# Vector Competence of Ixodid Ticks (Acari) for *Borrelia burgdorferi* as Determined With a Capillary-feeding Technique

*Xiaohong Li, PhD and Robert S. Lane, PhD*


## ABSTRACT

**Some factors that affect the vector competence of two ixodid ticks for *Borrelia burgdorferi* (Bb) were evaluated. The ticks included *Ixodes pacificus*, a proven competent vector and the primary bridge vector to humans in California, and *Dermacentor occidentalis*, an incompetent vector that rarely is found infected naturally with spirochetes. Adults of *I. pacificus* and *D. occidentalis* imbibed, on average, about $1.9\times10^5$ and $2.7\times10^5$ of Bb isolate CA4 in BSK-II medium, respectively, via a capillary-feeding technique. Spirochetes persisted in the midgut diverticula of both ticks for 5 months, but significantly more adult *D. occidentalis* died after infection. Despite the large spirochetal dose, none of the *D. occidentalis* (n=20) and only one of the *I. pacificus* (n=20) subsequently developed generalized tissue infections. When capillary-infected ticks were put on susceptible rabbits, only *I. pacificus* successfully transmitted spirochetes. Between 5 and 7 days after attachment to rabbits, the distribution of spirochetes within these ticks differed. Spirochetes in *I. pacificus* penetrated the midgut and disseminated to the salivary glands via the hemolymph in 30% to 40% of ticks, whereas dissemination did not occur in *D. occidentalis*, and spirochetes disappeared altogether from the midguts of 83% to 95% of ticks in different trials. These findings suggest that the vector incompetence of *D. occidentalis* is due to a midgut barrier that prevents spirochetal dissemination, to a borreliacidal factor associated with its feeding activities, or to both. We conclude that the capillary-feeding technique is a useful tool for studying the vector competence of ticks for Bb and for infecting ticks to be used in experimental transmission studies.**


**Key words:** *Ixodes pacificus, Dermacentor occidentalis, Borrelia burgdorferi*

The Lyme disease spirochete, *Borrelia burgdorferi*, is maintained and distributed in endemic foci primarily by ticks in the genus *Ixodes*.[1] In the western United States, *B. burgdorferi* is perpetuated in enzootic cycles involving ticks that do not bite humans (eg, *I. neotomae*, *I. spinipalpis*), woodrats, and other small mammalian hosts.[2-5] *Ixodes pacificus*, a worrisome biter of humans and an efficient vector of *B burgdorferi*,[3,6,7] also contributes to the chain of infection and serves as a bridge vector to people. In addition, several ticks in other genera occasionally have been found infected with spirochetes in this region of the country, including *Dermacentor occidentalis*, *Dermacentor variabilis*, and *Haemaphysalis leporispalustris*.[8-11] Of these, *D. occidentalis* and *D. variabilis* attach to humans, but experimental studies have demonstrated that both ticks are incompetent vectors of Lyme disease spirochetes.[3,7,12-16]

The vector competence of ticks for microbial agents is influenced by various intrinsic and extrinsic factors.[17] Although specific biologic factors influencing the vector competence of ticks for *B. burgdorferi* have not been identified,[18] growth-promoting factors may be present in *Ixodes* vectors that are absent in other tick genera, or

*From the Division of Insect Biology; Department of Environmental Science, Policy and Management; University of California at Berkeley.*

*This project was funded in part by grant AI22501 from the National Institutes of Health (R.S.L.).*

*Address correspondence to Xiaohong Li, Washington State University, Tree Fruit Research Center, 1100 N. Western Ave, Wenatchee, WA 98801.*

conversely, growth-inhibiting factors may occur in other tick genera that are lacking in *Ixodes* species.[19]

As an initial step toward elucidating intrinsic factors that may be responsible for the vector competence of *I. pacificus*, we compared the dissemination of *B. burgdorferi* in this tick with that in *D. occidentalis* after introducing spirochetes via a capillary-feeding technique.[20] We sought to determine the susceptibility and survivability of both species of ticks after ingestion of spirochetes to discover the route of spirochetal dissemination in infected ticks fed to partial repletion on rabbits versus unfed ticks. Also, we determined whether capillary-infected ticks are capable of transmitting spirochetes to rabbits.

## MATERIALS AND METHODS

### Lyme spirochetes

*B. burgdorferi* isolate CA4 (passage 6), which was derived from an adult *I. pacificus*, was used to infect laboratory-reared and spirochete-free ticks with the capillary feeding technique. The antigenic and genetic characteristics of this isolate have been described previously.[21,22] The capillary feeding method was modified slightly from that described by Burgdorfer.[20] In brief, a nonheparinized capillary tube (75 mm long by 1.4 mm in diameter) was heated medially, and the two ends of the tube were drawn apart gently to create a narrower central portion. The medial section was then broken to fashion two capillary tubes having diameters of about 1.4 mm and 0.6 mm at their respective nonheated and heated ends.

The spirochetal suspension was prepared by inoculating known quantities of motile spirochetes into antibiotic-free BSK-II medium and adding adenosine triphosphate to a concentration of 1.5 mM. A Petroff-Hausser counting chamber (Hausser Scientific Partnership, Horsham, Pa) was used to count spirochetes. After the capillary tube was embedded horizontally on a plasticine cuboid (about 10×10×4 mm), the hypostome of the tick was inserted into the narrow (0.6 mm diameter) end of the tube. The apparatus was put in a covered petri dish lined with moistened filter paper and held at 34°C for 2 to 3 hours while the ticks fed. Ticks gained up to 50% of their prefed weights during the feeding period. Ticks that gained less than 30% of their prefed weights were not used in the trials described subsequently.

### Source of ticks

Host-seeking adults of *I. pacificus* and *D. occidentalis* were collected from low vegetation in Tilden Regional Park, Berkeley, Calif, with a 1-m² tick-drag composed of white flannel. We used ticks from Tilden Regional Park because of their easy accessibility and the fact that the adults from there have rarely (*I. pacificus*) or never (*D. occidentalis*) been found to contain spirochetes.[11] Prior to experimentation, ticks were maintained at 21°C in a light:dark cycle of 12 hours:12 hours and relative humidities of 95% (*D. occidentalis*) or 98% (*I. pacificus*) for 1 to 2 weeks. Only motile ticks that seemed healthy were used in the capillary feeding trials.

### Effects of artificial feeding

To compare the survivability of fed and unfed females, 48 *I. pacificus* were capillary fed BSK-II culture medium containing $2.8\times10^8$ spirochetes per mL; 40 unfed females served as controls. Likewise, 113 *D. occidentalis* females were fed identically and 45 females were not fed as controls. In addition, 59 *D. occidentalis* females were fed only culture medium because a preliminary trial revealed that females fed a spirochetal suspension experienced a higher mortality than ticks that were not fed. All ticks were placed inside plastic vials (about 8 ticks/vial), which were held at the same conditions noted previously. The number of ticks that died monthly was recorded.

To determine the approximate number of spirochetes that were imbibed by each species of tick, 30 *I. pacificus* and 50 *D. occidentalis* females were weighed separately before and after capillary feeding. The average weight gains were 0.67 mg (SD=0.99) for *I. pacificus* and 1.004 mg (SD=3.78) for *D. occidentalis*. Because the density of BSK-II medium is 1.0176 g/mL, each *I. pacificus* and *D. occidentalis* female imbibed, on average, approximately $1.9\times10^5$ and $2.7\times10^5$ spirochetes, respectively.

The survival of capillary-fed versus unfed nymphal ticks also was determined. The survivability of 22 fed versus 17 nonfed *D. occidentalis* nymphs and of 62 fed and 16 nonfed *I. pacificus* nymphs was monitored weekly instead of monthly because capillary-fed nymphs had higher mortality rates than adult ticks. The amount of feeding material imbibed by a nymphal tick could not be determined because the weights of nymphs before and after feeding were too small to measure accurately. Spirochetes were delivered to *D. occidentalis* nymphs in 8% filtered rabbit serum instead of BSK-II medium because many *D. occidentalis* adults died after imbibing BSK-II medium alone. Nymphs were held at either 98% (*I. pacificus*) or 95% (*D. occidentalis*) relative humidity.

### Persistence of spirochetes in adult ticks

To determine how long *B. burgdorferi* could survive in capillary-fed *I. pacificus* adults, the hemolymph and midgut diverticula of ticks were examined for the presence of spirochetes by direct immunofluorescence according to previously described methods at 12 days, 3 months, and 5 months after feeding.[23] Also, portions of the midgut and salivary glands were put into BSK-II medium in isolation attempts. *D. occidentalis* adults were

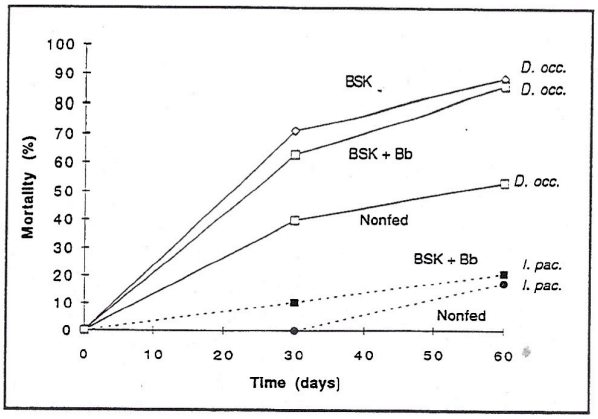


*Fig 1: Prevalence of* D. occidentalis (D. occ.) *and* I. pacificus (I. pac.) *adults that died after imbibition of BSK-II (BSK) medium alone or BSK-II medium plus* B. burgdorferi *(Bb) via a capillary feeding method. Nonfed ticks served as negative controls.*

likewise examined at 2, 4, and 5 months after feeding. To minimize contamination from other tissues, especially the midgut diverticula, the salivary glands were washed four times with phosphate-buffered saline before placement in BSK-II.

### Transmission of spirochetes

Sixty days after capillary feeding, two spirochete-infected *I. pacificus* females and two male ticks that were not infected were put on each of four female New Zealand white rabbits inside feeding capsules. An identical trial was conducted with *D. occidentalis*. All eight rabbits were assayed for spirochetal infection by tick-xenodiagnosis 4 weeks after infected adult ticks had fed to repletion on them. About 30 spirochete-free *I. pacificus* larvae or 20 *D. occidentalis* larvae from laboratory colonies were put on the rabbits that had been exposed to infected adult *I. pacificus* or *D. occidentalis*. After repletion and drop-off, xenodiagnostic ticks were tested for spirochetes by direct immunofluorescence and by culturing their tissues 4 to 5 weeks after the transstadial molt. Moreover, six ear-punch biopsies (4 mm in diameter, three per ear) and three pieces of skin from adult tick feeding sites were put into BSK-II medium. Serum obtained from each rabbit 6 weeks after infected adult ticks fed on them was tested for antispirochetal antibodies by indirect immunofluorescence.[24] The B-31 strain of *B. burgdorferi* was used as antigen.

The infectivity of capillary-fed nymphs of *I. pacificus* and *D. occidentalis* for rabbits also was tested. The density of spirochetes that nymphs were fed was $1.4\times10^8$/mL. Because capillary-fed nymphs of both species experienced a higher mortality rate than adult ticks, we placed fed nymphs on rabbits within 24 hours after they had ingested spirochetes. After repletion on rabbits and the subsequent transstadial molt, the midguts, hemolymph, and salivary glands of the resultant *I. pacificus* (n=15) and *D. occidentalis* (n=12) adults were examined for spirochetes by direct immunofluorescence and by culturing them in BSK-II medium. To determine if nymphal ticks transmitted spirochetes while feeding, ear-punch biopsies and serum obtained from each rabbit 5 weeks after tick detachment were assayed for infection, as described previously.

### Spirochetal dissemination after a blood meal

To ascertain the course of spirochetal dissemination in adult *I. pacificus* after blood feeding, 20 infected female and 20 noninfected male ticks were put inside four feeding capsules (five females/five males per capsule) on each of four rabbits 3 to 4 weeks after capillary feeding. Five or 6 days after the ticks had attached, the partially fed ticks were removed manually from each rabbit.

Because *D. occidentalis* females experienced such a high mortality after capillary feeding, adults were put on rabbits 3 days after feeding rather than several weeks later as was done in the case of *I. pacificus*. Before placement on rabbits, adult ticks were exposed to one of three spirochetal densities ($4.6\times10^8$/mL, $1.7\times10^8$/mL, or $9.7\times10^7$/mL). Female ticks in these groups imbibed, on average, approximately $4.5\times10^5$, $1.7\times10^5$, and $9.6\times10^4$ spirochetes. Four feeding capsules were fastened on each of four rabbits to confine ticks. One rabbit received 28 ticks infected with about $4.5\times10^5$ spirochetes apiece, another rabbit received 24 ticks infected with about $1.7\times10^5$ spirochetes each, and the remaining two rabbits each received 12 ticks infected with about $9.6\times10^4$ spirochetes apiece. Partially replete ticks were removed manually on either the sixth or seventh day after attachment. Ticks whose hypostomes were damaged during removal were discarded. Partially fed *I. pacificus* or *D. occidentalis* females were dissected immediately after they were removed from animals. Tick tissues and hemolymph, and rabbit ear-biopsy tissues or sera, were assayed for spirochetal infection by the same methods outlined previously for the first series of experiments.

### Data analysis

Fisher's exact test (two-tailed) was used to test for differences in prevalence of infection or mortality rates.[25]

## RESULTS

### Survival of capillary-fed adult and nymphal ticks

The mortality rates of capillary-fed *I. pacificus* females at 30 and 60 days after feeding did not differ significantly from those of the nonfed controls ($P$=0.13

and 0.91, Fig 1). *D. occidentalis* females fed BSK-II medium alone or BSK-II medium plus spirochetes experienced significantly higher mortality rates than nonfed ticks ($P<0.001$), but no difference in mortality rates was seen between females fed BSK-II only and those fed BSK-II plus spirochetes at 30 or 60 days after feeding ($P=0.35$ and 0.87). Among nymphal ticks, significantly more capillary-fed *I. pacificus* and *D. occidentalis* died at 6 and 21 days after feeding than nonfed nymphs ($P<0.01$). Mortality between capillary-fed nymphs of *I. pacificus* and *D. occidentalis* did not differ significantly (Fig 2).

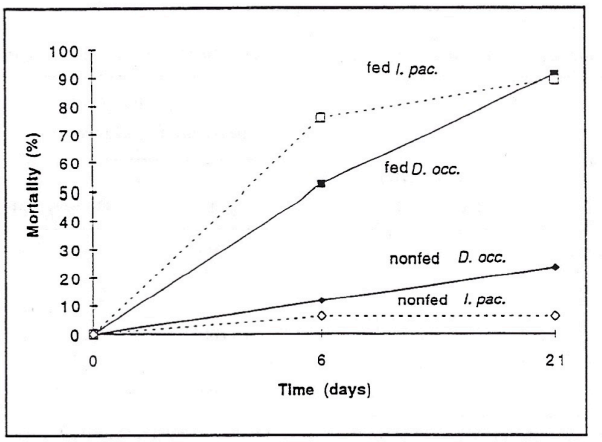


*Fig 2: Prevalence of* D. occidentalis (D. occ.) *and* I. pacificus (I. pac.) *nymphs that died after imbibition of 10% rabbit serum in phosphate-buffered saline plus* B. burgdorferi *via a capillary feeding method. Nonfed ticks served as negative controls.*

### Survival of spirochetes in capillary-fed adult ticks

The midguts of all *I. pacificus* and *D. occidentalis* females were found to contain spirochetes 5 months after capillary feeding. Among *I. pacificus* females, the prevalence of hemolymph-test-positive ticks at 12 days (1/14, 7.1%) versus 3 months (1/15, 6.7%) after feeding did not differ significantly. The prevalence of infection in salivary glands washed in phosphate-buffered saline 12 days (1/14, 7.1%) and 3 months (2/17, 11.8%) after feeding also was comparable. In contrast, nonwashed salivary glands were more likely to be infected than those that were washed 3 months after capillary feeding ($P=0.0056$, Table 1).

Except for one hemolymph-positive *D. occidentalis*, spirochetes were not detected in the hemolymph or salivary glands of capillary-fed *D. occidentalis* (Table 1).

### Transmission of spirochetes to rabbits by capillary-infected ticks

Two of four rabbits exposed to spirochete-infected *I. pacificus* females seroconverted; one of these rabbits also tested positive by ear-punch biopsy, and the other yielded infected xenodiagnostic ticks (Table 2). Overall, 24% of the xenodiagnostic ticks that had fed as larvae on both infected rabbits acquired and transstadially passed spirochetes. In contrast, none of four rabbits fed upon by capillary-fed *D. occidentalis* females became infected (Table 2).

In trials involving capillary-infected nymphal ticks, 15 of 50 (30%) *I. pacificus* and 12 of 64 (19%) *D. occidentalis* put on separate rabbits fed to repletion and later molted. Three (20%) of the resultant *I. pacificus* and none of the *D. occidentalis* adults contained spirochetes in their midguts. Neither rabbit was found to be infected by ear-punch biopsy or indirect immunofluorescence examination of their sera (Table 3).

### Spirochetal dissemination during blood feeding

The prevalence of spirochetal infection in capillary-fed female *I. pacificus* (n=15) and *D. occidentalis* (n=6) before blood feeding was 100%. Five or 6 days after attaching to rabbits, the midguts of 20 *I. pacificus* females were positive by direct immunofluorescence, but only 1 of 15 females tested concurrently by culture yielded spirochetes. The prevalence of spirochetal infection in *D. occidentalis* females that had been fed one of three dosages of spirochetes was significantly lower ($P<0.001$) than that of *I. pacificus* (Table 4), but the *D. occidentalis* females did not differ significantly among themselves ($0.53<P<1$). None of the midguts from *D. occidentalis* females produced positive cultures, regardless of the spirochetal dosage to which they had been subjected.

Hemolymph from 20 of 49 (41%) female *I. pacificus* were direct-immunofluorescence-test positive, and washed salivary glands from 3 of 10 (30%) ticks and 1 of 15 (7%) ticks were positive, respectively, by direct immunofluorescence and culture. Spirochetes were not detected in the hemolymph or salivary glands of *D. occidentalis* females. Cultures of ear-punch biopsies of rabbits fed upon by either species of tick did not yield spirochetes; however, all four rabbits fed upon by *I. pacificus* seroconverted.

## DISCUSSION

The capillary-feeding method is a simple technique for experimentally infecting ixodid ticks with microbial agents for use in vector competence studies. Burgdorfer[20] used a modified version of the glass capillary tube technique developed by Chabaud[26] to infect several species of ticks with microbial agents including the spirochete *Leptospira pomona*. Since then, European investigators have used this method to evaluate the vector efficiency of two *Ixodes* ticks for the Lyme disease spirochete.[27-29] Here we report for the first time the use of the capillary-feeding method to evaluate the factors

*Table 1*
*Prevalence of Spirochetal Infection in Various Tissues of* D. occidentalis *and* I. pacificus *Adults at Various Intervals After Capillary Feeding**

| Species | Time after feeding (days) | No. positive (%) by direct immunofluorescence | | No. positive (%) by culture | | |
|---|---|---|---|---|---|---|
| | | Midgut | Hemolymph | Washed salivary gland | Nonwashed salivary gland | Midgut |
| *I. pacificus* | 12 | 15/15 (100) | 1/14 (7.1) | 1/14 (7.1) | 5/13 (38.5) | 15/15 (100) |
| | 90 | 17/17 (100) | 1/15 (6.7) | 2/17 (11.8) | 8/17 (47.1) | 17/17 (100) |
| | 150 | 3/3 (100) | Not available | 2/3 (66.7) | Not available | 1/3 (33.3) |
| *D. occidentalis* | 60 | 11/11 (100) | 1/11 (9.1) | 0/11 (0) | 0/11 (0) | 10/11 (90.9) |
| | 120 | 1/1 (100) | 0/1 (0) | 0/1 (0) | Not available | 1/1 (100) |
| | 150 | 4/4 (100) | 0/4 (0) | 0/4 (0) | Not available | 4/4 (100) |

**Tissues were tested for spirochetes by direct immunofluorescence or by culture in BSK-II medium.*

*Table 2*
*Infectivities of Capillary-fed* I. pacificus *and* D. occidentalis *Females for New Zealand White Rabbits**

| Tick species | Rabbit no. | Positive by | | Tick xenodiagnosis† No. positive/no. tested (% positive) | |
|---|---|---|---|---|---|
| | | Indirect immunofluorescence (Serum) | Culture (Ear-punch biopsy) | BSK-II | Direct immunofluorescence |
| *I. pacificus* | 1 | — | — | 0/20 | 0/20 |
| | 2 | — | — | 0/20 | 0/20 |
| | 3 | + | + | 0/34 | 2/34 (5.9) |
| | 4 | + | — | 6/19 (31.6) | 8/19 (42.1) |
| *D. occidentalis* | 1 | — | — | 0/17 | 0/17 |
| | 2 | — | — | 0/10 | 0/10 |
| | 3 | — | — | 0/10 | 0/10 |
| | 4 | — | — | 0/16 | 0/16 |

**Determined by indirect immunofluorescence, ear-punch biopsy, and tick xenodiagnosis.*
†*After the transstadial molt, the midgut diverticula of xenodiagnostic nymphs were assayed for spirochetes using direct immunofluorescence or culture in BSK-II medium.*

that may affect the efficiency of a proven competent (*I. pacificus*) versus that of an incompetent vector (*D. occidentalis*) for *B. burgdorferi*.[3,6,7] The Western black-legged tick, *I. pacificus*, is a competent experimental vector of *B. burgdorferi* and the primary bridge vector to humans in the far Western United States.[3,7,30] Unlike *D. occidentalis*, which is incapable of acquiring and transstadially passing Lyme disease spirochetes, *I. pacificus* can efficiently acquire, maintain, and transmit *B. burgdorferi* after having fed on naturally or experimentally infected rodents.[3,6,7,31]

Our findings corroborate those of previous studies and suggest that the vector incompetence of *D. occidentalis* may be due to a midgut barrier to spirochetal dissemination, to physiologic changes during blood feeding that are inimical to spirochetes, or to both factors. Thus, spirochetes imbibed by *I. pacificus* females during capillary feeding disseminate to the salivary glands via the hemolymph in about 7% of ticks within 12 days. Further, if such ticks are placed on rabbits, 30% to 40% develop disseminated infections within 5 or 6 days of attachment. Similarly, *Ixodes ricinus* females infected via the capillary method develop systemic infections within a few days after their placement on rabbits.[27,28] For instance, spirochetes were detected in the hemolymph of 2 of 11 *I. ricinus* capillary-infected females 2 days after attachment to rabbits and in the salivary glands of 6 of 51 ticks after 3 days of attachment.[27]

In marked contrast, spirochetes in the midgut diverticula of artificially fed *D. occidentalis* females do not disperse to other tissues with or without a subsequent blood meal, which portends the existence of a midgut

*Table 3*
*Spirochetal Prevalence in Various Tissues of* I. pacificus *and* D. occidentalis *Females Infected By Capillary Feeding Before Partial Engorgement on New Zealand White Rabbits*

| | No. (%) positive by direct immunofluorescence | | | No. (%) positive by culture in BSK-II | | | Infectivity for rabbits | |
|---|---|---|---|---|---|---|---|---|
| Tick species | Midgut | Hemolymph | Washed salivary glands | Midgut | Front legs* | Washed salivary glands | Ear-punch biopsy | Serum IFA |
| *I. pacificus* | 3/15 (20) | 0/15 | 0/15 | 0/15 | 0/15 | 3/15 (20) | Negative | Negative |
| *D. occidentalis* | 0/12 | 0/12 | 0/12 | 0/12 | 0/12 | 0/12 | Negative | Negative |

**Front legs were amputated to obtain hemolymph, and the amputated legs were cultured in BSK II medium in isolation attempts.*

*Table 4*
*Tissue Tropisms of* B. burgdorferi *in Capillary-fed* I. pacificus *and* D. occidentalis *Females Within 5 to 7 Days After Attachment to New Zealand White Rabbits*

| | No. (%) positive by direct immunofluorescence | | | No. (%) positive by culture in BSK-II | | |
|---|---|---|---|---|---|---|
| Tick species (Spirochetal dose)* | Midgut | Hemolymph | Washed salivary glands | Washed salivary glands | Front legs† | Midgut |
| *I. pacificus* (high) | 20/20 (100) | 20/49 (40.8) | 3/10 (30) | 1/15 (6.7) | Not available | 1/15 (6.7) |
| *D. occidentalis* (high) | 1/20 (5) | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 |
| *D. occidentalis* (medium) | 2/15 (13.3) | 0/15 | 0/15 | 0/15 | 0/15 | 0/15 |
| *D. occidentalis* (low) | 2/12 (16.7) | 0/12 | 0/12 | 0/12 | 0/12 | 0/12 |

**Ticks were fed one of three spirochetal densities: $4.6\times10^8$/mL (high), $1.7\times10^8$/mL (medium), or $9.7\times10^7$/mL (low).*
*†Front legs were amputated to obtain hemolymph, and the amputated legs were cultured in BSK-II medium in isolation attempts.*

barrier. Also, the fact that most *D. occidentalis* females lose their midgut-restricted spirochetal infections by the fifth to seventh day after attachment indicates that intrinsic physiologic changes during the feeding process may be destructive to spirochetes.

We postulate therefore that *D. occidentalis* contains a borreliacidal factor that is released into the midgut diverticula during tick feeding, either from the salivary glands or from enzymes released by secretory cells lining the midgut. Other investigators have shown that many complex physiologic and biochemical changes occur in the midgut of ticks during blood feeding.[32] For example, studies on the digestive enzymes of ticks demonstrated that protease is present in the digestive cells or lumen of the midgut.[33-37] If some midgut enzymes induced during blood feeding are species-specific, either quantitatively or qualitatively, and are detrimental to spirochetes, they may contribute to the vector incompetence of certain ticks. Moreover, the viability of *B. burgdorferi* varies when incubated with cell cultures derived from different tick species.[38]

*Dermacentor variabilis* and *Amblyomma americanum* can acquire *B. burgdorferi* while feeding on infected rodents, but both ticks are incapable of maintaining spirochetes or transmitting them to susceptible animals.[12,13] The limited viability of spirochetes in these ticks after ingestion of blood is similar to that of *D. occidentalis* in the present study.

Although cultured spirochetes are widely used in experimental studies, the repertoire of surface proteins and the associated infectivity of cultured versus uncultured *B. burgdorferi* are not identical.[39,40] To minimize this potential shortcoming, we used low-passaged spirochetes and treated both ticks similarly during experimentation. Even so, spirochetes in adult *I. pacificus* and *D. occidentalis* behaved differently while these ticks fed on rabbits, and only *I. pacificus* transmitted *B. burgdorferi*.

Nymphal ticks of both species suffered higher mortality rates than adult ticks when fed spirochetes artificially, whereas BSK-II medium alone adversely affected the survivability of adult *D. occidentalis*. Consequently, experiments with the nymphal ticks and adult *D. occi-*

*dentalis* need to be repeated using an innocuous diluent (eg, defibrinated mouse blood) and lower doses of spirochetes. We used inocula containing approximately $10^8$ cells/mL to infect ticks, which resulted in adult ticks ingesting more than $10^5$ spirochetes.

In comparable vector competence studies with European vector ticks, *I. ricinus* and *Ixodes hexagonus*, Gern and coworkers[28,29] exposed ticks to lesser concentrations of spirochetes ($10^5$ to $10^6$ cells/mL) cultured in BSK-II medium with no apparent untoward effects.

In comparison, naturally or experimentally infected vectors of *B. burgdorferi* contain only about $10^2$ or $10^3$ spirochetes in the unfed state.[19,41] For example, the mean number of spirochetes present in experimentally infected *I. dammini* (now *I. scapularis*) was estimated to be less than 300 for nymphal and about 4000 for adult ticks,[19] and the median density of *B. burgdorferi* in naturally infected adult ticks from intensely zoonotic sites in coastal Massachusetts was 1925.[41] Further experimental studies are necessary to determine the minimum threshold of spirochetes that various enzootic or bridge vectors must ingest to acquire, transstadially pass, and transmit *B. burgdorferi*.

We conclude that the capillary-feeding method is highly suitable for this purpose because previously cultured and characterized microbial agents can be efficiently delivered to ticks in known concentrations with minimal expense and effort.

**Acknowledgments:** We thank Esther Omi-Olsen for her technical assistance.

## REFERENCES

1. Lane RS, Piesman J, Burgdorfer W. Lyme borreliosis: relationship of its causative agent to its vectors and hosts in North America and Europe. *Annu Rev Entomol.* 1991;36:587-609.

2. Lane RS, Brown RN. Wood rats and kangaroo rats: potential reservoirs of the Lyme disease spirochete in California. *J Med Entomol.* 1991;28:299-302.

3. Brown RN, Lane RS. Lyme disease in California: a novel enzootic transmission cycle of *Borrelia burgdorferi*. *Science.* 1992;256:1439-1442.

4. Maupin GO, Gage KL, Piesman J, et al. Discovery of an enzootic cycle of *Borrelia burgdorferi* in *Neotoma mexicana* and *Ixodes spinipalpis* from northern Colorado, an area where Lyme disease is nonendemic. *J Infect Dis.* 1994;170:636-643.

5. Lane RS, Keirans JE. *Ixodes spinipalpis* (Acari: Ixodidae): a potential enzootic vector of *Borrelia burgdorferi* in California. In: Needham GR, Mitchell R, Horn DJ, Welbourn WC. *Acarology IX: symposia.* Columbus, Ohio: Ohio Biological Survey; 1997 (in press).*

6. Piesman J. Standard system for infecting ticks (Acari: Ixodidae) with the Lyme disease spirochete, *Borrelia burgdorferi* . *J Med Entomol.* 1993;30:199-203.

7. Lane RS, Brown RN, Piesman J, Peavey CA. Vector competence of *Ixodes pacificus* and *Dermacentor occidentalis* (Acari: Ixodidae) for various isolates of Lyme disease spirochetes. *J Med Entomol.* 1994;31:417-424.

8. Lane RS, Burgdorfer W. Spirochetes in mammals and ticks (Acari:Ixodidae) from a focus of Lyme borreliosis in California. *J Wildl Dis.* 1988;24:1-9.

9. Lane RS, Lavoie PE. Lyme borreliosis in California: acarological, clinical, and epidemiological studies. *Ann N Y Acad Sci.* 1988;539:192-203.

10. Lane RS, Loye JE. Lyme disease in California: interrelationship of ixodid ticks (Acari), rodents, and *Borrelia burgdorferi*. *J Med Entomol.* 1991;28:719-725.

11. Lane RS. Risk of human exposure to vector ticks (Acari: Ixodidae) in a heavily used recreational area in northern California. *Am J Trop Med Hyg.* 1996;55:165-173.

12. Piesman J, Sinsky RJ. Ability of *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma americanum* (Acari: Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (*Borrelia burgdorferi*). *J Med Entomol.* 1988;25:336-339.

13. Mather TN, Mather ME. Intrinsic competence of three ixodid ticks (Acari) as vectors of the Lyme disease spirochete. *J Med Entomol.* 1990;27:646-650.

14. Mukolwe SW, Kocan AA, Barker RW, Kocan KM, Murphy GL. Attempted transmission of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) (JD1 strain) by *Ixodes scapularis* (Acari: Ixodidae), *Dermacentor variabilis*, and *Amblyomma americanum*. *J Med Entomol.* 1992;29:673-677.

15. Oliver Jr JH, Chandler Jr FW, Luttrell MP, et al. Isolation and transmission of the Lyme disease spirochete from the southeastern United States. *Proc Natl Acad Sci.* 1993;90:7371-7375.

16. Sanders Jr FH, Oliver Jr JH. Evaluation of *Ixodes scapularis*, *Amblyomma americanum* and *Dermacentor variabilis* (Acari: Ixodidae) from Georgia as vectors of a Florida strain of the Lyme disease spirochete, *Borrelia burgdorferi*. *J Med Entomol.* 1995;32:402-406.

17. Lane RS. Competence of ticks as vectors of microbial agents, with an emphasis on *Borrelia burgdorferi*. In: Sonenshine DE, Mather TN, eds. *Ecological Dynamics of Tick-borne Zoonoses.* New York, NY: Oxford University Press, 1994:45-67.

18. Mather TN. The dynamics of spirochete transmission between ticks and vertebrates. In: Ginsberg HS, ed. *Ecology and Environmental Management of Lyme Disease.* New Brunswick, NJ: Rutgers University Press; 1993:43-60.

19. Piesman J, Oliver JR, Sinsky RJ. Growth kinetics of the Lyme disease spirochete (*Borrelia burgdorferi*) in vector ticks (*Ixodes dammini*). *Am J Trop Med Hyg.* 1990;42:352-357.

20. Burgdorfer W. Artificial feeding of ixodid ticks for studies on the transmission of disease agents. *J Infect Dis.* 1957;100:212-214.

21. Lane RS, Pascocello JA. Antigenic characteristics of *Borrelia burgdorferi* isolates from ixodid ticks in California. *J Clin Microbiol* 1989;27:2344-2349.

22. LeFebvre RB, Lane RS, Perng GC, Brown JA, Johnson RC. DNA and protein analysis of tick-derived isolates of *Borrelia burgdorferi* from California. *J Clin Microbiol.* 1990;28:700-707.

23. Burgdorfer W, Lane RS, Barbour AG, Gresbrink RA, Anderson JR. The western black-legged tick, *Ixodes pacificus*: a vector of *Borrelia burgdorferi*. *Am J Trop Med Hyg.* 1985;34:925-930.

24. Lane RS, Manweiler SA. *Borrelia coriaceae* in its tick vector, *Ornithodoros coriaceus* (Acari: Argasidae), with emphasis on transstadial and transovarial transmission. *J Med Entomol.* 1988;25:172-177.

25. Zar JH. *Biostatistical Analysis*, 2nd ed. Englewood Cliffs, NJ: Prentice Hall; 1984.

26. Chabaud AG. Sur la nutrition artificielle des tiques. *Annals of Parasitology.* 1950;25:42-47.

27. Monin R, Gern L, Aeschlimann A. A study of the different modes of transmission of *Borrelia burgdorferi* by *Ixodes ricinus*. In: Stanek G, Kristoferitsch W, Pletschette M, Barbour AG, Flamm H, eds. *Lyme borreliosis II. Zbl Bakteriol Med Microbiol Hyg Suppl 18.* New York: Gustav Fischer Verlag; 1989;18:14-20.

28. Gern L, Zhu Z, Aeschlimann A. Development of *Borrelia burgdorferi* in *Ixodes ricinus* females during blood feeding. *Ann Parasitol Hum Comp.* 1990;65:89-93.

29. Gern L, Toutoungi LN, Hu CM, Aeschlimann A. *Ixodes (Pholeoixodes) hexagonus*, an efficient vector of *Borrelia burgdorferi* in the laboratory. *Med Vet Entomol.* 1991;5:431-435.

30. Clover JR, Lane RS. Evidence implicating nymphal *Ixodes pacificus* (Acari: Ixodidae) in the epidemiology of Lyme disease in

California. *Am J Trop Med Hyg.* 1995;53:237-240.

31. Peavey CA, Lane RS. Transmission of *Borrelia burgdorferi* by *Ixodes pacificus* nymphs and reservoir competence of deer mice (*Peromyscus maniculatus*) infected by tick-bite. *J Parasitol.* 1995;81:175-178.

32. Coons LB, Rosell-Davis R, Tarnowski BI. Blood meal digestion in ticks. In: Sauer JR, Hair JA, eds. *Morphology, Physiology and Behavior Biology of Ticks.* Chichester, Mass: Ellis Horwood; 1986:248-279.

33. Akov S. Blood digestion in ticks. In: Obenchain FD, Galun R, eds. *Physiology of Ticks.* Oxford, England: Pergamon Press; 1982:197-211.

34. Rebeiro JMC. The midgut hemolysin of *Ixodes dammini* (Acari: Ixodidae). *J Parasitol.* 1988;74:532-537.

35. Agyei AD, Herbert IV, Runham NW. Histochemical localization of acid phosphatase and non-specific esterase in the midguts of two species of tick, *Boophilus microplus* and *Rhipicephalus appendiculatus*, as determined by light microscopy. *Parasitol Res.* 1991;77:629-634.

36. Vundla WRM, Brossard M, Pearson DJ, Labongo VL. Characterization of aspartic proteinases from the gut of the tick, *Rhipicephalus appendiculatus Neuman*. *Insect Biochem Mol Biol.* 1992;22:405-410.

37. Gough JM, Kemp DH. Acid phosphatase in midgut digestive cells in partially fed females of the cattle tick *Boophilus microplus*. *J Parasitol.* 1995;81:341-349.

38. Kurtti TJ, Munderloh UG, Ahlstrand GG, Johnson RC. *Borrelia burgdorferi* in tick cell culture: growth and cellular adherence. *J Med Entomol.* 1988;25:256-261.

39. Schwan TG, Burgdorfer W, Garon CF. Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of in vitro cultivation. *Infect Immun.* 1988;56:1831-1836.

40. Norris SJ, Howell JK, Garza SA, Ferdows MS, Barbour AG. High- and low-infectivity phenotypes of clonal populations of in vitro-cultured *Borrelia burgdorferi*. *Infect Immun.* 1995;63:2206-2212.

41. Brunet LR, Spielman A, Telford SR III. Density of Lyme disease spirochetes within deer ticks collected from zoonotic sites. *Am J Trop Med Hyg.* 1995;53:300-302.

# The Role of Publicly Owned Properties in the Transmission of Lyme Disease in Central New Jersey

*Terry L. Schulze, PhD and Robert A. Jordan, PhD*


## ABSTRACT

**Using an ecological index to generate a relative ranking of sites regarding potential and actual Lyme disease transmission risk, 610 public parks, recreation areas, and public school properties were surveyed during the summer of 1993. The majority of surveyed sites (56.4%) were judged to pose low potential risk; only 60 sites (9.8%) were identified as posing a high potential risk, requiring additional assessment to estimate actual population densities of infected ticks.**

**High-risk sites typically were large, multiple-use parks and recreation areas located in areas of lower human population density. Parks and recreation areas in populated coastal areas generally were smaller and more developed. Publicly owned land may be surveyed effectively for Lyme disease transmission risk using an existing ecological index and tick survey techniques, allowing surveillance and intervention efforts to be targeted toward areas posing significant risk of transmission.**


**Key words:** public parks, recreation areas, Lyme disease

## INTRODUCTION

Despite general acceptance that the majority of Lyme disease cases are the result of exposure to infected black-legged ticks at or near the patient's place of residence,[1-3] other studies have suggested substantial transmission risk among visitors, workers, and nearby residents of some parks and recreation areas.[4-6] None of these studies adequately characterized habitats and use within the areas studied and, owing to the labor intensity inherent in surveying large areas for ticks, the number of study sites was limited. As a result, the majority of parks and recreational areas are never assessed, leaving the public unaware of potential risk. No studies of transmission risk associated with school properties have been published.

*From the Division of Communicable Diseases, New Jersey Department of Health and Senior Services (Dr Schulze), Trenton; and Freehold Area Health Department (Dr Jordan), New Jersey.*

*The current work was supported by a Cooperative Agreement (U50/CCU206567-05) between the New Jersey Department of Health and Senior Services and the Centers for Disease Control and Prevention.*

*Address correspondence to Terry L. Schulze, PhD, Division of Communicable Diseases, NJ Department of Health and Senior Services, NJ Department of Health, CN 360, Trenton, NJ 08625.*

Recognizing the limitations resulting from the level of effort required to perform tick surveys, an ecological assessment index (Index) was designed to predict potential Lyme disease transmission risk based on the presence, amount, and accessibility of vegetation associations capable of supporting *Ixodes scapularis* Say and its hosts.[7] The results of that pilot study suggested that the Index provides a rapid, accurate method to identify areas at risk for the transmission of Lyme disease.

Through a broad-scale assessment program, the study attempted to test and refine the efficacy of the Index throughout a county where Lyme disease is endemic. Such research is the first step toward the creation of cost-effective management procedures to reduce the incidence of Lyme disease in people using public properties by reducing exposure to infected *I. scapularis*. We report here the results of a survey of all public land in Monmouth County, NJ, for Lyme disease transmission risk.

## METHODS

### Site selection

Publicly owned land in Monmouth County, NJ, served as study areas for this project. Since Lyme disease became reportable in New Jersey in 1980,