VANGUARD® crLyme: Chimeric Recombinant Vaccine Technology for Broad-Spectrum Protection Against Canine Lyme Disease

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Summary

• VANGUARD® crLyme is the first and only canine chimeric recombinant Lyme vaccine, containing an OspA protein plus a second specialized chimeric protein composed of antigenic material from 7 types of OspC.
• The selective design process used to develop VANGUARD® crLyme also helps minimize the amount of extraneous material in the vaccine, thus potentially helping minimize injection reactions or other adverse effects.
• In a challenge study, beagles were vaccinated with VANGUARD® crLyme (two 1-mL SC doses 3 wk apart) or saline (controls) and then challenged 3 weeks later with wild-caught ticks likely infected with Borrelia burgdorferi (10-day challenge).\(^1\)
  - VANGUARD® crLyme vaccinates demonstrated a 93.7% reduction (\(P < 0.0001\)) in B. burgdorferi infection incidence (based on C6 antibodies), and dramatically reduced incidence of inflammation/lesions in joints and skin.
• A multi-site safety study involving 620 dogs vaccinated with VANGUARD® crLyme determined that post-vaccination abnormal health events occurred at a low rate and were mild, transient, and associated with the normal immune response to vaccination.\(^2\)
• Veterinarians deploying the chimeric recombinant technology of VANGUARD® crLyme can be confident they are administering the most advanced Lyme vaccine to their patients.

Lyme disease remains one of the most common health threats for dogs in the US. The disease is caused by Borrelia burgdorferi bacterial spirochetes that are transmitted to dogs through the bite of Ixodes spp. ticks.\(^3,4\) The disease threat can vary by location due to variability in opportunities for exposure to the infected carrier tick. For instance, a survey reported the prevalence of seropositive dogs in various regions.\(^5\) In Northeast states the regional mean was 11.6% (range of 7.1% in NY to 19.8% in MA) while in Midwest states the regional mean was 4.0% (<1% to 10.2% in WI). Canine Lyme disease manifests with arthritis-induced lameness, anorexia, fever, lethargy, lymphadenopathy and, in some cases, fatal glomerulonephritis.\(^6,7\)

Vaccination for Lyme disease is a routine protocol for many veterinary practitioners, especially those located in endemic regions. In general, Lyme vaccines have focused
on 2 particular types of antigens that *B. burgdorferi* spirochetes express, both classified as outer surface proteins (Osp). One type, ‘OspA’, is expressed when the bacteria occupy the tick midgut. However, once a tick feeds on blood, the microbes migrate to tick salivary glands, reduce expression of OspA, and begin expressing the other vaccine critical antigen, ‘OspC’. Once in the dog, the spirochetes establish infection and soon reduce OspC expression. Available Lyme vaccines work by prompting a vaccinated dog to generate antibodies against OspA and, depending on the vaccine, potentially OspC as well. The OspA antibodies attack bacteria in the midgut of an attached tick, while the OspC antibodies predominantly engage microbes in the dog (however, they can also have activity in the tick).

While this seems like a sound strategy for immunological control of Lyme disease, several problems have emerged:

- Whole-cell bacterins are often reactive in the dog, thus generating some safety concerns.
- Some vaccines may contain only the OspA component (including one vaccine formulated as a recombinant subunit product), leaving vaccinated dogs unprotected for OspC antigens.
- Recombinant technology has been applied to some Lyme vaccines in an effort to help limit the amount of extraneous proteins in the products and thereby potentially helping reduce reactivity. However, until the application of chimeric technology to Lyme vaccination, development of a recombinant OspC vaccine was not possible.
- OspC expression is genetically diverse. Over 30 distinct ospC phyletic types have been identified in recent years, and immune responses elicited by OspC antigens are specific to particular phyletic types. Furthermore, since ticks can be infected with multiple *B. burgdorferi* organisms, several types of OspC can be expressed as a result of a single tick bite and subsequent feeding. Any OspC-based vaccine designed for broad protection must include protective epitopes derived from OspC variants most commonly associated with mammalian infection, but ‘cocktails’ consisting of multiple OspC proteins have not proven effective.

Fortunately, biotechnology involving the construction of specific chimeric recombinant OspC proteins appears to offer a promising approach for the development of broadly protective vaccines.

**Vanguard® crLyme**

Vanguard® crLyme, from Zoetis, is the first and only canine chimeric recombinant Lyme vaccine. Unlike other vaccines, Vanguard® crLyme contains a recombinant OspA protein plus a second specialized chimeric protein composed of antigenic material from 7 types of OspC. As a result of these 2 proteins, Vanguard® crLyme represents the most advanced vaccine technology that veterinarians can administer to their patients.

The particular proteins in Vanguard® crLyme were specifically selected and designed to help elicit a targeted immune response to *B. burgdorferi* bacteria, thus helping provide broad-spectrum protection with low reactivity. This was accomplished by incorporating a wide variety of OspC antigens commonly seen in canine infections into a single chimeric protein, thereby distinguishing Vanguard® crLyme as the only Lyme vaccine to contain antigenic material from multiple variants of OspC. In addition, this selective design process also helped minimize the amount of extraneous material in the vaccine, thus helping contribute to low incidences of injection reactions or other adverse effects.

Vanguard® crLyme is licensed for vaccination of dogs 8 weeks of age or older as an aid in preventing clinical disease and subclinical arthritis associated with *B. burgdorferi*. The dose rate is 1 mL administered by subcutaneous (SC) injection. Healthy dogs should initially receive 2 doses administered 3 weeks apart, and annual revaccination with a single dose is recommended. Vaccination of pregnant bitches should be avoided. Vanguard® crLyme is available in packages containing either 25 or 50 single-dose, 1-mL vials of vaccine (refrigerate at 2-7°C, do not freeze).

Two studies investigated the efficacy and safety of Vanguard® crLyme as part of the research conducted by Zoetis in support of product licensing by USDA.
Efficacy Study

Experiment Design

A laboratory research study evaluated the efficacy of VANGUARD® crLyme for helping prevent B. burgdorferi infection.1 The study involved 36 healthy beagles (18 males, 18 females) between 8 and 9 weeks of age with no history of Lyme disease vaccination and negative Lyme C6 serology.* After randomly selecting 4 animals to serve as nonvaccinated/noninfested sentinels, the remaining 32 dogs were assigned to 2 treatment groups (randomized complete block design, blocked by date of birth and litter; 16 blocks of 2 dogs) as follows:

- VANGUARD® crLyme, n=16;
- Controls (saline, adjuvanted with adsorbent aluminum hydroxide to match VANGUARD® crLyme formulation), n=16.

The ‘vaccination phase’ of the study (Figure 1) involved SC administration of 1 mL of the respective treatments on study day 0 (right dorsal scapular area), with a second dose administered 3 weeks later on day 21 (left dorsal scapular area).

The ‘challenge phase’ of the study commenced on day 42 (21 days after second vaccination) by exposing all dogs to a challenge tick infestation. Fifteen pairs of field-caught Ixodes scapularis ticks likely infected with B. burgdorferi were placed in infestation chambers on the dorsal midline of each dog and allowed to feed for 10 days. After this period, each beagle was treated with a topical acaricide.

Dogs were observed daily for clinical signs of Lyme disease (e.g., ataxia, lameness, depression, lethargy) after infestation until study conclusion on days 154/155. Individual serum samples were collected on days 0, 21, 35, 77, 117, and 153. Samples were qualitatively tested (positive/negative) for B. burgdorferi C6 antibodies (SNAP 4Dx Plus Test, IDEXX) and quantitatively tested for OspA and OspC antibodies by ELISA. Skin biopsies were collected on 3 occasions (days 78, 118, 154/155), cultured, and analyzed by polymerase chain reaction (PCR) for detection of B. burgdorferi.

All dogs were humanely euthanized on day 154/155 and samples collected for histopathological evaluation of skin and synovial joint tissues for lesions (arthritis) associated with borreliosis. Lesions were scored for severity using a scale of 0 to 5 (0=no lesion, 5=extensive number or size of lesions).

Data were statistically analyzed by appropriate standard methods using each dog as the experimental unit. Back-transformed least squares (LS) means were reported, with statistical significance recognized at $P \leq 0.05$. All daily observations and laboratory evaluations of test samples were conducted by personnel without knowledge of treatment group assignments. The study was conducted in accordance with the Zoetis Institutional Animal Care and Use Committee.

Results

Mean antibody titers of dogs to OspA and OspC over the course of the study are summarized in Figure 2. All animals were seronegative at study initiation, and control dogs remained seronegative until

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Figure 1 – Summary of study design and timeline.

*C6 peptide is expressed when B. burgdorferi infect a mammalian host. Detection of C6 peptide is widely accepted for diagnosis of Lyme disease spirochete infection in dogs, regardless of vaccine history (not present in vaccine formulations).
Dogs vaccinated with VANGUARD® crLyme demonstrated humoral responses to both OspA and OspC within 3 weeks.

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Vanguard® crLyme vaccination conferred a significant 93.7% reduction (P < 0.0001) in the incidence of B. burgdorferi infection.

Results of histopathological evaluations (Table 1) indicate that control animals experienced frequent incidences (56.3%-87.5%) of joint or skin lesions associated with B. burgdorferi infection (‘mild’ or greater severity). Notably, lesions/inflammation were absent or ‘minimal’ in vaccinates and sentinels (minimal changes observed in the joints of some dogs were considered incidental and not related to vaccination or experimental challenge). Based on microscopic analysis, VANGUARD® crLyme prevented the presentation of synovitis and hypodermitis (with perineuritis and perivasculitis) associated with B. burgdorferi.

Efforts to identify dogs with borreliosis were not successful (based on skin biopsy culture/isolation or PCR detection of B. burgdorferi C6 antibodies).

The primary efficacy variable for this study was protection from infection based on the absence of B. burgdorferi C6 antibodies on any of 3 post-challenge sample dates. All animals were healthy and seronegative to B. burgdorferi C6 antibodies prior to challenge. However, post-challenge outcomes summarized in Figure 3 show that all control dogs (100%, 16/16) were positive for B. burgdorferi C6 antibodies, compared to only 1 vaccinated dog (6.3%, 1/16) that was transiently positive on a single occasion (30 days post-challenge). Thus, VANGUARD® crLyme vaccination conferred a significant 93.7% reduction (P < 0.0001) in the incidence of B. burgdorferi infection.

Table 1 – Histopathology results.

<table>
<thead>
<tr>
<th>Inflammationa graded 2 or higher in...</th>
<th>Control</th>
<th>VANGUARD° crLyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subdermis (%)</td>
<td>87.5</td>
<td>0</td>
</tr>
<tr>
<td>1 joint (%)</td>
<td>81.3</td>
<td>0</td>
</tr>
<tr>
<td>2 or more joints (%)</td>
<td>56.3</td>
<td>0</td>
</tr>
</tbody>
</table>

a Lesion severity grades of 0=absent (no lesion found), 1=minimal (very few or very small), 2=mild (few or small), 3=moderate (moderate number or moderate size), 4=marked (many or large), 5=severe (extensive number or extensive size)

Figure 2 – OspA and OspC antibody titers of dogs receiving 2 doses of VANGUARD° crLyme vs controls.

Figure 3 – Incidence of B. burgdorferi infection (as indicated by C6 antibodies).

Joint or skin lesions associated with B. burgdorferi infection were absent or minimal in VANGUARD® crLyme vaccinates.

Dogs vaccinated with VANGUARD® crLyme demonstrated humoral responses to both OspA and OspC by day 21 (3 weeks after the first vaccination), with a peak in geometric mean titers at day 35 (2 weeks after the second vaccination). No increase in humoral response to OspC antigen was observed in vaccinates after challenge, suggesting that VANGUARD® crLyme prevented B. burgdorferi transmission from infected ticks to vaccinated dogs.

After challenge. In contrast, VANGUARD® crLyme vaccinates demonstrated humoral responses (P < 0.0001) to both OspA and OspC by day 21 (3 weeks after the first vaccination), with a peak in geometric mean titers at day 35 (2 weeks after the second vaccination). No increase in humoral response to OspC antigen was observed in vaccinates after challenge, suggesting that VANGUARD® crLyme prevented B. burgdorferi transmission from infected ticks to vaccinated dogs.

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Efforts to identify dogs with borreliosis were not successful (based on skin biopsy culture/isolation or PCR detection of B. burgdorferi C6 antibodies).
*B. burgdorferi*, with or without the signs of clinical disease). Only 3 of the control dogs developed a Lyme-related clinical sign, such as post-challenge lameness. As expected, all sentinel dogs remained seronegative for C6, OspA, or OspC antibodies during the study, as well as any signs of Lyme disease or relevant histopathology.

**Safety Study**

**Experiment Design**

Additional research conducted for licensure of VANGUARD® crLyme included a multi-site field-safety study that evaluated local and systemic reactions in dogs for at least 10 days post-vaccination. The study involved 620 healthy dogs (321 male/299 female) associated with 11 small animal veterinary practices and 2 commercial breeders located in 3 distinct geographic regions of the US (Northeast n=256; Midwest n=272; Southeast n=92). Regions represented in the study were selected based on the prevalence of Lyme disease and the practice of routine *B. burgdorferi* vaccination in those areas. The dogs represented a wide age range with 203 ≤ 8 weeks of age and 417 ≥ 9 weeks of age (up to 17 yr).

Each enrolled dog was administered 1 mL of VANGUARD® crLyme by the SC route, followed by a second vaccination 3 to 4 weeks later.

Dogs were observed for approximately 10 to 20 minutes after each vaccination for any ‘immediate’ abnormal health events (e.g., anaphylaxis, allergic edema, convulsion, emesis, injection site pain/rubbing/licking/paraesthesia, urticaria, vocalization at administration, etc.). All dogs were subsequently observed over a 10-day period for any ‘late’ abnormal health events (e.g., anorexia, diarrhea, hyperthermia, vomiting, etc.). Nine dogs did not fully complete the study (variety of reasons, e.g., noncompliance, etc.). None of the withdrawals were related to an abnormal health event related to VANGUARD® crLyme administration.

<table>
<thead>
<tr>
<th>Abnormal health event</th>
<th>Percent of injections</th>
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<tbody>
<tr>
<td><strong>Immediate post-vaccination reactions</strong> (&lt;30 min post-vaccination; n=1231 injections)</td>
<td></td>
</tr>
<tr>
<td>Injection site edema</td>
<td>0.08%</td>
</tr>
<tr>
<td>Injection site paraesthesia</td>
<td>0.24%</td>
</tr>
<tr>
<td>Vocalization at administration</td>
<td>0.73%</td>
</tr>
<tr>
<td><strong>Late post-vaccination reactions</strong> (&gt;30 min–10 d post-vaccination; n=1232 injections)</td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>0.08%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0.16%</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>0.08%</td>
</tr>
<tr>
<td>Injection site edema</td>
<td>3.81%</td>
</tr>
<tr>
<td>Injection site pain</td>
<td>0.32%</td>
</tr>
<tr>
<td>Lethargy</td>
<td>0.24%</td>
</tr>
<tr>
<td>Muscle tremor</td>
<td>0.08%</td>
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</table>

**Table 2 – Frequency of adverse reactions.**

Post-vaccination abnormal health events observed during the study (Table 2) were mild, transient, and associated with the normal immune response to vaccination. The majority of late abnormal health events involved injection site edema (3.81%, 47/1232 vaccinations). Most of these (38 of 47) were reported at a single breeder site. These facilities typically used veterinarians or technicians to perform observations for late abnormal health events, as opposed to dog owners. Thus, reports for late abnormal health events were expected to be higher from commercial breeder sites. Notably, no injection site edema was reported at another commercial breeder site used in the study (personnel at both sites received the same pre-study training).

Study results demonstrated that VANGUARD® crLyme was safe in dogs of various ages when administered as a 1-mL SC primary dose followed by a second dose 3 to 4 weeks later. The low incidence of reactivity or other adverse effects was expected since VANGUARD® crLyme is an inactivated (non-live) product specifically designed to help minimize the amount of extraneous material in the vaccine.
Conclusions

As the first and only chimeric recombinant Lyme vaccine, VANGUARD® crLyme represents a significant advance for Lyme disease protection technology. VANGUARD® crLyme contains 2 proteins that provide antigens for both OspA (found in the tick) and 7 types of OspC (found in the dog), thus helping provide broad-spectrum protection with low reactivity.

Research results support the adoption of VANGUARD® crLyme for use in Lyme prevention protocols. An efficacy study found that a 2-dose VANGUARD® crLyme regimen reduced the incidence of *B. burgdorferi* infection in tick-challenged dogs by over 93%, and dramatically reduced the incidence of inflammation/lesions in joints and skin. Furthermore, a multi-site field study confirmed the safety of VANGUARD® crLyme, with only very low incidences of reactivity or abnormal health events observed following vaccination.

Veterinarians deploying the chimeric recombinant technology of VANGUARD® crLyme can be confident they are administering the most advanced Lyme vaccine to their patients. In addition, VANGUARD® crLyme is backed by the Zoetis Companion Animal Immunization Support Guarantee, thus providing further rationale for adopting the novel vaccine into their practice.

References