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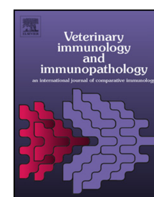

Demonstration of the ability of a canine Lyme vaccine to reduce the incidence of histological synovial lesions following experimentally-induced canine Lyme borreliosis

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ABSTRACT

Lyme disease in dogs can be effectively prevented by vaccination against antigens expressed by the spirochete *Borrelia burgdorferi* during transmission by the tick vector *Ixodes* sp. Lyme vaccine efficacy has traditionally been based on indicators of infection following wild-caught tick challenge whereas most other types of vaccine are required to demonstrate protection from clinical signs of disease. In this vaccination-challenge study we sought to demonstrate the ability of a nonadjuvanted, outer surface protein A (OspA) vaccine to protect from infection and to prevent synovial lesions consistent with Borreliosis.

Thirty, purpose-bred beagles were randomly divided into vaccinated and unvaccinated groups. The vaccinated group was administered two subcutaneous doses of a nonadjuvanted, purified, *Borrelia burgdorferi* OspA vaccine at a 21- day interval. Dogs were challenged by wild-caught, *B. burgdorferi*-infected ticks (*Ixodes scapularis*). Clinical signs, serology, *Borrelia* isolation and PCR evaluated antemortem vaccine efficacy. Postmortem histopathological analysis of synovial tissue was compared to antemortem infection status.

Borreliosis was demonstrated by *Borrelia* isolation from skin biopsies in 13 out of 15 unvaccinated dogs. All unvaccinated dogs' Western blot profiles were consistent with infection. Two of 15 vaccinated dogs had at least one positive spirochete culture which cleared 91 days post-challenge, and Western blot profiles were consistent with vaccination alone.

No dogs, vaccinated or unvaccinated, exhibited clinical signs consistent with borreliosis. Based on a histopathological cumulative joint scoring system (CJS), all unvaccinated dogs had synovial lesions indicative of Lyme disease. Only one of the vaccinated dogs had a CJS that was greater than the statistical cut off score for the absence of synovial lesions. There was high correlation between clinical histopathology and spirochete isolation.

Infection with *B burgdorferi* may produce inconsistent clinical signs of lameness. Histopathological changes in joints from infected dogs are reliable indicators of borreliosis and correlate well with other indicators of infection. This model provides support that vaccination with a nonadjuvanted, purified OspA vaccine offers protection from *Borrelia* infection and the resulting synovial lesions that can lead to clinical signs of lameness.

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1. Introduction

Lyme disease in dogs is caused by the spirochete *Borrelia burgdorferi*, transmitted by the tick vector *Ixodes* sp. Several canine vaccines have been developed that induce a humoral immune response in dogs (Topfer and Straubinger, 2007), and to successfully prevent the transmission of the causative agent during tick feeding challenge (Wikle et al., 2006; LaFleur et al., 2009). Up until recently, demonstration of efficacy of these vaccines has been based on prevention of infection following wild-caught tick challenge (Wikle et al., 2006). Infection is established via culture of the spirochetes and/or DNA from skin biopsies at the site of tick attachment. However, the paradigm has been shifting towards demonstration of

Abbreviations: OspA, outer surface protein A; CJS, cumulative joint score.

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vaccine efficacy based on clinical signs of disease. This has proven to be challenging, given that less than 10% of infected dogs appear to manifest clinical signs consistent with Lyme disease (Levy and Magnarelli, 1992), and then only after a period of 2–5 months incubation (Littman et al., 2006).

Clinical models for experimental canine Lyme borreliosis that have been developed, up until recently, have practical limitations for vaccine development studies. Appel (Appel et al., 1993) was able to successfully produce lameness and persistent infection in specific pathogen free dogs infected at 6 weeks of age at 2–5 months post exposure. Development of clinical signs was apparently age-related. Lameness was observed in only 1 in 4 of 13 week-old seropositive dogs and no lameness was seen in seropositive dogs greater than 6 months of age.

However, mild polyarthritis was observed in infected dogs showing no clinical signs of disease. Histopathological lesions in an experimental model were further characterized by Summers et al. (Summers et al., 2005). They were able to produce clinical signs of lameness in 39/62 (63%) in dogs infected at 6 weeks of age between 6 and 22 weeks post-infection. However, this required the examination of 10 joints per animal in which characteristic arthritis lesions were observed in 3–6 joints whether or not clinical lameness was evident.

These models have several limitations with respect to vaccine testing. Efficacy must be demonstrated in animals that are at the vaccine labels minimum age at vaccination, with challenge to follow at a **prescribed** time after completion of the vaccination protocol. Thus, in order to establish vaccine efficacy at a minimum age of 9 weeks, the age at which most juvenile dogs are expected to be immunologically competent, dogs would not be subject to challenge until approximately 18 weeks of age. Secondly, because of the numbers of animals required to achieve statistical significance between vaccinates and unvaccinated controls, examination of 10 joints per animal presents logistical challenges. In a more recent model (Susta et al., 2012), a histopathological joint lesion scoring system was developed that, in the absence of clinical signs of lameness, correlates well with other accepted indicators of infection, i.e. spirochete isolation from infected tissue, amplified gene sequences (PCR), and Western blot analysis of serum antibody. In this model, the number of joints per animal required for examination was reduced without sacrificing sensitivity in the ability to distinguish between infected and non-infected animals.

In this study we use this Lyme disease model in a vaccination-challenge study to demonstrate the ability of nonadjuvanted, purified outer surface protein A (OspA) vaccine, derived from a bacterial recombinant vector to protect from *Borrelia* infection and subsequent synovial lesions consistent with borreliosis.

2. Materials and methods

2.1. Animals

Thirty, purpose-bred, specific pathogen free, male (n = 17) and female (n = 13) Beagle dogs (Liberty Research, Inc., Waverly, NY 14892) were used in the study. All dogs had received routine core vaccination (canine distemper virus, canine adenovirus, parainfluenza, canine parvovirus) but no previous vaccination for Lyme disease. Dogs were acclimated to the housing facility and handling procedures prior to entering the study. Dogs were 7–8 months of age at first vaccination and weighed between 7.8 and 14.4 kg. All were deemed clinically healthy based on physical examination. All animals were managed and handled in compliance with Merial Institutional Animal Care and Use Committee approvals, and any applicable federal and local regulations.

Dogs were housed separately by sex and weight (3–4 dogs/run), and commingled with respect to vaccination status in conventional housing until tick challenge. Four days prior to challenge they were transferred to the isolation facility and placed in their randomized, individual runs the day before challenge. After challenge, all dogs were treated with a topical insecticide, and returned to conventional housing. Dogs were fed an appropriate commercially available adult dog food and water was provided *ad libitum*.

2.2. Procedures

2.2.1. Blinding and randomization

The animals were randomly assigned to each treatment group by a factor of litter and gender. SAS Enterprise Guide (EG Version 9.1) was used for producing the randomization table. Treatments were administered in accordance with the randomization table. All personnel performing clinical assessments, sample collection, laboratory analyses and challenge were blinded to the animal/treatment group assignments.

2.2.2. Vaccination

Dogs were vaccinated with nonadjuvanted, purified *B. burgdorferi* OspA antigen derived from a bacterial recombinant vector. The OspA antigen corresponds to the active ingredient of recombitek® Lyme (Merial, Inc., **Duluth, GA**) and was formulated at the minimum protective dose. It was administered in a volume of 1 **mL,** by subcutaneous injection, in the right and left flank, on Days 0 and 21, respectively. Control animals remained unvaccinated.

2.2.3. Tick challenge

Thirty-four days following the second vaccination, 13 female and 12 male *B. burgdorferi*-infected ticks (*Ixodes scapularis*) were confined within a 5-cm diameter plastic capsule and secured with elastic adhesive over the thorax of each dog. Ticks were allowed to feed to repletion and/or detachment (approximately 5–7 days). The tick infection rate for *B. burgdorferi* was estimated to be 57% as determined by the supplier (URI Center for Vector-Borne Disease, Kingston, RI 02881).

2.3. Sample collection

2.3.1. Blood

Approximately 10 **mL** of blood were collected for Borrelia antibody titer determination prior to the first and second vaccination, 21 days following the second vaccination, just prior to challenge and at 87 days following challenge. Blood was collected into serum-separator tubes **(Becton, Dickinson and Co., Franklin Lakes NJ)** from the jugular vein of unsedated dogs using a 21 g ¾" Surflo® butterfly catheter (Terumo, Tokyo, Japan), processed into serum by centrifugation, and **stored at 20 °C** until assay.

2.3.2. Biopsy

Skin biopsies were collected 29, 57, and 91 days following tick exposure Two 4-mm skin punch (Miltex, Inc., York, PA) biopsies were taken aseptically from all dogs within the 5 cm diameter area covered by the tick enclosure device. Biopsies were obtained under butorphanol (2 mg/kg; **Reckitt Benckiser Healthcare (UK) Ltd., Hull, England)** and dexmedetomidine (0.04 mg/kg; **Orion Corporation, Espoo, Finland**) sedation and biopsy sites were closed with skin staples **(Henry Shein Animal Health, Dublin OH**). Biopsy samples for spirochete isolation (culture) were chilled on ice and shipped on cold packs on the day of collection, via overnight courier, to the testing laboratory. Duplicate biopsy samples (PCR) were maintained at ambient temperature and transported immediately to the testing laboratory.

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