 2 sets of primers. The first primer pair, Ehr521 and Ehr747, targets a 247-bp portion of the 16S rRNA gene of aoHGE,³⁰ and the second primer pair, MSP3F and MSP3R, targets a 334-bp portion of the *p44* gene of aoHGE.¹⁶ Positive control DNA was extracted from the spleen of a C3H/HeJ mouse that had been exposed to *I. scapularis* ticks from Spooner, Wisconsin.¹⁶

RESULTS

From February through October 1999, a total of 155 animals of 6 different species were trapped (Table 1). The most common species trapped at the study site were *Peromyscus maniculatus* (deer mouse), *Neotoma mexicana* (Mexican wood rat), and *Microtus orchogaster* (prairie vole) with 80, 41, and 17 animals, respectively. We found that 82, 73, 54, 33, and 12% of the *M. orchogaster*, *N. mexicana*, *P. maniculatus*, *Sylvilagus nuttallii*, and *Peromyscus difficilis* we captured were positive for *B. bissetii* by ear biopsy culture. The DNA of the aoHGE was present in 39% of *N. mexicana*, 21% of *P. maniculatus*, and 7% of *M. orchogaster*. Only *M. orchogaster* was infected with *B. microti*, with 87% of the animals positive by PCR analysis.

A total of 392 *I. spinipalpis* ticks (346 larvae and 46 nymphs) were removed from *M. orchogaster*, *N. mexicana*, *P. maniculatus*, *Reithrodontomys megalotis*, and *P. difficilis* in the study area (Table 2). Prevalence of *I. spinipalpis* ranged from 71% of *M. orchogaster* infested with an average of 4.2 ticks per infested animal (standard deviation [SD] = 4.5) to 33% of *R. megalotis* infested with an average of 1.0

TABLE 2
Prevalence and mean density of *Ixodes spinipalpis* on infested rodents at the foothills study site

Species name	No. of rodents examined	Infested with <i>I. spinipalpis</i> *		
		Larvae, % (mean \pm SD)	Nymphs, % (mean \pm SD)	Total, % (mean \pm SD)
<i>Microtus orchogaster</i>	17	59 (4.5 \pm 5.2)	38 (1.0 \pm 0.0)	71 (4.2 \pm 5.2)
<i>Peromyscus maniculatus</i>	80	58 (4.5 \pm 5.2)	5 (4.5 \pm 5.2)	60 (5.5 \pm 7.0)
<i>Peromyscus difficilis</i>	8	38 (1.7 \pm 1.1)	0 (0.0 \pm 0.0)	38 (1.7 \pm 1.1)
<i>Neotoma mexicana</i>	41	24 (3.7 \pm 3.4)	29 (2.8 \pm 1.9)	49 (3.4 \pm 2.2)
<i>Reithrodontomys megalotis</i>	3	33 (1.0)	0 (0.0 \pm 0.0)	33 (1.0)

* SD = standard deviation. Data are expressed as the percentage infected with ticks (mean number of ticks per infested animal \pm SD).

tick per infested mouse (Table 2). A single *S. nuttallii* was examined for ticks: 100 *I. spinipalpis* were removed (1 adult male, 8 nymphs, and 91 larvae) as well as 20 *Haemaphysalis leporis-palustris* (1 adult male, 18 nymphs, and 1 larva). A single replete *I. spinipalpis* nymph was also collected from one yellow-breasted chat (*Icteria virens*).

In addition to the *I. spinipalpis* found on wild-caught rodents, a total of 301 fleas in 5 species were collected from *M. orchogaster*, *N. mexicana*, *P. maniculatus*, and *P. difficilis*. A sample of the fleas was identified from each host species. Fleas were collected from 53% of *M. orchogaster* (9 of 17 infested; mean per infested animal \pm SD, 5.7 \pm 6.9) with 38% being *Orchopeas neotomae* and 62% being *Orchopeas leucopus*. Fifteen percent of *P. maniculatus* (12 of 80) were infested with *Aetheca wagneri*, *O. neotomae*, and *Neopsylla inopina*, with 56, 33, and 11% of identified fleas, respectively, with a mean of 3.9 fleas per infested *P. maniculatus* (SD = 7.6). Fifty-six percent of *N. mexicana* (23 of 41) were flea infested, with 94% being *O. neotomae* and 6% *Malaraeus telchinus*; *N. mexicana* were infested with a mean of 8.6 fleas (SD = 27.9). Twenty-five percent of *P. difficilis* were infested with *A. wagneri*, with a mean per infested animal of 3.0 (SD = 1.4). Fleas were not found on *Reithrodontomys megalotis* ($n = 3$).

A total of 244 SPF mice were used as sentinel animals during the course of the study. Forty-one different sites in the study area housed sentinel mice at up to 10 different time periods between March and October 1999. Data on the percentage of time that a mouse was at a site with a particular characteristic are given in Table 3. The most commonly used sites were described as being under or near shrubs

TABLE 3

Habitat characteristics of sites where sentinel specific-pathogen-free mice were located

Characteristic	Percentage*
Among or under shrubs	83
In shade throughout the day	64
Proximity to a wood rat nest	55
In or among rocks	52
In a grassy area	28
Partially sunny during the day	26
In direct sun throughout the day	10
Slope	
East	43
North	20
On top of a hill	20
South	18

* Sites were characterized for >1 characteristic. Therefore, percentages add up to >100.

(83%), in a shady area (64%), near a wood rat nest (55%), in or among rock outcroppings (52%), and on an east-facing slope (43%). Sentinel mice at 63% ($n = 26$) of the 41 sites attracted *I. spinipalpis* ticks during at least one sample period.

Over the 10 sample periods, 19% (46 of 244) of the sentinel mice were infested by 132 questing *I. spinipalpis* (122 larvae and 10 nymphs) (Figure 2), with a mean of 2.8 ticks per infested mouse (SD = 4.4). Peak infestations of sentinel mice occurred in April, May, and August–October, with up to 45 and 20% of sentinel mice being infested by questing *I. spinipalpis*, respectively.

A significant increase in the proportion in sentinel mice infested with *I. spinipalpis* from the March to the April–May collections was seen (chi-square = 7.9, degree of freedom [df] = 1, $P = 0.005$). There was then a significant decrease in the proportion of mice infested with *I. spinipalpis* from the April–May to the June–July collections (chi-square = 15.5, df = 1, $P < 0.0001$). From June–July to the August–October collections, a significant increase in the proportion of sentinel mice infested with *I. spinipalpis* was found (chi-square = 5.6, df = 1, $P < 0.02$). A significant difference in the proportions of sentinel mice infested with *I. spinipalpis* between the March and the June–July collections (the 2 low points for infestation) was not found (chi-square = 0.01, df = 1, $P > 0.9$).

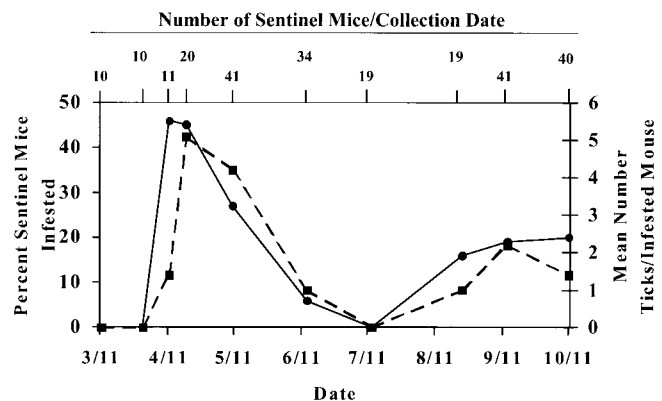


FIGURE 2. Seasonal prevalence and density per infected animal of *Ixodes spinipalpis* on specific-pathogen-free sentinel mice. A bimodal pattern is seen for both prevalence and density with peaks in April–May and September. The percentage of sentinel mice infested is shown by the solid line, and the mean number of ticks per infested mouse is plotted by the dashed line. Numbers of sentinel mice for each collection date are shown on the upper x-axis.

TABLE 4

Association between habitat characteristics and the presence of *Ixodes spinipalpis* on specific-pathogen-free sentinel mice

Characteristic	Relative risk	95% confidence interval	Chi-square	P
Grass	0.45	0.22–0.90	5.00	0.025
Sun	0.16	0.02–1.12	4.37	0.036
Shrubs	3.14	1.03–9.57	4.31	0.038
East-facing slope	0.55	0.31–0.98	3.74	0.052
Proximity to a wood rat nest	1.45	0.86–2.44	1.55	0.212

Mean numbers of *I. spinipalpis* ticks per infested sentinel mouse also showed a bimodal pattern, with peaks in April and September, with 5.1 and 2.2 ticks, respectively, per infested mouse. The maximum number of ticks found on a sentinel mouse was 26 and with a mode of 1 per infested mouse. Questing *I. spinipalpis* were not found during the 2 sample periods in March, but questing activity increased dramatically in April. Questing ticks were not found during the July sample period. In addition, 3 sentinel mice attracted fleas; 2 of these mice were infested with *M. telchinus* and one with *O. neotomae*.

In order to determine the time of day during which *I. spinipalpis* will quest, sentinel mice were replaced every morning at 8 AM, as well as every evening at 5 PM, at 2 sites during 3 days. Five questing *I. spinipalpis* were captured in this experiment. Three ticks attached to mice that were in the field between 8 AM and 5 PM, with the other 2 attaching between 5 PM and 8 AM.

Sites where questing ticks were found had a positive significant association with shrubs (relative risk [RR] = 3.14, $P = 0.038$) and negative significant associations with being in sun-exposed (RR = 0.16, $P = 0.036$) or grassy areas (RR = 0.55, $P = 0.025$) (Table 4). Perhaps of greater significance was the lack of a significant association for questing ticks on sentinel mice that were housed in immediate proximity to wood rat nests (RR = 1.45, $P = 0.212$). Two sentinel mice from which fleas were collected had been stationed at a site immediately adjacent to a wood rat nest, and 1 sentinel mouse had been at a site > 20 feet from the nearest wood rat nest.

Five of the 46 sentinel mice became infected with either *B. bissettii* or aoHGE, with one mouse becoming infected with both *B. bissettii* and aoHGE (Table 5). The *B. bissettii*-infected mouse had been fed upon by a single *I. spinipalpis* nymph, as had the aoHGE and *B. bissettii* coinfecting mouse. Only *I. spinipalpis* larvae had fed on the aoHGE-infected sentinel mice.

DISCUSSION

The potential for any arthropod to transmit a pathogen is dependent on 2 basic parameters: the infection rate in the arthropod and the probability of the arthropod coming into contact with a susceptible host. High infection rates of *B. burgdorferi sensu lato* have been reported in *I. spinipalpis* collected in California,^{8,9} Oregon,¹⁰ and Colorado.¹¹ Furthermore, this tick has also been shown to be competent for transmission of both aoHGE¹⁶ and *B. microti*¹⁷ However, this tick has been characterized as nidicolous, and therefore it

TABLE 5

Infection of specific-pathogen-free sentinel mice with aoHGE and *Borrelia bissettii* after infestation by questing *Ixodes spinipalpis* ticks*

Mouse	Infection with:		No. of <i>I. spinipalpis</i> fed:	
	<i>B. bissettii</i>	aoHGE	Larvae	Nymphs
S5	–	+	1	0
S15	–	+	3	0
S31	–	+	5	0
S42	–	+	1	0
S8	+	–	0	1
S38	+	+	0	1

* aoHGE = agent of human granulocytic ehrlichiosis; – = negative; + = positive.

was assumed to be unimportant in the transmission of human pathogens because nidicolous ticks have limited opportunities to encounter humans.

Occasional reports of this tick being found on humans in Canada,¹⁹ New Mexico,¹¹ and Oregon,¹⁸ as well as being found on birds,¹⁸ led to a reexamination of the host-seeking potential of *I. spinipalpis*. In this study, *I. spinipalpis* were found on *N. mexicana*, *P. maniculatus*, *P. difficilis*, *M. orchogaster*, *S. nuttallii*, and the yellow-breasted chat. Finding *I. spinipalpis* on both *S. nuttallii* and the yellow-breasted chat suggests that *I. spinipalpis* may quest outside rodent nests.

The finding that 19% of sentinel mice attracted questing *I. spinipalpis* was unexpected. Even more surprising was the fact that 63% of sentinel sites, including some > 20 feet away from the closest wood rat nest, attracted questing *I. spinipalpis* at some time between April and October. Questing *I. spinipalpis* attached to sentinel hosts both in the day and at night. There was a significant positive association between where questing *I. spinipalpis* ticks were found and the presence of shrubs, as well as significant negative associations between questing *I. spinipalpis* and a site that was sunny and grassy. These results were similar to those found by Adler and others,³³ who reported a positive association of *I. scapularis* immature stages on *Peromyscus leucopus* with woody vegetation and a negative association with herbaceous vegetation. It may be that the association of shrubs with questing *I. spinipalpis* reflects the preferred habitat of wood rats and that *I. spinipalpis* may detach from wood rats while outside the rodents' nests, where it could subsequently contact other potential host species.

Even more significant was the lack of a notable association between questing ticks and immediate proximity to a wood rat nest. If *I. spinipalpis* were truly nidicolous, one would expect a significant positive association with proximity to wood rat nests.

The use of sentinel mice allowed investigations on the biology of *I. spinipalpis* that are not possible by other means. In addition to allowing the characterization of the questing behavior and distribution of this tick in nature, this approach also affords a mechanism for performing natural transmission experiments. In this study, SPF mice become infected with both aoHGE and *B. bissettii* after their infestation with *I. spinipalpis* in the field. Laboratory studies have previously shown that *I. spinipalpis* is capable of transmitting these pathogens.^{34,16} This study demonstrated that *I. spinipalpis* does indeed transmit aoHGE and *B. bissettii* under natural

conditions. Furthermore, the observation of transmission of aoHGE by *I. spinipalpis* larvae raises the possibility that transovarial transmission of the aoHGE occurs by *I. spinipalpis* and warrants further investigation.

Ixodes spinipalpis has only occasionally been reported on humans. The data reported in these studies indicate that questing *I. spinipalpis* may frequent shrubby areas away from rodent nests. Its more widespread distribution in shrubby habitats, together with the increasing settlement of humans where *I. spinipalpis* exists, could increase the rate of contact between humans and this tick. Although direct human-*I. spinipalpis* contact may remain infrequent, *I. spinipalpis* may play an important role in maintaining *B. burgdorferi sensu lato* (including *B. bissettii*), aoHGE, and *B. microti* in enzootic cycles where a bridging vector may be responsible for transmission of these pathogens to humans.

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