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Glass Capillary Tube Feeding: A Method for Infecting Nymphal *Ixodes scapularis* (Acari: Ixodidae) with The Lyme Disease Spirochete *Borrelia burgdorferi*

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ABSTRACT We evaluated an artificial capillary feeding method to infect nymphal *Ixodes scapularis* (Say) ticks with *Borrelia burgdorferi*, the causative agent of Lyme disease. Thirty to 70% of the nymphs were infected after feeding for 2.5 h from glass capillary tubes filled with a solution of spirochetes. Capillary infection was stable and persisted in the nymphs for at least 10 d after feeding. Capillary feeding also maintained natural vector competence patterns because *I. scapularis* ticks acquired infection unlike *Dermacentor variabilis* (Say), which did not become infected. Capillary infected *I. scapularis* nymphs were capable of transmitting the infection to naive mice although not as efficiently as naturally infected nymphs. The capillary infection method is convenient and is a better alternative to syringe inoculation as a means of infecting animals with *B. burgdorferi*.

KEY WORDS *Borrelia burgdorferi*, *Ixodes scapularis*, ticks, Lyme disease, artificial feeding, capillary feeding

LYME DISEASE IS caused by the tick-borne spirochete, *Borrelia burgdorferi*. Over the past three decades, Lyme disease has emerged to become the leading vector-borne infection in North America (Anonymous 2000) and Europe. The tick vectors of the Lyme disease agent are members of the “*Ixodes ricinus*/per-sulcatus complex” (Keirans et al. 1992) that are widely distributed in the Nearctic and Palaearctic regions of the world (Lane et al. 1991). In North America, the vectors of *B. burgdorferi* include the blacklegged tick, *I. scapularis* (Say) in the eastern and central United States and the western blacklegged tick, *I. pacificus* (Cooley & Kohls) in the western United States (Burgdorfer et al. 1991). The majority of human Lyme disease cases in the United States are caused by exposure to *B. burgdorferi*-infected *I. scapularis* ticks.

Tick transmission, which is the natural method of infection, is rarely used as the method of infection in laboratory based animal models of Lyme disease. Because *B. burgdorferi* can be cultured in artificial media, laboratory animals are usually infected by syringe-injecting cultured bacteria. Recent studies point to major differences between tick and syringe challenge.

Culture grown spirochetes and spirochetes delivered by ticks produce different antigens (Schwan 1996, de Silva and Fikrig 1997). Consequently, laboratory mice infected by tick bite have a different immune response than mice infected by syringe inoculation (Gern et al. 1993). Tick-borne and cultured bacteria also differ in their ability to evade host immune responses (de Silva et al. 1998). To accurately reproduce events that occur during and after transmission of *B. burgdorferi*, animal models of Lyme disease should be based on animals infected by tick bite.

The *B. burgdorferi* transmission cycle can be reproduced in the laboratory by feeding larval ticks on infected mice (*Mus musculus*), allowing the engorged larvae to molt and subsequently feeding the infected nymphs on naive mice. However, rearing infected ticks in the laboratory is both expensive and time consuming, and has certain limitations. Host animals must be purchased and infected 2–4 wk before larval feeding. When larvae are placed on infected mice, it is difficult to monitor pathogen uptake. Following the larval blood meal, a period of 4–6 wk is necessary for the larvae to molt and another 2–4 wk must elapse before the molted nymphs are prepared to feed.

Although artificial feeding methods have been widely used with fast feeding hematophagous insects and argasid ticks, the method has been more difficult to adapt to the slow feeding ixodid ticks, which create a feeding pool in the host's skin, secrete cement to bond their mouthparts to the wound site and feed for several days continuously (Sonenshine 1991, Young et

All use of animals in this research was done in accordance with protocols approved by the Old Dominion University Animal Use and Care Committee Protocols on 17 March 1999 and the University of North Carolina Animal Use and Care Committee 3 June 1999.

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al. 1996). A variety of artificial infection techniques have been tested for ixodid ticks, including feeding chambers with natural or artificial membranes, enema infusion, hemocoelic inoculation, and capillary feeding. Feeding chambers with membranes have been used to infect *Dermacentor*, *Amblyomma*, *Ixodes*, and *Rhipicephalus* ticks with different bacterial and protozoan parasites (Howarth and Hokama 1983, Waladde et al. 1995, Young et al. 1996, Barre et al. 1998, Burkot et al. 2001). However, membranes are difficult and expensive to prepare as well as maintain without contamination and decomposition (Chabaud 1950). Intrahemocoelic injections have been used in a few studies to introduce pathogens into ticks (Turell et al. 1997; Johns et al. 2001, 2002). However, this method is not ideal for *B. burgdorferi* because the bacteria are introduced into the hemocoel rather than the lumen of the gut where they normally reside. Enema infusion has also been attempted to introduce bacteria into ticks but the method creates injuries and often kills the ticks (Turell et al. 1997).

Capillary feeding was originally described as a method for feeding ixodid ticks by Chabaud and subsequently used by Burgdorfer to introduce pathogens into hard ticks (Chabaud 1950, Burgdorfer 1957). More recently, the method has been used in a variety of studies to infect *I. ricinus* L., *I. hexagonus* (Leach) and *I. pacificus* ticks with *B. burgdorferi*, and also to introduce *Ehrlichia chaffeensis* into Ixodid ticks (Moinin et al. 1989; Hu et al. 1992; Gern et al. 1993, 1994; Toutoungi and Gern 1993; Hu et al. 1996; Li and Lane 1996; Rechav et al. 1999). However, the method has not been described in detail and it is not clear how parameters such as the duration of capillary feeding, the volume of fluid ingested, and the density of bacteria in the feeding solution affect the ability of ticks to acquire and transmit infection. Furthermore, to our knowledge, no attempt has been made to infect *I. scapularis* by capillary feeding.

In this article, we describe studies to develop glass capillary feeding as a method for infecting nymphal *I. scapularis* ticks with *B. burgdorferi*. We also describe studies on the ability of capillary-infected ticks to maintain the infection and transmit the infection to naive mice.

Materials and Methods

Bacteria. A cloned isolate of *B. burgdorferi* B31 (CDC, Fort Collins, CO) was used in these experiments. Cultures were grown in BSK II media (Barbour 1984) and harvested at a density $\leq 5 \times 10^7$ cells/ml. *B. burgdorferi* cultures were counted using a Petroff-Hauser counting chamber and dark field microscopy. All artificially and naturally infected ticks used in the study were infected with this cloned isolate of B31.

Tick feeding solutions. To create a capillary feeding solution an appropriate amount of a mid-log phase *B. burgdorferi* culture was transferred to a microcentrifuge tube and centrifuged at $2,000 \times g$ for 10 min. The supernatant was removed and the bacteria were resuspended at the desired density by gentle vortexing

in fresh BSK II media or $1/2 \times$ BSK II and $1/2 \times$ Shen's tick saline (Oliver et al. 1972). The concentration of bacteria in the feeding solution ranged from 3×10^6 to 5×10^7 cells/ml in different experiments. Similar solutions without bacteria were used as negative controls for capillary feeding.

Ticks. *Dermacentor variabilis* (Say) were colonized as described by Sonenshine (Sonenshine 1993). All life stages were fed on rabbits and the fed ticks were kept at $26^\circ\text{C} \pm 1^\circ\text{C}$ and $92 \pm 1\%$ RH. In capillary feeding experiments with *D. variabilis*, only adult females that had partially fed on a rabbit for 4–5 d were used. *I. scapularis* was colonized from ticks collected near Armonk, NY, fed on rabbits and the spirochete-free progeny reared to adults. Capillary feeding was done with *I. scapularis* unfed nymphs, partially fed nymphs removed from mice after 17 h, and partially fed adult females removed from rabbits after 4–5 d. Partially fed ticks were removed from anesthetized mice or rabbits with fine tipped forceps. Engorged ticks were collected daily as they fell into the water, placed on absorbent paper, rinsed with water and saved for analysis.

Mice. Female C3H HENner mice (4–6 wk) were purchased from the National Institute of Health. Mice were used for tick transmission studies. Ticks were placed on the back and shoulders of mice after anesthetizing the mice with a 50 μl intraperitoneal injection of ketamine/xylazine (25 mg/ml ketamine and 7.6 mg/ml xylazine). The mice were housed individually on stainless steel grids over water.

Capillary Feeding *B. burgdorferi* to Ticks. Two standard microscope slides were assembled using double sided tape such that the upper slide's long edge was recessed 6 mm from the lower slide's long edge (Fig. 1A). Double-sided tape was placed along the recessed edge on the upper slide. Ten unfed or partially fed *I. scapularis* ticks were placed on the tape, dorsal side down, with the forward third of the tick's body extending beyond the recessed edge of the upper slide. Single-sided tape was attached to the ventral surfaces of the ticks and to the tape on the slides to further immobilize the ticks (Fig. 1A). The slide assembly with immobilized ticks was then taped into a 100 by 15 mm culture dish base or lid with modeling clay affixed along the edge of the dish opposite the tick's mouthparts. Small indentations were made in the modeling clay in line with the mouthparts of each tick. Five microliter glass micropipets (Fisher, Pittsburgh, PA) were filled with the prepared feeding solution by immersing one end of the tubes (at a 45° angle) into the feeding solution. Capillary action drew the fluid into the glass tube. The filled capillary tubes were positioned individually so that one end was close to the tick's mouthparts and the other end was pressed into the corresponding indentation in the modeling clay. Using a dissecting microscope and fine-tipped forceps, each fluid-filled capillary was inserted over the tick's hypostome and palps. This process was repeated until all ticks had filled capillary tubes positioned over their mouthparts. (Fig. 1B) The feeding apparatus was placed in a sealed plastic container at 37°C and lined

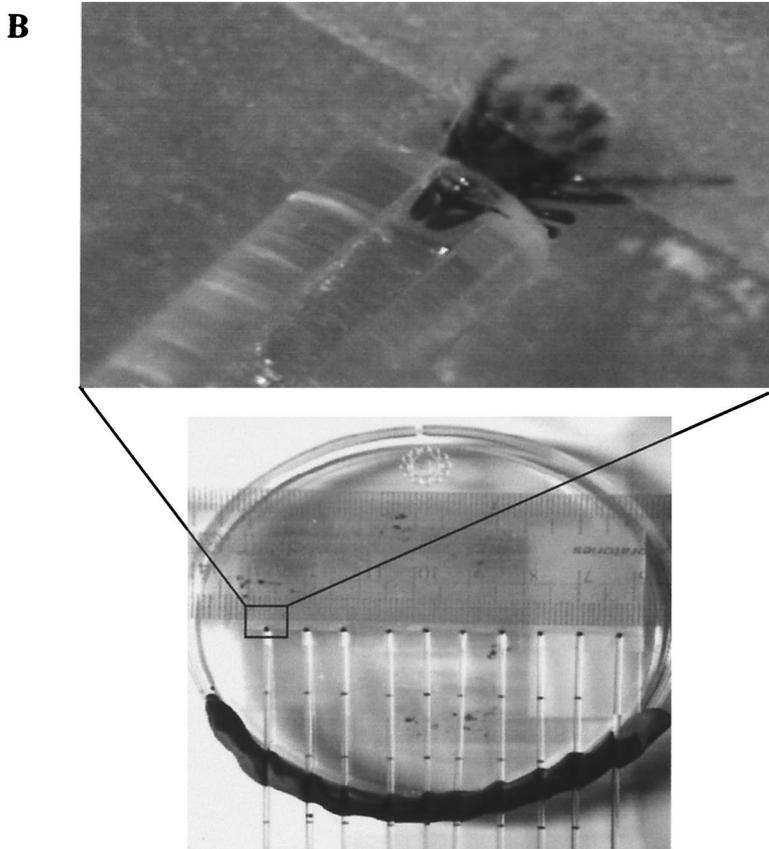
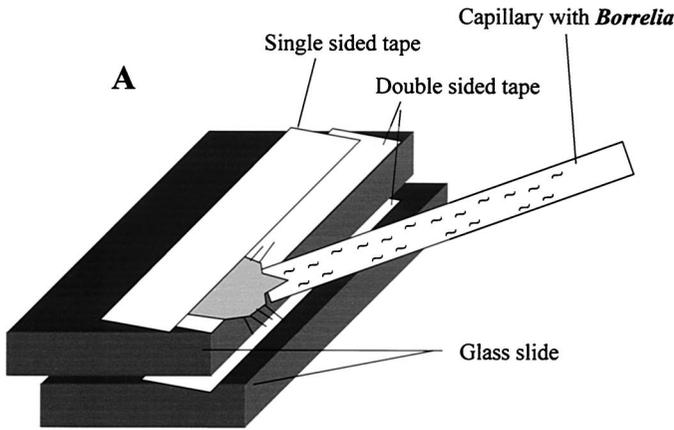


Fig. 1. Method used to capillary feed groups of ticks. (A) Diagram of method used to immobilize ticks. (B) a close up photograph of a nymph with capillary attached to hypostome. See *Materials and Methods* section for details.

with wet paper towels to maintain humidity and prevent tick dehydration.

After capillary feeding, the immobilized ticks were recovered from the humidifying chamber and examined under a dissecting microscope to assess the placement of the capillary tubes. Ticks with detached capillary tubes were noted and later discarded. Ticks with capillaries that remained properly positioned were collected for the experiments. To collect the ticks, the

capillaries were first removed and discarded. The upper layer of tape was removed and the ticks were gently lifted off the lower tape using fine-tipped forceps, placed on absorbent paper, rinsed with water to wash off any external feeding solution residue, and saved for analysis or placement on host animals.

Measurement of Fluid Uptake by Capillary Feeding Ticks. Ticks were allowed to feed from capillaries containing a solution of ^{14}C (uniformly labeled)

Table 1. Summary of radioactive fluid uptake by living and dead nymphal *I. scapularis*

| | Live nymphs (24 ticks) | | Dead nymphs (16 ticks) | |
|------------------------------------|--|--|--|--|
| | Active fluid uptake (≥ 100 CPM) | No active fluid uptake (< 100 CPM) | Active fluid uptake (≥ 100 CPM) | No active fluid uptake (< 100 CPM) |
| No. of ticks | 13/24 (54%) | 11/24 (46%) | 0/16 (0%) | 16/16 (100%) |
| Mean radioactivity CPM (\pm SD) | 656 (399) | 41 (32) | NA | 4 (11) |
| Mean volume μ l (\pm SD) | 0.03 (0.02) | 0 (0) | NA | 0 (0) |

NA, not applicable.

amino acids (NEN, Boston, MA) in BSK II medium. The radioactivity of the solution was 24,459 DPM/ μ l. Following capillary feeding for 90 min, the ticks were removed and checked to ensure that they were still alive. Surviving ticks were decontaminated and assayed for radioactivity as described below. To control for radioactive fluid contaminating the surface of ticks, capillary tubes were filled with radioactive fluid and placed over the hypostome of dead nymphs. To remove surface radioactivity, ticks (both living and dead) were washed in Counts Off (New England Nuclear, Boston, MA) until radioactivity could no longer be detected in the wash. Following decontamination, the ticks were minced with fine scissors in 100 μ l PBS solution and the contents transferred to liquid scintillation cocktail solution glass mini-vials for radioassay. Measurement of 14 C radioactivity was done with a Beckman LS 1710 Liquid Scintillation Counter (Beckman, Fullerton, CA). The volume of fluid taken up by the ticks was determined by comparing the radioactivity in the tick specimens with that of a known volume of radio-labeled amino acid solution in BSK II. Quench correction was omitted since there was no detectable color change following sample preparation (Knoche 1991).

Spirochete Detection in Ticks. A direct immunofluorescence assay (DFA) was used to confirm *B. burgdorferi* infection in ticks. Ticks were individually minced with micro-surgical scissors (Roboz Surgical Instrument, Washington, DC) and fine-tipped forceps in a drop of phosphate buffered saline (PBS) on a pre-cleaned or silylated microscope slide (PGC Scientifics, Frederick, MD). Scissors and forceps were decontaminated in 70% ethanol between samples to avoid cross contamination. After mincing, the cuticle fragments were removed and the slide preparation was air-dried then acetone fixed for 10 min. The slides were stained with a fluorescein isothiocyanate (FITC) conjugated goat anti-*Borrelia* polyclonal antibody (KPL, Gaithersburg, MD), diluted 1:150 in 5% fetal bovine serum and PBS for 1 h at room temperature. Following the antibody incubation, the slides were washed three times for 5 min. each with PBS and allowed to air dry after the final wash. A cover slip was mounted with Aqua Poly/Mount (Polysciences, Warrington PA) and the spirochetes were visualized by fluorescence microscopy. Ticks naturally infected with the B31 strain were used as a positive control and uninfected ticks or those capillary fed a solution without spirochetes were used as a negative control.

Ticks were also tested for infection by culturing in BSK II media. Individual ticks were placed in micro-centrifuge tubes and surface sterilized by soaking in 3% hydrogen peroxide for 10 min followed by 70% ethanol for 10 min. After removal of the ethanol, the ticks were homogenized in 25–50 μ l BSK II media using a disposable, sterile pestle (Fisher, Pittsburgh, PA). The homogenate and tick exoskeleton were transferred to a 6 ml culture of BSK II media also containing rabbit serum (Sigma, St. Louis, MO) and *Borrelia* antibiotic mixture (Sigma). Cultures were placed at 32°C for 1–2 wk and assessed by dark field microscopy for the presence of spirochetes.

Feeding of Capillary-Fed Ticks on Mice and Detection of *B. burgdorferi* in Murine Hosts. To determine whether capillary fed ticks would attach to mice, a group of 42 nymphal ticks that had capillary fed for periods ranging from 15 min to 1 h were placed on mice and allowed to attach and feed to repletion. The control consisted of six unfed nymphs from the tick colony placed on other mice at the same time and allowed to feed to repletion. To determine whether capillary infected ticks transmit *B. burgdorferi*, the tick-infested mice were tested for infection by serology (Western blot) and organ culture as previously described (de Silva et al. 1998).

Results

Measurement of Fluid Uptake by Nymphal Ticks. Of the 25 living unfed nymphs used in the radioassay experiment, 24 were alive at the end of capillary feeding. Fifty four percent (13/24) of these ticks had > 100 CPM in their tissues ranging from 155 to 1,658 CPM (mean = 656 ± 399 CPM) (Table 1). This represents an average fluid uptake of 0.03 ± 0.018 μ l (Table 1). In contrast, no significant radioactivity was found in the dead nymphs indicating that the radioactivity in the live nymphs was due to active uptake and not surface contamination.

Effect of Duration of Capillary Feeding on the Ability of Nymphal Ticks to Acquire *B. burgdorferi* Infection. To optimize capillary infection of ticks, nymphal ticks were capillary fed for different time periods before testing for infection. One group of unfed *I. scapularis* nymphs was capillary fed a solution containing *B. burgdorferi* (5×10^6 cells/ml) for 2.5 h while another group of ticks were fed for 13.0 h (Table 2). After capillary feeding, the ticks were allowed to attach to naive mice and feed to repletion before

Table 2. *B. burgdorferi* Infection of nymphal *I. scapularis* ticks by capillary feeding

| Nymphal ticks ^a | Capillary feeding ^b | | Nymphal infection ^c | | |
|----------------------------------|--------------------------------|---------|--------------------------------|--------------|-------------------|
| | Solution | Time, h | No. tested | No. infected | % infected |
| Unfed | +Bb | 2.5 | 10 | 6 | 60.0 ^d |
| Unfed | +Bb | 13.0 | 7 | 5 | 71.4 ^d |
| Unfed | -Bb | 13.0 | 2 | 0 | 0.0 |
| Unfed naturally infected | Not capillary fed | | 3 | 3 | 100.0 |
| Partially fed | +Bb | 4.0 | 9 | 6 | 66.7 ^d |
| Partially fed | -Bb | 4.0 | 5 | 0 | 0.0 |
| Partially fed naturally infected | Not capillary fed | | 8 | 8 | 100.0 |

^a Uninfected nymphal ticks that had not fed or that had partially fed on mice for 17 h were used in the capillary feeding experiments. Naturally infected nymphs were used as positive controls in both groups.

^b Nymphal ticks were capillary fed for different times on BSK media alone or BSK media with spirochetes (5×10^6 cells/ml). The naturally infected nymphs were not capillary fed.

^c Nymphs were tested for infection by DFA and/or culture.

^d The proportion of capillary infected nymphs in the unfed group (11/17) and the partially fed group (6/9) were not significantly different from one another (Fisher exact two-tailed test, $P > 0.05$).

testing for infection. The blood meal stimulates bacterial growth (de Silva and Fikrig 1995), thus amplifying small numbers of bacteria that may have entered the tick during capillary feeding. Ticks that were capillary fed for 2.5 h had a similar infection rate to ticks that were capillary fed for 13.0 h (60 compared with 71.4%) (Table 2). In another experiment nymphal ticks were capillary fed for shorter periods of time (15 min, 30 min and 1 h) before testing for infection. Even following these short exposures to solutions of *B. burgdorferi*, 33.3–50% of the ticks were infected. (Table 3). Prolonged capillary feeding (>2.5 h) did not increase infection prevalence among nymphal ticks and capillary feedings as short as 15 min resulted in 37.5% of the nymphs becoming infected. Furthermore, capillary feeding did not interfere with subsequent feeding on mice because similar proportions of capillary fed and unfed ticks attached and fed on mice (data not shown).

Experiments were performed to determine if partially blood fed nymphal ticks, already stimulated to feed by their previous host attachment, were able to acquire spirochetes by capillary feeding more efficiently than unfed ticks. Nymphal ticks were fed on naive mice for 17 h, removed from the mice and immediately capillary fed a solution with *B. burgdorferi* for 4 h. Partially blood fed and unfed nymphs had

a similar prevalence of infection (60.0 versus 66.7%) when they were capillary fed a solution of bacteria (Table 2).

Transmission of *B. burgdorferi* by Capillary-Infected Nymphal Ticks. Capillary-infected nymphs were placed on three mice (three to five ticks per mouse) and allowed to feed to repletion to determine if the ticks could transmit spirochetes to the mice. Two of the three mice exposed to capillary-infected ticks acquired *B. burgdorferi* infection (Table 3). Thus, capillary-fed ticks were capable of transmitting the infection to susceptible mice, although such transmission was not 100% efficient (Table 3).

Maintenance of *B. burgdorferi* in Nymphs Following Capillary Infection. Experiments were done to determine if ticks infected by capillary feeding retained the infection and could transmit the bacteria if feeding on host animals was delayed. One group of capillary-infected ticks was placed on mice immediately after capillary feeding, while another group was held for 10 d before placement on mice. The tick infection prevalence in both groups was similar (32 compared with 35%). Thus, capillary-infected nymphs maintained the infection for at least 10 d. Both groups of capillary fed ticks attached to mice at a similar frequency (80 compared with 100%). Both groups

Table 3. Effect of duration of capillary feeding on infection of *I. scapularis* nymphs with *B. burgdorferi* and transmission of infection to mice

| Nymphal ticks ^a | Capillary feeding ^b | | <i>B. burgdorferi</i> Infection ^c | | | Mouse infection ^d | |
|----------------------------|--------------------------------|------------|--|--------------|-------------------|------------------------------|---------|
| | Solution | Time (min) | No. tested | No. infected | % infected | WB | Culture |
| Unfed | +Bb | 60 | 9 | 3 | 33.3 ^e | - | - |
| Unfed | +Bb | 30 | 10 | 5 | 50 ^e | + | + |
| Unfed | +Bb | 15 | 8 | 3 | 37.5 ^e | + | + |
| Unfed | -Bb | 30 | 6 | 0 | 0 | - | - |
| Unfed naturally infected | Not capillary fed | | 3 | 3 | 100 | + | + |

^a Unfed, uninfected nymphal ticks were used for capillary feeding. Unfed, naturally infected nymphs were used as a positive control.

^b Nymphal ticks were capillary fed for different times with BSK/Shen's alone or BSK/Shen's with spirochetes (4.4×10^7 cells/ml).

^c Nymphs were tested for infection by DFA.

^d Mice were individually tested for infection 2 wk after ticks detached by collecting sera for Western blot (WB) and by culturing spleen and bladder in BSK II media.

^e In pairwise comparisons, the infection prevalence in groups fed for 15, 30 60 mins was not significantly different from one another (Fisher exact two-tailed test, $P > 0.05$).

Table 4. Long term maintenance and transmission of *B. burgdorferi* by capillary-infected nymphs

| Nymphal ticks ^a | Capillary feeding solution ^b | Time of placement on mice ^c | No. ticks engorged/ No. ticks placed (%) ^d | No. ticks infected/ No. ticks engorged (%) ^e | No mice infected/ Total no. mice (%) ^f |
|----------------------------|---|--|---|---|---|
| Unfed | +Bb | Immediate | 20/25 (80) ^g | 7/20 (35) ^h | 4/5 (80) ⁱ |
| Unfed | +Bb | Held 10 days | 25/25 (100) ^g | 8/25 (32) ^h | 3/5 (60) ⁱ |
| Unfed naturally infected | Not capillary fed | Not applicable | 5/5 (100) | 5/5 (100) | 1/1 (100) |

^a Unfed, uninfected nymphal ticks were used for capillary feeding. Unfed, naturally infected nymphs were used as a positive control.

^b Nymphal ticks were capillary fed for 1 h with BSK/Shen's containing spirochetes (4.6×10^7 cells/ml).

^c Capillary fed ticks were either placed immediately on mice or held for 10 d prior to placement.

^d A total of 25 Capillary fed ticks from each group was placed on mice (five ticks per mouse). Engorged ticks were collected from individual mice and data pooled within each group. One mouse received five naturally infected ticks and served as a positive control.

^e Engorged ticks were tested for infection by DFA.

^f Mice were individually tested for infection 2 wk after ticks detached by collecting sera for Western blot and by culturing spleen and bladder in BSK II media.

^{g,h,i} Pairwise comparisons within these groups did not reveal any significant differences between the immediate and 10 day held nymphs. (Fisher exact two-tailed test, $P > 0.05$).

were also able to transmit the infection to naive mice (Table 4).

Salivary Gland Infections in Ticks Infected by Capillary Feeding. Naturally infected nymphs are efficient at infecting mice and, in most cases, a single infected nymph is capable of infecting a mouse (Burkot et al. 1994, des Vignes et al. 2001). In experiments with capillary-infected nymphs, mice sometimes failed to acquire infection even though the nymphs fed to repletion (Tables 3 and 4). Experiments were performed to determine if capillary-infected nymphs had bacteria that multiplied and invaded the tick salivary glands as efficiently as spirochetes in naturally infected ticks. A group of nymphs were capillary fed and immediately placed on a mouse. Another group of naturally infected nymphs were also placed on a mouse. Both group of ticks were allowed to feed on the mice for sixty hrs at which point they were forcibly removed from the mice. Salivary glands and guts were dissected and examined for spirochetes by DFA. All the guts (10/10) and 90% (9/10) of the salivary glands from naturally infected nymphs contained spirochetes. In contrast, only 29% (6/21) of the guts and 14% (3/21) salivary glands from capillary-infected ticks had spirochetes. Thus, significantly fewer capillary fed nymphs had gut infections and salivary gland infections compared with naturally infected nymphs (Fisher exact two-tailed test, $P < 0.001$). Some of the infected guts from capillary-infected ticks had large numbers of spirochetes indicating that the small number of bacteria entering the gut had multiplied during the blood meal. Salivary gland invasion was not efficient in capillary fed nymphs that had gut infections because only three of the six nymphs with gut infections had bacteria in the salivary glands. Furthermore, infected salivary glands from capillary fed ticks had fewer bacteria than salivary glands from naturally infected nymphs collected at the same time point (A.B. and A.deS., unpublished data).

Contrasts in Ability of *I. scapularis* and *D. variabilis* Adults to Acquire *B. burgdorferi* Infection by Capillary Feeding. Female *I. scapularis* and *D. variabilis* adult ticks were partially fed on a rabbit, forcibly removed and capillary fed a solution of spirochetes as

described previously. To determine whether the ticks acquired *B. burgdorferi*, the ticks were surface sterilized and guts were removed for assay by DFA and culture in BSK II. In *I. scapularis*, infection was found in gut samples from 10 of 18 specimens. In contrast, in the *D. variabilis* specimens, no evidence of *B. burgdorferi* infection was found in the guts of nine specimens examined.

Discussion

Gern et al. were able to infect adult *I. ricinus* ticks using the capillary method (Gern et al. 1990). These investigators also used the capillary method to demonstrate that adult and nymphal *I. hexagonus* ticks were competent vectors of *B. burgdorferi* in the laboratory (Gern et al. 1991). The capillary feeding method has also been used to successfully infect adult *I. pacificus*, the main vector of Lyme disease in the western United States (Li and Lane 1996).

Here we have demonstrated that nymphal *I. scapularis* ticks can be infected with *B. burgdorferi* by capillary feeding. Capillary fed ticks were capable of maintaining the infection for at least 10 d and transmitting the spirochetes to mice. Ticks were infected as soon as 15 min after capillary feeding and the peak prevalence of infection (60%) was observed following 2.5 h of capillary feeding. More lengthy capillary feedings or increasing the concentration of bacteria in the feeding solution did not significantly increase the proportion of infected ticks. Based on these studies we recommend using unfed *I. scapularis* nymphs and feeding them a solution of spirochetes at a concentration of at least 5×10^6 cells/ml for 2.5 h to obtain optimal numbers of infected nymphs. It is also important to note that these experiments were performed with a single strain (B31) and the procedure may have to be optimized for other strains.

In spite of the different parameters we varied, the infection prevalence ranged from 30–70%. Similar results were obtained by Gern et al. (1991) who observed an infection prevalence of 66.6% with *I. hexagonus* nymphs that were capillary fed. When we measured fluid uptake by capillary fed ticks, 46% of the

ticks did not take up fluid and these ticks may be responsible for infection prevalence remaining below 70% in all our experiments. The ticks that did take up fluid consumed an average of 0.03 μ l of BSK II medium. Kurtenbach et al. also observed a similar volume (0.05 μ l) of fluid taken up by capillary fed *Ixodes ricinus* nymphs (Kurtenbach et al. 1994). The observation that the amount of liquid consumed by nymphs was small is not surprising given the artificial nature of the feeding procedure. Furthermore, we placed the palps of the feeding ticks inside the glass tube because it was easier to fit the narrow opening of the tube over all the mouth parts. However, under natural feeding conditions the palps remain outside the host and our placement of the palps inside the tube may have inhibited fluid uptake. The feeding solutions we used contained spirochetes at a density between 5×10^6 to 5×10^7 /ml and a nymph consuming 0.03 μ l would have received an inoculum of 150–1,500 bacteria which was sufficient for establishing an infection in the tick.

A single naturally infected nymph is capable of infecting susceptible rodents (Burkot et al. 1994, des Vignes et al. 2001). We observed several instances in which a capillary-infected nymph fed to repletion on a mouse without transmitting the infection. When naturally infected nymphs feed on a host, the spirochetes multiply within the tick gut (de Silva and Fikrig 1995). About 48 h into the blood meal some spirochetes move from the gut to the salivary glands and enter the host dermis (Ribeiro et al. 1987, Zung et al. 1989, Piesman 1993). In the experiments reported here we observed a greater proportion of infected salivary glands in naturally infected nymphs compared with capillary infected nymphs indicating that the differences in transmission may be due to differences in the ability to invade the salivary glands of the nymphs. Naturally infected nymphs acquire the infection at the larval stage and the spirochetes survive in the tick for several months to over a year before the nymphal blood meal (Yuval and Spielman 1990). In the case of capillary-infected nymphs, the nymphal blood meal occurred soon after (2 h to 10 d) acquisition. This brief period may not have been sufficient for the *Borrelia* to adapt to the internal environment of the nymphal ticks and may have contributed to the reduced efficiency of transmission. Nonetheless, mice exposed to as few as three capillary-infected nymphs became infected.

Perhaps, not surprising was the observation that adult female *D. variabilis*, considered as an incompetent vector of *B. burgdorferi* (Mather and Mather 1990; Piesman and Sinsky 1988), failed to maintain infection after imbibing infectious spirochetes. These data are also in agreement with recent studies from our laboratory demonstrating that *D. variabilis* are capable of mounting an effective immune response to clear *B. burgdorferi* unlike *I. scapularis* ticks which are immunotolerant (Johns et al. 2001). Our results are also similar to results obtained by Li and Lane (1996) who used the capillary method to demonstrate that adult *I. pacificus* is a competent vector and *D. occidentalis* (Marx) an incompetent vector. In capillary fed *D.*

occidentalis ticks, spirochetes were present in the gut but the bacteria were unable to disseminate beyond the gut. In the case of *D. variabilis*, we did not observe spirochetes in the gut. Li and Lane (1996) used spirochetes at a density of 2.8×10^8 /ml whereas we used 10–100-fold less concentrated solutions of bacteria, which may explain the differences in our results.

The capillary feeding method is simple and of particular interest for the small *Ixodes* nymphs because it is less invasive than other methods such as enema or intrahemocoelic injection. In our hands both enema and intrahemocoelic injections resulted in the majority of nymphs dying, most likely due to tissue trauma (data not shown). Capillary feeding also permits experimental manipulations that are otherwise not possible. Individual ticks can be infected with clonal populations of bacteria as well as different strains of spirochetes. The inoculum size entering the tick can be altered to determine the infectious dose. Spirochetes that are not infectious to mice can be tested for their phenotype in the vector. Individual ticks can be co-infected with multiple strains or species of tick-borne pathogens. Molecules that enhance or inhibit tick infection can be tested by introducing them along with the inoculum. We hope that this simple method will be more widely used to study interactions between pathogens and their ixodid tick vectors.

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