RECOMBITEK® LYME

MEANS PURE.

TARGETED
LYME
PROTECTION

RECOMBITEK® Lyme has all the protection dogs need, and none of the antigens they don't.

RECOMBITEK® lyme

LYME DISEASE: A GROWING, PREVENTABLE THREAT

Lyme disease: the threat against dogs continues to rise

121% Increase In Cases

82% US Tickborne Human Cases[4]

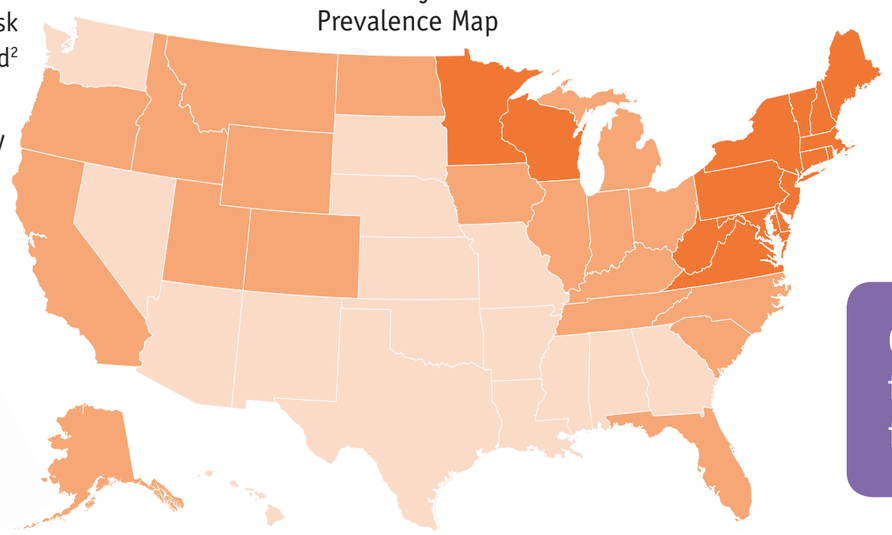
- Lyme disease cases in dogs have increased between 2012 and 2019[1]
- Since the late 1990s, reported human cases of Lyme disease in the US have tripled[2]
- A positive correlation between Lyme disease incidence in humans and seroprevalence in dogs has been demonstrated[3]
- Diagnosis of Lyme disease is difficult: Most *Borrelia burgdorferi* seropositive dogs show no clinical signs, and infected dogs do not typically present acutely[5]

Lyme disease is on the move

Lyme disease has been detected in dogs in all 50 states and is common in the Northeast, upper Midwest, Mid-Atlantic, and West Coast.[1]

- The number of counties in the Northeast and upper Midwest considered high-risk for Lyme disease has more than tripled[2]
- Ticks that transmit Lyme disease have spread to geographic areas where they had not been seen before[2]
- Lyme disease is transmitted from the bite of what is commonly called the black-legged tick (or deer tick)—*Ixodes scapularis* in the Northeast and Midwest, *Ixodes pacificus* on the West Coast[6]

2019 Canine Lyme Disease Prevalence Map



Infection Risk

No Data — High Risk

CAPC, Companion Animal Parasite Council

Lyme disease mode of transmisson

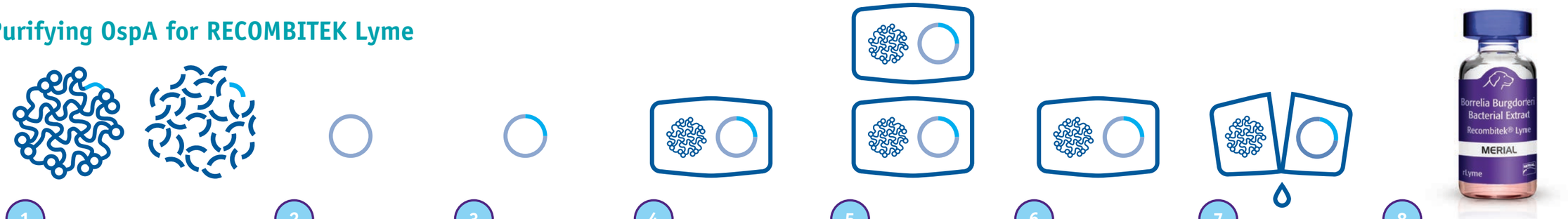
- *B. burgdorferi*, the causative agent of Lyme disease is transmitted by ticks
- Transmission of *B. burgdorferi* typically occurs 36 to 48 hours or more after tick attachment[7]
- Outer surface protein A (OspA), the predominant surface protein expressed on *B. burgdorferi*, is involved in the attachment of spirochetes to the tick's midgut[8]
- During feeding, the warmth of the new environment causes spirochetes to downregulate their expression of OspA and start expressing OspC; spirochetes then migrate from the midgut to the salivary glands and subsequently infect the mammalian host[8]
- The OspA lipoprotein is highly conserved between *B. burgdorferi* species in the US; this homogeneity makes it an ideal vaccine target[9]
- OspC exhibits much greater heterogenicity than OspA: **Over 30 distinct OspC phyletic types** have been identified in the US, and immune responses elicited by OspC epitopes are phyletic specific[10]

OspA is the only vaccine antigen necessary to block transmission of *B. burgdorferi* from the tick to the dog.[11]

The only vaccine that contains lipidated OspA in a non-adjuvanted formulation

RECOMBITEK® LYME: TARGETED, PURE PROTECTION

Purifying OspA for RECOMBITEK Lyme



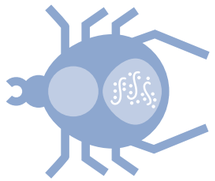
1. DNA of *Borrelia burgdorferi* spirochete with genetic code of OspA is fragmented by restriction enzymes.
2. Plasmid vector (free-floating DNA) is selected.
3. OspA DNA sequence is inserted into plasmid vector, creating recombinant plasmid.
4. Recombinant plasmid is inserted in host *E. coli*.
5. *E. coli* containing the recombinant plasmid multiplies in culture.
6. Recombinant plasmid directs *E. coli* to produce OspA.
7. *E. coli* is lysed; OspA is extracted and purified.
8. Purified OspA is used to manufacture RECOMBITEK Lyme Vaccine. (No adjuvant is needed.)

Non-adjuvanted formulation

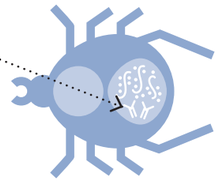
- RECOMBITEK Lyme is the only non-adjuvanted Lyme vaccine
- RECOMBITEK Lyme contains a highly purified lipidated OspA[12]
- RECOMBITEK Lyme uses only a pure protein without an adjuvant to stimulate immunity
- Unlike RECOMBITEK Lyme, whole-cell bacterin canine Lyme vaccines and the chimeric recombinant canine Lyme vaccine contain adjuvants to enhance the immune response[5]
  - Sensitization to an adjuvant may contribute to hypersensitivity reactions[13]
- Whole-cell bacterin Lyme vaccine contains more than 100 *B. burgdorferi* lipoproteins, inducing host inflammatory responses that may lead to pathologic changes responsible for the inflammation associated with infection[14,15]

What happens when an infected tick bites a dog protected by RECOMBITEK Lyme?

In the unfed tick, *B. burgdorferi* expresses OspA in the tick midgut.



As the tick feeds, OspA antibodies in the dog's blood enter the midgut and bind to OspA on the surface of *B. burgdorferi*.



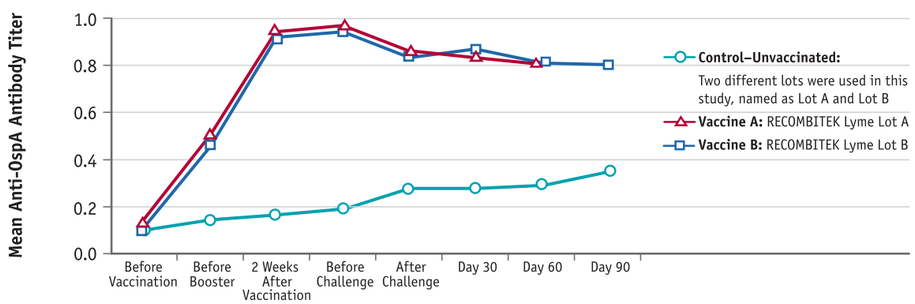
B. burgdorferi growth is arrested, migration to the salivary gland is blocked, and transmission is prevented.

PROVEN EFFICACY

RECOMBITEK Lyme blocked infection before transmission could occur[11]

- Randomized, blinded, controlled study designed to mimic natural tick challenge
- 100% of control dogs infected post-challenge
- Efficacy of RECOMBITEK Lyme was determined by:
  - Serology (ELISA)
  - Recovery of the infectious agent via skin biopsy culture, PCR analysis, western blot analysis and tick xenodiagnosis

RECOMBITEK Lyme induced a robust antibody response

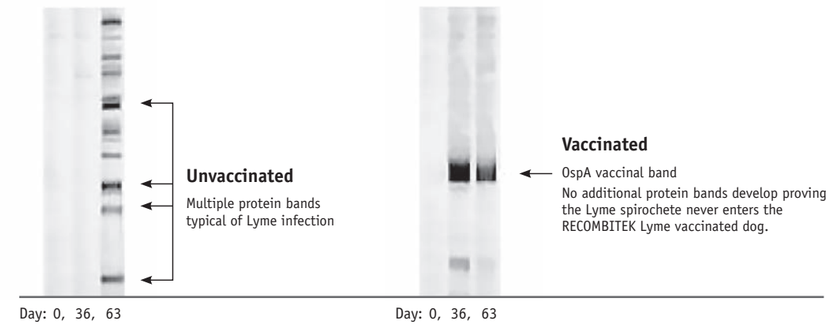


- Relative to controls, vaccinates responded with significantly high levels of anti-OspA antibodies that were maintained throughout the study
- Vaccinates showed no booster effect after challenge, demonstrating solid immunity
- The unvaccinated control group had low titers that increased in response to challenge and were maintained throughout the study

AAHA recommendation: Lyme vaccination is recommended for dogs with a known risk of exposure due to living in or visiting regions where risk of vector tick exposure is considered to be high, or where disease is known to be endemic.[16]

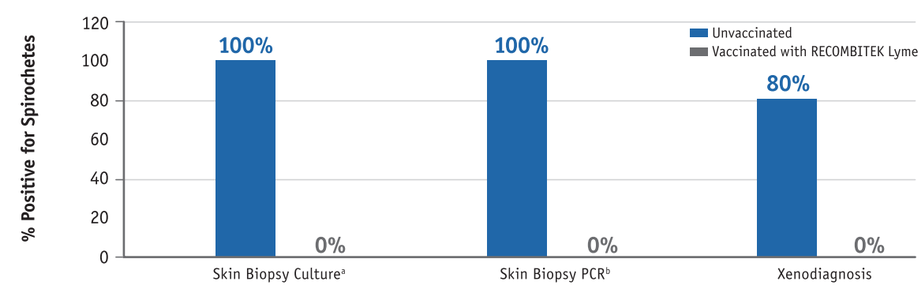
ELISA, enzyme-linked immunosorbent assay

RECOMBITEK Lyme prevented infection in the face of challenge



- All controls (10/10) had antibodies characteristic of infection with *B. burgdorferi* after challenge (day 63), whereas no antibodies developed to antigens that are specific for infection to *B. burgdorferi* in the vaccinates
- Vaccinated dogs demonstrated an OspA antibody pattern following vaccination (day 36) and tick challenge (day 63)
- This confirms that no infection occurred in the vaccinated dogs following challenge with ticks infected with *B. burgdorferi*

RECOMBITEK Lyme showed no evidence of infection in vaccinated dogs



[a] Post challenge days 30, 60, and 90
[b] Post challenge days 30 and 60

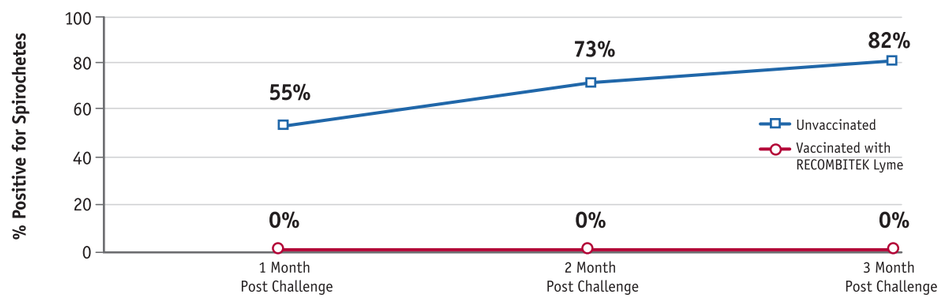
- In contrast to unvaccinated group, vaccinates had no isolation of spirochetes on skin biopsy and had no positive PCR results after challenge for up to 3 months and 60 days, respectively
- Xenodiagnosis demonstrated that all ticks recovered from vaccinated dogs were negative for *B. burgdorferi,* whereas 80% of ticks were positive for *B. burgdorferi* in unvaccinated controls

PROVEN 1-YEAR IMMUNITY AND STRONG IMMUNOGENICITY

RECOMBITEK Lyme demonstrated long-lasting protection in a live-tick challenge 366 days after vaccination[17]

100%
1-year Protection in Vaccinates

- Duration of immunity and safety for RECOMBITEK Lyme was evaluated in a randomized, blinded, placebo controlled study
- Dogs were challenged by exposure to naturally infected ticks 1 year (366 days) after initial vaccination series (2 doses administered 3 weeks apart)
- Spirochete reisolation from skin biopsies was used to assess vaccine efficacy at monthly intervals for 3 months after challenge



- Spirochetes were reisolated from 82% of the unvaccinated dogs at 3 months post challenge, whereas none of the vaccinated dogs had spirochete reisolation at any assessed time
- None of the vaccinated dogs had clinical signs of Lyme disease, whereas 18% of unvaccinated control animals showed clinical signs during the study
- No general adverse reactions to the vaccine were noted at any time during the study
- No vaccinates showed any signs of infection after challenge in both efficacy and duration of immunity study[a]

[a] Infection with *B. burgdorferi* does not always produce clinical signs in dogs[8]

RECOMBITEK Lyme features a uniquely constructed OspA antigen that provides strong immunogenicity[12]

- Natural spirochete-associated OspA is lipidated at the N-terminus of the amino acid sequence[18]
- Lipidation has been demonstrated as a determinant of immunogenicity for OspA[12]

Lipidation assessment of the OspA antigen of 2 recombinant canine Lyme vaccines[12]

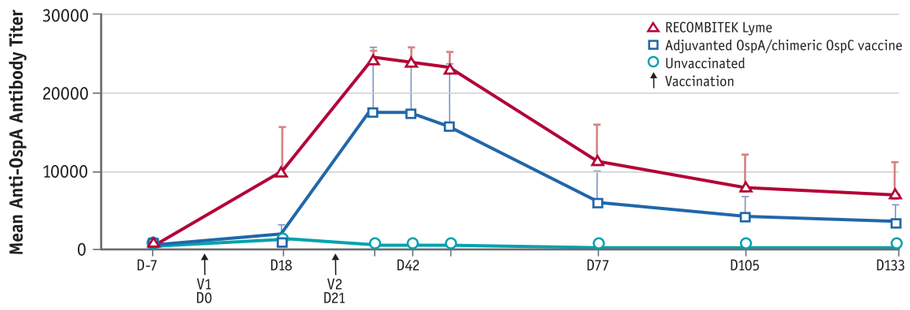
RECOMBITEK Lyme	Adjuvanted OspA/chimeric OspC vaccine
A mix of bi- and tri-lipidated forms	Non-lipidated
Molecular weight of 418 kDa; diameter: 18 nm	Molecular weight of 34 kDa; diameter: 6.4 nm

- Recombinant OspA lipidation and subsequent micelle formation might account for superior immunogenic profile of recombinant OspA compared with adjuvanted OspA/chimeric OspC vaccine[12]

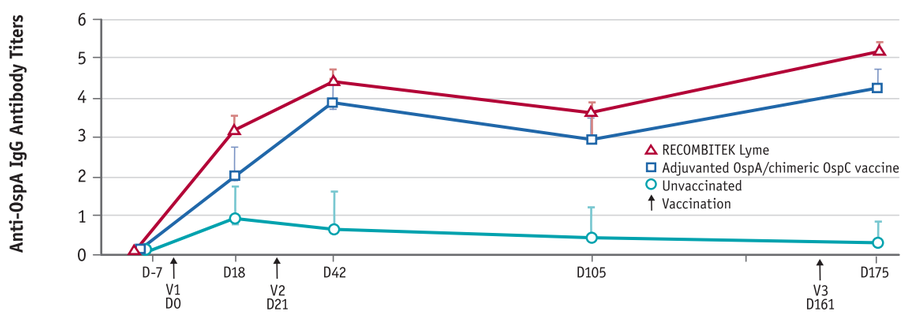
ROBUST, HOMOGENEOUS, AND HIGH-AVIDITY RESPONSE

Serological response and borreliacidal activity of RECOMBITEK Lyme vaccine and adjuvanted OspA/chimeric OspC vaccine[12]

RECOMBITEK Lyme produces a greater and more homogenous anti-OspA humoral response



- The response of RECOMBITEK Lyme following a single vaccination was significantly greater than that induced by adjuvanted OspA/chimeric OspC vaccine at day 18
- At subsequent time points the mean anti-OspA antibody titers in RECOMBITEK Lyme remained higher than the adjuvanted OspA/chimeric OspC vaccine (not statistically significant)



- Total IgG titers were significantly higher for RECOMBITEK Lyme vaccinates on days 18, 42, 105, and 175, compared with adjuvanted OspA/chimeric OspC vaccine
- There was a trend of more consistent anti-OspA (less dispersion) response for RECOMBITEK Lyme vaccinates compared with adjuvanted OspA/chimeric OspC vaccine, which was significant at day 175

RECOMBITEK Lyme elicits a higher avidity anti-OspA antibody response

- RECOMBITEK Lyme generated a significantly superior avidity response at days 18, 105, and 175, compared with adjuvanted OspA/chimeric OspC vaccine
- Vaccination with RECOMBITEK Lyme resulted in the production of large amounts of specific, high-avidity antibodies that increased after each vaccination
- Dogs vaccinated with adjuvanted OspA/chimeric OspC vaccine resulted in the production of specific antibodies with lower avidity that did not increase after subsequent vaccination

RECOMBITEK Lyme generates a greater concurrent increase in serum borreliacidal activity

- Post-vaccination borreliacidal activity of sera from dogs receiving RECOMBITEK Lyme vaccine were consistently and significantly higher on days 18, 35, and 175, compared with those from dogs that received adjuvanted OspA/chimeric OspC vaccine

Dogs receiving RECOMBITEK Lyme had higher levels of anti-OspA antibodies, higher avidity of anti-OspA antibodies, and greater concurrent increase in serum borreliacidal activity compared with dogs that received adjuvanted OspA/chimeric OspC vaccine

This vaccine is brought to you by the maker of a full line of vaccine products, including RECOMBITEK®, PUREVAX®, and IMRAB®

RECOMBITEK Lyme Guarantee

In the event that a dog is found to be Lyme positive after being properly vaccinated with RECOMBITEK® Lyme, Boehringer Ingelheim Animal Health USA Inc. will support testing with either the Cornell Canine Lyme Multiplex Assay or the IDEXX™ Quantitative C6 Antibody Test (please contact Veterinary Technical Solutions (VeTS) to set up support for testing preemptively). If a positive diagnosis is confirmed, Boehringer Ingelheim Animal Health USA Inc. will support urinalysis testing and treatment with appropriate antibiotics, such as amoxicillin or doxycycline. More in-depth support will be considered for testing and treatment of definitively diagnosed complications of Lyme disease on a case-by-case basis.

For full details, ask your Representative about our Satisfaction Guarantee on RECOMBITEK or call our Veterinary Technical Solutions team at 1-888-637-4251 opt 1.

RECOMBITEK® LYME MEANS PURE

RECOMBITEK Lyme has all the protection dogs need and none of the antigens they don't

- Effective protection that blocks *Borrelia burgdorferi* while it's still in the tick[11]
- THE ONLY canine Lyme vaccine that is non-adjuvanted
- THE ONLY recombinant Lyme vaccine that contains lipidated OspA[12]
- Proven 1-year immunity and a robust, homogeneous, and high avidity immune response[12]

TARGETED LYME PROTECTION

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To learn more about RECOMBITEK vaccines and how they can benefit your patients, contact your Sales Representative or your Distributor Representative, call Customer Care at 1-888-637-4251 or visit BI-CONNECT.com

Ask your Representative about our Satisfaction Guarantee on RECOMBITEK.

Boehringer Ingelheim

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