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Original Investigation

Experimental infection of dogs with *Borrelia burgdorferi* sensu stricto using *Ixodes scapularis* ticks artificially infected by capillary feeding

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Abstract Specific pathogen-free dogs were experimentally infected with *Borrelia burgdorferi* sensu stricto using nymphal or adult female *Ixodes scapularis* ticks artificially infected with spirochetes by capillary feeding. The ticks were capillary fed *B. burgdorferi* isolate 610, previously isolated from a dog with Lyme disease and grown in BSK medium. This isolate induced clinical signs in the dogs similar to those for dogs infested with ticks naturally infected with *B. burgdorferi*. Adult ticks were more efficient than nymphs in transmitting spirochetes to the dogs. One of five dogs infested with nymphal ticks capillary fed *B. burgdorferi* was skin biopsy culture and serologically positive, and demonstrated lameness. In contrast, all five dogs infested with adult female ticks that had been capillary fed with *B. burgdorferi* were culture and serologically positive,

with one dog developing lameness. The immunoblot profiles of dogs challenged with female ticks infected by capillary feeding (8 weeks post challenge) were similar to immunoblots (4 weeks post challenge) from dogs challenged with naturally infected females collected in the field. These studies demonstrated that *B. burgdorferi* cultured in BSK medium can be capillary fed to either nymphal or adult female ticks under laboratory controlled conditions for the purpose of transmitting the spirochete to dogs during the tick's blood meal. This tick infection system would be useful for a controlled and defined challenge of vaccinated and non-vaccinated dogs for proper evaluation of vaccine efficacy, which is difficult to achieve using field-collected ticks. Furthermore, this system may also be useful for investigation of the pathogenesis of Lyme disease, evaluation of the pathogenicity of new isolates of *B. burgdorferi*, or evaluation of antibiotic therapy.

Keywords Lyme disease · *Borrelia burgdorferi* sensu stricto · *Ixodes scapularis* ticks · Experimental infection · Capillary feeding

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Introduction

Borrelia burgdorferi sensu stricto, the spirochete that causes Lyme disease in the United States, is transmitted to a mammalian host mainly by *Ixodes scapularis* ticks [7]. The spirochete also causes Lyme borreliosis in dogs [19, 27, 33, 34, 35, 58], where the disease can be divided into an early and late stage [29]. The most common clinical sign of early Lyme disease is sudden on set of lameness with swelling in the affected joints [9, 23, 27, 28, 29, 34, 51]. Associated clinical signs of lethargy, fever, anorexia, arthralgia, lymphadenopathy, and generalized pain are also observed during this stage. The ELISA is mostly used to document exposure of dogs to *B. burgdorferi* and IgG antibodies are detectable 4–6 weeks after exposure [39]. During the early stage the spirochetes are susceptible to antibiotics. If left untreated, neurological, cardiac, renal disease, and arthritis, are potential manifestations of the late-stage disease as the organisms can take residence in areas not accessible to humoral and cellular immune responses or antibiotics. Remaining organisms may then multiply and be responsible for sites of inflammation, resulting in recurrent episodes of arthritis and other associated clinical signs [39, 55]. Antibiotic administration, orally or intravenous, fails to eliminate a persistent infection in dogs but may prevent and cure joint disease [55]. However, persistently infected dogs when subsequently treated therapeutically with an immunosuppressant (e.g., prednisone) may develop severe polyarthritis [56].

Vaccines that induce antibodies to outer surface proteins (Osp) of *B. burgdorferi*, specifically OspA and B, can provide protection against infection and disease in dogs [11, 53]. To evaluate efficacy of these vaccines, vaccinated as well as non-vaccinated control dogs must be challenged with virulent organisms inoculated by a tick bite [1, 17, 24, 41]. Needle inoculation of cultured spirochetes to evaluate vaccine efficacy is unsatisfactory because it fails to reproduce the natural challenge and *B. burgdorferi* cultured in liquid medium can change their outer surface antigen profile and lose infectivity [49, 59]. The diverse environmental changes that spirochetes experience, especially at the time of transmission from one host to the other, induces differential expression of genes important to spirochete survival in each host. Most notable is the change in the expression of genes that code for immunogenic outer surface proteins, OspA and OspC [13, 50]. At the time of transmission from the tick to the mammalian host spirochetes up-regulate *ospC* expression and down-regulate *ospA* expression [50]. Thus, in dogs, as in humans, a primary immune response is directed against those spirochetal proteins expressed during the blood meal, e.g., OspC, and rarely to OspA [1, 53, 56]. Furthermore, pharmacologically active substances in tick saliva are also transmitted and contribute to the infectivity and establishment of the spirochete [22]. Thus, a tick bite challenge is necessary for proper evaluation of vaccine efficacy [11, 47, 48]. Infected ticks used for such challenges have either been field collected from Lyme disease endemic areas or laboratory-reared ticks experimentally infected with *B. burgdorferi* under controlled conditions. Ticks collected from the field suffer from the shortcoming that they can vary in their levels of spirochetal infection and strain identity [12, 20, 25, 40]. They may also harbor other canine pathogens such as ehrlichial or babesial species that can complicate or negate a proper tick challenge [36, 54]. The artificial infection of pathogen-free ticks in the laboratory provides a controlled challenge system free of interfering disease agents and specific for the *B. burgdorferi* isolate chosen. The method of capillary feeding ticks [5, 6, 32, 46] has been successfully used to artificially infect ticks with several different tick-borne pathogens. It has also been used to evaluate changes in outer surface proteins of *B. burgdorferi* fed to ticks and to evaluate vaccine efficacy in rodents [15, 16, 17].

The objective of our research was to compare the host immune response of beagle dogs infected with *B. burgdorferi* via the bite of ticks that were capillary fed *B. burgdorferi* sensu stricto strain 610 with field-collected ticks having acquired spirochetes naturally by feeding on a spirochetemic host. We also determined if the dogs infested with the capillary fed ticks became infected and demonstrated clinical signs of canine Lyme disease. Here we present evidence that the immune response of dogs was similar following infection with either capillary fed or naturally infected

ticks. We also present evidence that ticks that have been capillary fed spirochetes are capable of transmitting the spirochete to dogs and that spirochetes transmitted this way can cause clinical canine Lyme borreliosis.

Materials and methods

Bacteria

B. burgdorferi sensu stricto isolate 610 was recovered from a skin biopsy specimen of a dog infested with ticks collected from a Lyme disease endemic area in rural LaCrosse, Wisconsin. Isolate 610 was identified as *B. burgdorferi* sensu stricto by polymerase chain reaction (PCR) [31, 38]. Identification was also confirmed using the OspA-specific monoclonal antibody (mAb) H5332 [3]. Isolate S-1-10 from a white-footed mouse trapped in the LaCrosse Wisconsin endemic area, [30] was used to prepare antigen for immunoblots and the whole cell ELISA assays. Isolate S-1-10 was selected for use as a standard in the ELISA and Western immunoblots because of its infectivity and similarity to B31, the type strain for *B. burgdorferi* sensu stricto; the *ospA* gene sequences of B31 and S-1-10 are identical [30]. S-1-10 was also chosen because it originated from the same geographical area [31] as the field-collected ticks that were used in the preliminary study (see below), during which isolate 610 was obtained. We used isolate 610 for capillary feeding trials because of its apparent ability to induce clinical signs of Lyme borreliosis, including lameness, in a dog. BSKH medium [45] (Sigma Chemical Co., St. Louis, MO) was used to grow spirochetes for capillary feeding ticks. BSK medium [2] was used to isolate spirochetes from tissues of dogs. Isolates S-1-10 and 610 were cultured at 32°C or 34°C and stored frozen in BSKH medium with 10% glycerol at −70°C or liquid nitrogen until used. Both isolates had been passaged less than ten times after isolation from a dog (610) or receipt from Dr. Lovrich (S-1-10).

Ticks

I. scapularis adult and nymphal ticks used in these studies were from a laboratory colony in the fourth to sixth generations [24]. Ticks were confirmed free of *B. burgdorferi* by PCR analysis of tick tissues, by ear biopsy cultures of hamsters used to feed the ticks, and by phase-contrast microscopic analysis of tick midguts.

Capillary feeding of *B. burgdorferi* to adult and nymphal ticks

B. burgdorferi isolate 610 passage three was cultured in BSK-H medium, grown to mid-log phase, centrifuged, and resuspended in fresh BSK-H medium to a concentration of 1×10^8–$1.5X10^8$ cells/ml. Ticks were immobilized in petri dishes with their dorsal side down using double-sided tape. Micro-tip capillary pipettes were made using a capillary pipette puller (Sutter Instrument Co., San Rafael, CA). The capillary pipette tip was broken off to fit loosely over the tick's hypostome, and filled with a spirochete suspension. The pipette was then placed carefully over the hypostome and the tick was allowed to imbibe the suspension while housed in a humid chamber at 34°C. Feeding ticks were examined with a dissecting microscope and those with a distended abdomen and anal excretion, evidence that the ticks had imbibed spirochetes, were transferred to sterile vials and stored at 28°C for 1 week. Only those ticks that had ingested spirochetes and excreted BSK medium were used in the subsequent challenge (see next section); thus, all of the ticks placed on the dogs had ingested spirochetes and had putative borrelial infections of the gut.

Tick challenge of dogs

All dogs were handled according to USDA animal welfare guidelines. Each dog was infested with 30 nymphal ticks or 10–11 adult females with 6–7 male ticks. Males were added to ensure that females had mated and to maximize the feeding response and hence the challenge. *I. scapularis* females mate off and on the host and mated females are more likely to engorge to repletion and to transmit spirochetes. Furthermore, *I. scapularis* must feed for at least 3 days to transmit *B. burgdorferi* to the host [43]. Ticks, randomly selected, were placed in small petri dishes. Petri dishes were taped to a shaved area on the dorsal-anterior area of the thorax of each dog. The dishes were further secured by placing a tape wrap around the thorax. Nymphal or adult female ticks were allowed to feed for 6–7 days to reach full engorgement. After feeding the ticks were collected and the presence of *B. burgdorferi* confirmed by either microscopy or culture of midgut tissue in BSK medium. Two infection studies were conducted. In one study, 30 nymphal ticks infected with isolate 610 were placed on each of five, 3-month-old beagles of mixed sex from Harlan Sprague Dawley, Inc. (Madison, WI). Blood samples were collected from dogs at the time of tick placement, at 2, 3, and 4 weeks, and then every 4 weeks to week 28 post tick placement. In the second study, 11 adult female ticks infected with isolate 610 and 7 male ticks were placed on each of five approximately 4-month-old beagles of mixed sex acquired from Liberty Research, Inc. (Waverly,

NY). Blood samples were collected from dogs at the time of tick placement, at 1, 2, 3, and 4 weeks, and then every 4 weeks to week 32 post-tick placement.

ELISA and Western immunoblot

Sera from blood samples collected in each study were tested by ELISA and Western immunoblot. Antibody response to antigens of *B. burgdorferi* was determined using a modification of a whole cell ELISA used by the Regional Animal Health Laboratory (Baron, WI). Briefly, mid to late log phase cultures of isolate S-1-10 were inactivated with binary ethyleneimine (BEI). Following neutralization of BEI with sodium thiosulfate, the cells were washed by centrifugation three times with sterile saline. The total protein content of the inactivated organisms was determined by bicinchoninic acid (BCA) protein assay (Pierce Co., Rockford, IL). Wells of Immulon 3 plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated with whole cell antigen at 0.3 µg in 100 µl per well of 0.1 M carbonate buffer, pH 9.6. Plates were incubated in a humid chamber at 4°C for 15–17 h. Following incubation, unreacted sites were blocked with 0.15 M phosphate-buffered saline (PBS) solution containing 5% nonfat dried milk and incubated in a humid chamber for 60 min at 37°C. Wells were emptied and 50 µl of twofold dilutions of test sera were added to wells of the Immulon 3 plates. Test sera were from dogs challenged using ticks capillary fed *B. burgdorferi* isolate 610. Dog sera, serially diluted in PBS containing 0.05% Tween-20 (PBS-TW), were added to duplicate wells and incubated in a humid chamber for 60 min at 37°C. Positive and negative canine control sera were included on each plate. Plates were washed three times with saline containing 0.05% Tween-20. Bound antibody was detected by adding 50-µl aliquots of peroxidase-labeled goat anti-dog IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). The plates were incubated in a humid chamber for 60 min at 37°C and washed three times with PBS-TW. Bound antibody was visualized with *o*-phenylenediamine chromogen substrate. The substrate was prepared by dissolving 30 mg *o*-phenylenediamine in a 0.051 M dibasic sodium phosphate, 0.024 M citric acid, 0.012% hydrogen peroxide solution. Substrate was added at 100 µl/well and allowed to react for 4 min. A 2 N sulfuric acid solution (50 µl/well) was used to stop the reaction and the optical density of each well was determined at 490 nm using an ELISA reader. The titer was defined as the reciprocal of the last dilution that gave an optical density of 30% of the peak optical density.

For the Western immunoblot analysis, *B. burgdorferi* isolate S-1-10 was grown in BSK medium at 32°C. Cultures in the mid to late logarithmic phase of growth were harvested by centrifugation (15,000 *g*, 4°C, 30 min), and washed three times with sterile saline (0.85% NaCl, pH 7.2).

Suspensions of approximately 1×10^8 cells were boiled 9 min in reducing sample buffer (0.06 M TRIS-HCl, pH 6.8, 2% sodium dodecyl sulfate, 10% glycerol, 5% mercaptoethanol, 0.001% bromophenol blue) and electrophoresed at 25 mA for 2–3 h on a 10% SDS-polyacrylamide gel [26]. Proteins were transferred onto an Immobilon PVDF membrane (Millipore Corp., Bedford, MA) by modification of the procedure described by Towbin et al. [57]. To block unreactive sites the PVDF membranes were incubated for 90 min at 22°C in 20 mM TRIS (pH 7.2), 150 mM NaCl, and 5% nonfat dried milk. Membrane strips were incubated with canine sera or mAb to OspA (H5332) [4] diluted 1:75 in the blocking solution for 60 min at 22°C. Strips were then washed twice in TBS containing 0.2% Triton X-100 and once in TBS. Bound antibody was detected by the addition of horseradish peroxidase-labeled anti-canine IgG (Kirkegaard & Perry Laboratories) or anti-murine IgG. Bound antibodies were visualized with a TMB membrane peroxidase substrate system (Kirkegaard & Perry Laboratories).

To approximate the sizes of the immunoblot-stained antigens, we used molecular weight standards containing prestained markers from Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA; phosphorylase B, 112 kDa; bovine serum albumin, 84 kDa; ovalbumin, 53.2 kDa; carbonic anhydrase, 34.9 kDa; soybean trypsin inhibitor, 28.7 kDa; lysozyme, 20.5 kDa) or GIBCO BRL (Life Technologies, Rockville, MD; myosin, H-chain, 200 kDa; phosphorylase B, 97 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 29 kDa; β-lactoglobulin, 18 kDa; and, lysozyme, 14 kDa).

Skin biopsies, clinical signs and necropsy

Skin biopsy specimens were taken from anesthetized (a local anesthetic of xylazine, butorphanol and tiletamine HCl-zolazepam HCl) dogs approximately 3 weeks following tick attachment and at the time of necropsy. Biopsies were taken from the tick attachment site and from a site distant to the tick bite site located in the posterior area of the dog. The sites were shaved, washed with Solvahex Surgical Scrub (Solvay Animal Health, Inc., Mendota Hts., MN), and rinsed thoroughly with sterile water. An elliptical incision was made through the dermal and subcutaneous skin layers. Approximately 0.5 g skin was placed in 9 ml BSK medium, homogenized, and two additional tenfold dilutions of the homogenate were made in 9 ml BSK medium. Cultures were incubated at 32°C for 6 weeks and were examined microscopically at weekly intervals for the presence of spirochetes. Spirochetes were confirmed as *B. burgdorferi* by indirect fluorescence antibody analysis using a *B. burgdorferi* mAb specific to OspA (H5332).

Dogs were observed daily following tick infestation for clinical signs of Lyme disease. Lameness, stiff-leggedness, or reluctance to bear weight on the affected limb, with or without swelling, and warmth at the affected joint was used as the primary clinical sign of Lyme disease. Dogs were also observed for lethargy and fever usually associated with lameness. Daily observations were made for 1 year.

To establish the extent of infection, dogs displaying lameness were euthanized humanely (Beuthanasia) and necropsied either at the end of a study or immediately following an episode of lameness. Tissue was taken from the elbow, carpus, knee, and tarsus joints. Approximately 0.5 g tissue from each joint was added to 9 ml BSK medium with an additional 10-fold dilution made in the same medium. These cultures were incubated and observed as previously described. The heart, spleen, kidneys, and bladder were separately homogenized in 50 ml BSK medium using a Stomacher (Seward Medical, London, England). A sample of the homogenate was further diluted 10- and 100-fold in BSK medium. All dilutions and a sample of the original organ homogenates were incubated at 32°C and observed for 6 weeks; positive cultures were confirmed as *B. burgdorferi* as described above.

Results

Immune response of dogs infected using naturally infected ticks

In a preliminary study, adult ticks collected in the field from a Lyme disease endemic area in LaCrosse, Wisconsin were used to challenge 15 dogs. Eight weeks post infestation, 14 (93.3%) of the dogs were immunoblot positive for *B. burgdorferi* (Fig. 1). One dog identified as 610, and from which isolate "610" was obtained, developed a severe case of recurrent arthritis, swollen joints, lethargy, and fever. Serological analysis and recovery of *B. burgdorferi* from skin, joints, heart, spleen, kidneys, and cerebrospinal fluid confirmed the diagnosis as Lyme borreliosis. Strong bands in the 40-kDa range, putative 39 and 41 kDa antigens and weak or absent immune responses in the region of Osp A, both indicative of an immune responses to *B. burgdorferi* induced by tick bite [1, 10, 53], revealed that 13 of the15 dogs were immune positive for *B. burgdorferi*. In addition, a series of bands with dominant reactivity was noted in the region of higher molecular weight standards bovine serum albumin (68 kDa) and phosphorylase B (97 kDa) region

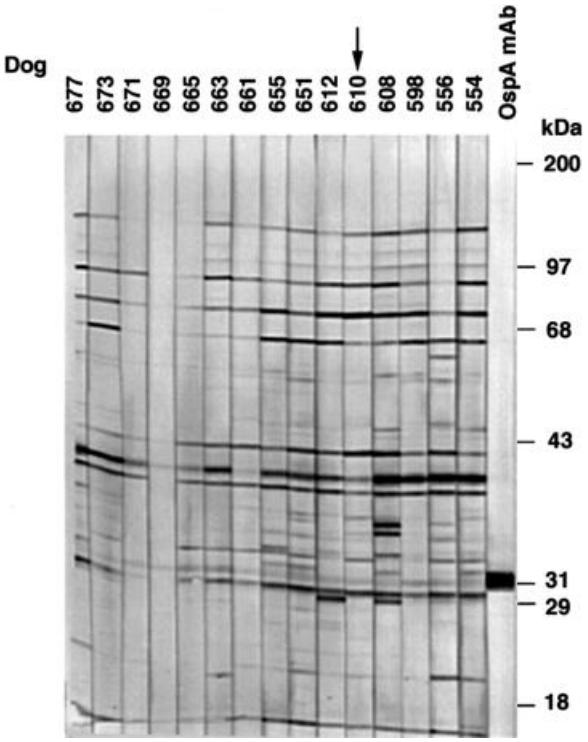

Fig. 1. Western immunoblot of serum from dogs challenged with naturally infected *Ixodes scapularis* ticks collected in rural LaCrosse, Wisconsin. An average of ten adult female ticks were allowed to feed on the dogs for 7 days. Sera were collected from the dogs approximately 4 weeks post challenge and reacted against S-1-10 antigen. *Arrow* points to lane depicting immune response of dog 610 from which isolate "610" was recovered. The prestained molecular weight standard indicated was from Life Technologies and the positions of the prestained proteins (in kDa) are shown on the *right*

Nymphal ticks capillary fed *B. burgdorferi*

Nymphal ticks infected with isolate 610 attached quickly and fed well. On day 6 the ticks were collected and examined for extent of feeding. An average of 12 ticks per dog had fed to engorgement. Only one of the five dogs (dog GCT) seroconverted and *B. burgdorferi* was isolated from skin biopsiy specimens taken at the tick bite site. A rise in the borrelial antibody titer of that dog was first detected 4 weeks post tick placement. The antibody response quickly elevated to an ELISA titer of 2,560 by 8 weeks (data not shown) and remained elevated for 3 months post tick placement at which time the dog exhibited an episode of lameness. The Western immunoblot of serum from the infected dog also indicated that the dog had mounted an immune response to *B. burgdorferi* by 8 weeks following infestation (Fig. 2). The presence of two bands in the 40-kDa region and absence of a band in the OspA region, again consistent with published reports [1, 10, 53], were taken as evidence of an immune response to *B. burgdorferi* transmitted by tick bite.

Bands were also present in the 100, 80, and 30-kDa ranges. Again, we noted a series of bands with dominant reactivity in the region of bovine serum albumin (84 kDa) and phosphorylase B (112 kDa) region

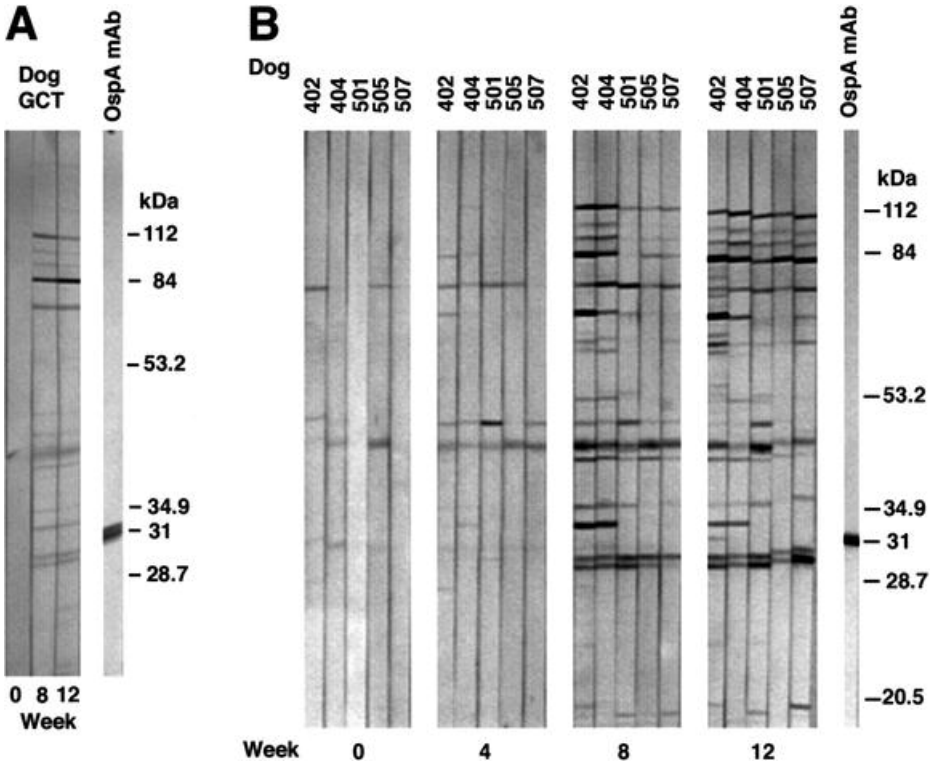

Fig. 2. Western immunoblots of sera from dogs challenged with *Ixodes scapularis* ticks capillary fed *Borrelia burgdorferi* sensu stricto, isolate 610. **A** Western immunoblot of serum from one dog (GCT), of five, that became serologically positive for *B. burgdorferi* when challenged with 30 laboratory reared *I. scapularis* nymphs infected with isolate 610 *B. burgdorferi* via capillary feeding. Serum was collected prior to tick challenge (0), 8 and 12 weeks after tick challenge. **B** Western immunoblot of sera from dogs, each individually, challenged with 11 laboratory reared adult *I. scapularis* ticks infected with *B. burgdorferi* isolate 610 via capillary feeding. Sera were collected prior to tick challenge (0), 4, 8 and 12 weeks post challenge. Antisera were tested against *B. burgdorferi* isolate S-1-10 antigen. Note that all five dogs were serologically positive by week 8. The positions of the prestained molecular weight markers from Bio-Rad Laboratories (in kDa) are shown on the right of each panel

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The other four dogs remained serologically negative for the duration of the study. The infected dog had a fever of 102.7°F, a swollen carpal joint and exhibited lameness in the right front leg approximately 3 months after tick feeding. Immediately following this lameness episode the dog was euthanized and necropsied. The elbow and carpal joints had excessive yellowish synovial fluid and *B. burgdorferi* was recovered from these joints and from the kidney. *B. burgdorferi* was also recovered from the skin at the tick bite site and the skin from the posterior area of the dog.

Adult ticks capillary fed *B. burgdorferi*

Three days following placement the infected adult female ticks had started to feed. When ticks were collected on day 7 an average of eight ticks per dog had fed to engorgement. Positive ELISA titers were detected in all five dogs by 4 weeks post placement of the adult ticks, indicating that all dogs were exposed to spirochetal antigens. Titers peaked by 8 weeks and remained elevated throughout the study. Immunoblots of sera collected 8 weeks post infestation showed Western banding profiles (Fig. 2) characteristic of animals infected with *B. burgdorferi* by tick bite [1, 10, 53]. Characteristic bands were present in the 40-kDa range, but no bands were present in the region of OspA. As with the dogs challenged with infected nymphs, we noted a series of bands with dominant reactivity in the region delineated by the bovine serum albumin and phosphorylase B molecular weight markers. These profiles were similar to those shown by dogs infected using naturally infected field-collected ticks (compare with Fig. 1) and demonstrated that all dogs had established *B. burgdorferi* infections. Furthermore, *B. burgdorferi* was isolated from skin biopsy samples taken from each dog.

One dog infested with capillary fed adult s exhibited two episodes of lameness in the right front leg approximately 3 months after tick placement. The dog had temperatures of 103.1 and 103.6°F during the episodes and swelling was observed in the metacarpal joint area. This dog was also necropsied following the episodes of lameness and *B. burgdorferi* was isolated from the tick bite site, the skin in the posterior area of the dog, and from the right and left carpal and elbow joints. Microscopic examination of synovial fluid from these joints indicated nonseptic inflammation consistent with immune-mediated polyarthritis. The other four dogs showed no signs of lameness or fever and were not necropsied.

Discussion

Vaccines for prevention against canine Lyme disease have been licensed based on efficacy studies in which vaccinated and nonvaccinated dogs were challenged with *B. burgdorferi*-infected ticks collected from the field. In fact, field-collected ticks have been the usual source of infection for studying the pathogenesis of Lyme borreliosis and for testing immune protective responses. However, ticks collected from the field may have low or variable infection rates, may be infected with more than one strain or species of *Borrelia*, and may transmit other canine pathogens that could interfere with proper evaluation of vaccine efficacy. In such studies the co-infection of ticks with other canine pathogens was considered and the ticks were tested for such pathogens. These

have included ehrlichial and babesial species along with the Powassan virus [1]. One study on the regulation of interleukin 8 in synovial fluid was complicated by the use of ticks co-infected with ehrlichia [54]. Such tests are limited by our knowledge of pathogens transmitted by the tick and adequate diagnostic tests for these extraneous pathogens. An alternative method is to use ticks reared on specific pathogen-free laboratory animals and infected with specific and characterized strains of the pathogen. The production of nymphal or adult ticks naturally infected via feeding on spirochetemic hosts is still somewhat imprecise and requires a lengthy process of blood meal digestion and subsequent molting of the tick resulting in highly variable infection rates [41, 42]. An alternative approach is to use ticks infected by feeding them spirochetal suspensions via a capillary tube. This method was first reported by Burgdorfer [6] and has been used by others recently [15, 16, 17, 46]. A method to facilitate the mass feeding of ticks for such studies has recently been described [5]. Until now host responses of dogs challenged with capillary fed ticks had not been compared with those of dogs challenged using naturally infected ticks. We have demonstrated that the immune responses of these dogs infected using capillary fed ticks were similar to those infected using ticks infected naturally by feeding on a spirochetemic host.

In this report we demonstrate capillary feeding as a method of transmitting *B. burgdorferi* to *I. scapularis* female or nymphal ticks obtained from a laboratory colony specifically free of *B. burgdorferi* and other canine pathogens. We have described the use of pathogen-free ticks infected with a specific *B. burgdorferi* isolate to characterize a specific infection in dogs. Ticks infected by this method successfully transmitted spirochetes to dogs during their blood meal. All dogs infested with infected adult female ticks became seropositive for *B. burgdorferi* as determined by Western immunoblot and ELISA. Our immunoblot banding patterns were similar to an infection caused by spirochetes inoculated into dogs by field collected ticks and those published elsewhere [14, 53]. Intense bands in the 31 (OspA) or 34 (OspB) -kDa regions, which is a characteristic immune response to *B. burgdorferi* administered by vaccination or needle inoculation of cultured spirochetes were not observed. In addition, we observed bands in the 40-kDa range, representing putative 39 and 41-kDa antigens. Both patterns are representative of an immune response elicited by tick transmitted infection in dogs [1, 10, 53]. The immunodominant bands in the region of the bovine serum albumin (84 kDa) and phosphorylase B (112 kDa) molecular weight markers were also present in the immunoblots shown by Chang et al. [10] and Shin et al. [52]. The significance of these immunogens of higher molecular weight in dogs remains to be defined. In a separate study we have successfully infected dogs using nymphs and adults capillary fed with another midwestern strain of *B. burgdorferi* sensu stricto, isolate JMNT, and obtained results similar to those with the

610 isolate (unpublished results). All of the dogs infected with the 610 isolate by adult female ticks seroconverted and were skin biopsy positive with one dog exhibiting lameness. Lameness due to Lyme disease is more commonly seen in younger dogs and is the predominant clinical sign for canine Lyme disease [1]. Only one of the five dogs challenged using nymphal ticks capillary fed 610 seroconverted, was skin biopsy positive, and exhibited lameness. Similarly, Appel et al. [1] reported that dogs exposed to infected field-collected *I. scapularis* nymphs had lower rates of seroconversion and disease frequency than those infected using adult female ticks. This may reflect the smaller number of spirochetes transmitted by nymphs during feeding [8]. Historical data indicate that dogs infected with field-collected ticks may become ill due to additional pathogens naturally present in such ticks, e.g., ehrlichial and babesial species along with the Powassan virus. It has been suggested that lameness may reflect the presence of secondary infections by babesia or ehrlichia [54]. Clearly, the role of such mixed infections in the clinical manifestation in dogs infected with *B. burgdorferi* needs to be examined. The use of ticks selectively infected with borrelia and other pathogens via capillary feeding offers one approach to testing this hypothesis.

The immune response of dogs inoculated subcutaneously with *B. burgdorferi* is quite different when compared to dogs naturally infected via the bite of an infected tick [1]. Most notable is a strong anti-OspA and -OspB response in dogs inoculated subcutaneously and the weak response in dogs infected via tick bite. This has caused researchers to question the validity of using needle inoculation with spirochetes to test efficacy of Lyme disease vaccines for dogs. Serological results, and recovery of *B. burgdorferi* from skin biopsy samples and joint tissues indicate that ticks infected in the laboratory under controlled conditions induce infection in dogs similar to field ticks. Lack of clinical signs may be related to the isolate used to infect ticks, which may not be of sufficient virulence or have the proper host-adaptive pathogenic characteristics suitable for establishing an infection resulting in lameness. Younger dogs of approximately 6 weeks of age have been found more likely to develop lameness than older dogs when challenged with field-collected ticks [1]. The dogs used in our studies were selected to be 3–4 months old at the time of challenge. In previous studies we have found lameness to occur in the age range of 6–21 months following challenge with field-collected ticks (unpublished data) with lameness most frequently occurring in the age range of 8–14 months. False positive results can be obtained when whole cell antigens are used in ELISA to detect canine borreliosis infections. Shin et al. [52] emphasized that ELISA tests using whole *B. burgdorferi* lysates must be treated with caution because of the cross-reactivity between borrelial and leptospiral serovars. However, they could be distinguished using immunoblots, indicating the need to couple ELISA with immunoblot

analysis, as performed in this study. Indeed, one dog that was found to be positive via ELISA was later found to be negative on the basis of immunoblot analysis. The results in our study confirmed those of Magnarelli et al. [37], as the pattern of reactivity to whole cell antigens was also highly variable for dog sera. The immunoblots showed distinct positive reactions with OspE (19 kDa) and OspF (29 kDa). However, the reactivities to OspA and OspB were infrequent and the number of dogs showing antibodies to OspC was low [37].

At 8 and 12 weeks, the general banding patterns of the immunoblots from the dogs challenged using capillary fed adult females were similar to the 4 week banding profiles of the dogs challenged with naturally infected field collected female ticks. Both sets showed strong bands in the 40 kDa range, location of putative P39 (39 kDa) and flagellin (41 kDa), and no bands in the region of OspA (31 kDa). Similar banding profiles were also noted in the higher molecular weight region delineated by the prestained proteins bovine serum albumin and phosphorylase B. Mice challenged with *I. ricinus* nymphs capillary fed *B. burgdorferi* sensu stricto (strain ZS7) gave prominent responses to P39, flagellin and a higher molecular weight antigen and a concomitant lack of response to OspA or OspB [17, 18]. At 4 weeks the immune responses of the dogs challenged with capillary fed adult females were weak, and this apparent delay in the immune response may reflect the kinetics and number of spirochetes inoculated during tick feeding. Field-collected adult ticks that are infected are likely to have acquired the spirochetes as larvae and/or nymphs. Also, field-collected nymphal and adult female *I. scapularis* can have disseminated infections with spirochetes already present in the salivary glands [44, 60]. Only a small number of spirochetes capillary fed to *I. ricinus* nymphs migrated from the gut to cause a systemic infection and invade the salivary glands [21]. The apparent lack or weak immune response shown by dogs challenged using capillary fed nymphs may also reflect the kinetics and number of spirochetes inoculated during nymphal feeding. Further studies are needed to characterize the tissue distribution and differential expression of antigens in spirochetes within capillary fed ticks during the blood meal. Such studies are needed to better understand the kinetics of the immune response and to identify the spirochetal immunogens revealed in the Western blots.

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