

# Efficacy of the canine Lyme vaccine in North America: a protocol for a systematic review and meta-analysis

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## Abstract

**Background:** Lyme disease is an emerging tick-borne disease in North America affecting both humans and animals. To reduce the risk of Lyme infection in dogs, tick preventives and vaccinations are commonly used. Although a number of vaccines are commercially available their efficacy is controversial, with reports citing anywhere from 50% to 100% efficacy. This protocol describes a review of the efficacy of the canine Lyme vaccine in Canada and the United States. To date, there are no systematic reviews or meta-analyses on this topic.

**Methods/Design:** The populations of interest are pet dogs in Canada and the United States. Outcomes of interest were divided into critical and non-critical outcomes based on clinical relevance. Critical outcomes include morbidity (e.g. lameness, pyrexia). Non-critical outcomes include seroconversion. The intervention of interest is any canine Lyme vaccine. Primary studies using observational or experimental designs will be eligible. Electronic searches will be conducted using MEDLINE (via PubMed), CAB Abstracts (via CAB Direct), Web of Science and Google Scholar (first 500 relevant abstracts) with no language restrictions. Experimental studies will be assessed for risk of bias using the Cochrane Collaboration's tool, and observational studies will be evaluated using the seven domains of bias within the Cochrane tool for the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I). We propose to conduct meta-analyses for critical and non-critical outcomes. Subgroup analyses for commercially available vaccines and for each vaccine subtype will be performed if data are available.

**Discussion:** This systematic review will provide a synthesis of current evidence regarding the efficacy of the canine Lyme vaccine in North America.

**Keywords:** Systematic review, Canine, Lyme, *Borrelia burgdorferi*, Vaccine efficacy

## Background

Lyme disease is an emerging tick-borne disease in North America affecting both humans and animals. The predominant causative agent of Lyme disease in North America is a spirochete bacterium, *Borrelia burgdorferi*, transmitted primarily via deer ticks (*Ixodes scapularis*) (Schuijt et al. 2011). The increasing incidence of Lyme cases in humans may be attributed at least in part to increased awareness and improved diagnosis of the condition, and an increasing prevalence and expanding distribution of ticks, which are most likely related to climate change (Ogden et al. 2006).

In humans, Lyme disease can be particularly difficult to diagnose and may have debilitating consequences if left untreated (Steere et al. 2004). Most dogs, however, do not develop acute clinical disease following infection; less than 5% of seropositive dogs exhibited limb/joint disorders in one study (Levy et al. 1992). Clinical signs of Lyme

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disease in dogs may include shifting lameness, anorexia, lethargy, pyrexia and/or lymphadenopathy.

There are a number of methods to reduce the risk of Lyme infection in dogs including tick preventives (acaricides), tick avoidance, and vaccination. There are limitations with acaricides, because they do not target immature tick stages, namely nymphs, which are capable of transmitting *Borrelia burgdorferi*. The efficacy of acaricides is also dependent on owner compliance, as they require frequent monthly applications. Tick avoidance is not always practical, or even possible. In theory, vaccination should provide an alternative means of protection against Lyme infection which does not depend on owner compliance or complete tick avoidance. There are a number of commercial Lyme vaccines available for dogs; however, their utility is considered controversial by internal medicine experts (Littman et al. 2006). Reported vaccine efficacies are variable, ranging from 50% to 100% (Littman et al. 2006, Chang et al. 1995, Chu et al. 1992, Rhodes et al. 2013).

As an emerging disease with a changing ecology, Lyme disease is a subject of significant concern for pet owners and veterinarians alike. To the best of our knowledge, and at the time of writing, no systematic reviews or meta-analyses evaluating the efficacy of the canine Lyme vaccine have been performed.

## **Methods/Design**

### **Study Registration**

This protocol has been published on the SYREAF (systematic reviews for animals and food) website at [www.syreaf.org](http://www.syreaf.org). The systematic review will be reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines (Liberati et al. 2009). This review protocol will follow the PRISMA-P guidelines for review protocols (Moher et al. 2015).

### **Review Question**

“What is the efficacy of vaccination for *Borrelia burgdorferi* in North American dogs when compared to no vaccine?” A secondary objective of this review will be to assess the efficacy of currently commercially available vaccines, with the intention of informing clinical decision-making. The following definitions will be used:

- 1) Vaccine effect:
  - a. Reduction of incidence of clinical illness among infected dogs
  - b. Reduction of incidence of infection among susceptible dogs
- 2) North America: Canada and United States
- 3) Currently commercially available: licensed for use in dogs in North America at the time of the review

### **Eligibility criteria for considering studies for this review**

#### ***Study designs eligible***

Eligible studies are primary research studies using experimental (natural or deliberate disease challenge) or analytical observational study designs. Although observational studies are prone to confounding bias and therefore provide a lower level of evidence than experimental designs, a carefully designed and well-executed observational study

may provide useful information on this topic. The real-life risk of exposure to Lyme disease and/or ticks is also more closely approximated by observational studies compared to certain experimental studies, such as challenge trials.

The following study designs are not eligible: ecological studies, descriptive studies (e.g. case reports, case series), reviews.

Studies must include outcomes assessing at least one measure of vaccine effect: reduction of incidence of clinical illness given infection, or reduction of incidence of infection given exposure.

### ***Participants eligible***

The population of interest is pet dogs in Canada and the United States. The review will be limited to North America, because Lyme is an evolving disease in this region. Mexico will be excluded because it is uncertain whether canine Lyme disease is endemic, and current research efforts are focused on measuring the prevalence and distribution of Lyme (Feria-Arroyo et al. 2014, Movilla et al. 2016) rather than intervention efficacy. Additionally, other species of *Borrelia* are responsible for Lyme disease elsewhere (e.g. Europe), so the vaccines used to control Lyme disease in those areas might not be relevant to the North American population.

### ***Exposures eligible***

Studies which report the use of a canine Lyme vaccine will be eligible. Monovalent and multivalent vaccines are both eligible. Commercially available and non-commercially available vaccines are eligible. Studies without a concurrent placebo or control group are not eligible.

### ***Outcome measures eligible***

We will extract outcomes from eligible studies performed on dogs that include a vaccinated and a comparator group (either unvaccinated or placebo). Vaccine effect will be assessed using critical outcome measures and non-critical outcome measures corresponding to the previously defined measures of vaccine effect, and are defined as follows:

- Critical: measures of morbidity, conditional upon infection with *Borrelia burgdorferi* or associated with a clinical diagnosis of Lyme (assessing reduction of incidence of clinical illness among infected individuals)
- Non-critical: measures of seroconversion, conditional upon exposure to *Borrelia burgdorferi* or associated with exposure to Lyme (assessing reduction of incidence of infection among susceptible individuals)

Critical measures of vaccine effect represent clinical outcomes that are relevant to practitioners and pet owners. These measures of morbidity will consist of the following: lameness following Lyme infection or associated with a clinical diagnosis of Lyme, anorexia following Lyme infection or associated with a clinical diagnosis of Lyme, pyrexia following Lyme infection or associated with a clinical diagnosis of Lyme, lymphadenopathy following Lyme infection or associated with a clinical diagnosis of Lyme, and lethargy following Lyme infection or associated with a clinical diagnosis of Lyme.

Although non-critical outcomes such as seroconversion are not direct measures of clinical outcomes, they represent a means by which to evaluate vaccine effect. Seroconversion will be captured as antibody response to spirochetes in randomized controlled trials and observational studies; in challenge trials, antibody response to the vaccine will be used to assess seroconversion.

Adverse effects are not strictly considered an outcome measure, but they will be recorded.

### **Search methods for the identification of studies**

Search strategies will be developed to identify relevant studies. A draft strategy was developed (**Appendix I**). The literature search will be limited from 1984, when the first case of Lyme disease-related arthritis in a dog was documented (Lissman et al. 1984). The search will not be limited by study design or language. Articles in French will be translated.

Electronic searches will be conducted using the following databases: MEDLINE (via PubMed), CAB Abstracts (via CAB Direct), Web of Science and Google Scholar (first 500 abstracts sorted on relevance). The search strategy will be adapted for each search resource, accounting for differences in syntax, indexing and functionality.

A search of the grey literature will also be undertaken. In addition to the grey literature retrieved through Google Scholar, theses and dissertations will be searched using Thesis Canada Portal and ProQuest Dissertations and Theses Database. The manufacturers of currently commercially available vaccines will be contacted to identify, and potentially access, unpublished data. Citation searches and reference list checking will be performed on relevant articles to ensure that potentially relevant studies have not been missed. Corresponding authors of eligible studies will be contacted for conference proceedings they have published, and responses received within one month of contact will be included.

Search results will be uploaded into EndNote™ bibliographical management software and duplicates will be removed. Documentation of all search strategies and results will be provided in the final report.

### **Selection of eligible studies**

Search results will be loaded into online systematic review software (DistillerSR®, Ottawa, ON, Canada). Two rounds of screening will be performed. The first round of screening will assess abstracts and titles for eligibility, and will be performed by two reviewers (NAV and MCM), both DVMs with post-graduate training in epidemiology and the methodology of systematic reviews. Titles and abstracts will be assessed for relevance using the following primary screening questions:

- 1) “Does the title and/or abstract describe primary research?”
- 2) “Does the title and/or abstract describe dogs being used as the study subjects?”
- 3) “Does the title and/or abstract describe a study evaluating a vaccine intervention against Lyme disease (*Borrelia burgdorferi*)?”

A reference will be excluded if both reviewers give a final decision to exclude (i.e. the reviewers agree that the answer to at least one question is “no”). Any conflicts will be

resolved by consensus. If there is a conflict that cannot be resolved, a third member of the review team (JMS) will arbitrate.

Abstracts in a language other than English and French will be translated using Google Translate and screened using the primary screening questions. Translation of articles will be performed only if a large number of articles are identified as eligible by the primary screening process.

A secondary screening process will involve the full text. In addition to confirming responses to the initial questions based on the full text, the following secondary screening questions will be used:

- 4) Was this study performed in Canada or the United States or was the primary author affiliation from Canada or the United States?
- 5) Is the study design eligible? (i.e. experimental and analytical observational studies are eligible, whereas ecological studies, descriptive studies and reviews are not)
- 6) Did the study include a concurrent comparator group (either a control group or placebo group)?
- 7) Did the study evaluate a measure of vaccine effect? (i.e. reduction of incidence of clinical illness given infection, or reduction of incidence of infection given exposure)

Exclusion of references during the secondary screening will be performed as for the primary screening process (i.e. an article is excluded if both reviewers agree that the answer to at least one question is “no”). Prior to both primary and secondary screening, reviewers NAV, MCM and JMS will undergo training to ensure consistency in screening and data extraction processes. Fifty references will be used for the pre-test of the primary screening tool, and five references will be used for the pretest of the secondary screening tool.

### **Data extraction from eligible studies**

Two of the three reviewers for this study (NAV, MCM, JMS) will perform data extraction independently. The following information will be extracted from each study: geographic location (country, province/state, region), study duration, season, year of publication.

The following population information will be extracted: mean age, and co-morbidities and associated treatments used to restrict the study population (inclusion or exclusion criteria).

For interventions, the type and subtype of vaccine, as well as the dose, frequency and method of administration will be recorded. Commercial availability of the vaccine will also be recorded, if noted in the article. For challenge trials, any co-interventions given to increase disease susceptibility will be extracted. If applicable, details of the disease challenge will be recorded. The method and the cutoff used to determine seroconversion will be extracted.

All outcomes will be extracted as dichotomous measures. For all eligible outcome measures, the effect size estimate comparing two groups will be extracted as an odds ratio. If the effect size is not reported, raw data will be extracted in order to calculate the odds ratio comparing the two treatment groups; the number of animals in each group will

be extracted, as well as the number of animals with and without the event in each group. A measure of precision will be extracted for all outcome measures.

In studies with natural disease exposure (i.e. randomized controlled trials and observational studies), the criteria used to associate morbidity measures with Lyme infection will be extracted. Information about the control of confounders and additional covariates in the data analysis will be extracted. Confounders may include the use of tick preventives and the real risk of exposure (e.g. a hunting dog in a Lyme-endemic area). In the case of effect modifiers, the outcome measure for each level of the modifier will be extracted. The data extraction form will be pre-tested using five references.

### **Risk-of-bias assessment for eligible studies**

Two of the three reviewers for this study (NAV, MCM, JMS) will perform the risk of bias assessment. The Cochrane Collaboration's Risk of Bias tool will be used to assess risk of bias for experimental studies (Higgins and Green 2011). Observational studies will be assessed for risk of bias using the following seven bias domains from Cochrane's tool for the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I) (Sterne et al. 2016):

- Bias due to confounding
- Bias in selection of participants into the study
- Bias in measurement of interventions
- Bias due to departures from intended interventions
- Bias due to missing data
- Bias in measurement of outcomes
- Bias in selection of the reported result

Each domain will be assessed as follows:

1. Low risk of bias (the study is comparable to a well-performed randomized trial with regard to this domain)
2. Moderate risk of bias (the study is sound for a non-randomized study with regard to this domain but cannot be considered comparable to a well-performed randomized trial)
3. Serious risk of bias (the study has some important problems in this domain)
4. Critical risk of bias (the study is too problematic in this domain to provide any useful evidence)
5. No information on which to base a judgment about risk of bias for this domain

In general, risk of bias will be assessed for each outcome. However, observational studies will be assessed at the study level for confounding bias and for bias in the measurement of outcomes (dependent on the criteria used to associate morbidity with Lyme infection). An example of a serious risk of bias due to confounding in an observational study would be if the use of tick preventives was not accounted for. The pretest for the risk of bias will be performed using five references each for observational and experimental studies.

### **Process for data extraction and risk of bias assessment**

Prior to data extraction, a data extraction form will be created and pilot-tested by data extractors on five studies. Based on feedback, the extraction form will be modified to ensure ease of use and completeness. Two of the three reviewers for this study (NAV, MCM, JMS) will complete data extraction independently. Disagreements will be resolved by consensus. If consensus cannot be reached, a third reviewer will arbitrate.

If data in the study are unclear, incomplete or missing, the authors of the study will only be contacted if the study is less than five years old. This cutoff of five years has been made to increase the likelihood of making contact with study authors. If certain data cannot be extracted, this will be noted in the final report.

A risk of bias assessment form will be created based on Cochrane Collaboration's Risk of Bias tool (experimental studies) and ROBINS-I (observational studies), and will be pilot-tested on five experimental and observational studies each. The assessment form will be modified as needed. Two of the three reviewers for this study (NAV, MCM, JMS) will complete risk of bias assessment independently. Disagreements will be solved as for data extraction.

### **Strategy for data synthesis**

The findings will be summarized with a meta-analysis that will be conducted using R or Cumulative Meta-analysis software. A separate meta-analysis is planned for each study outcome (e.g. each morbidity outcome, seroconversion). Outcomes from experimental studies and observational studies will be analyzed separately; natural and deliberate disease exposure studies will also be analyzed separately. Random effects models will be used for all outcomes. Summary effect measures will be reported as summary odds ratios. Heterogeneity will be assessed using Cochran's Q test and I-squared (Higgins and Thompson 2002).

Possible sources of heterogeneity may include: geographic region, season, vaccine subtype (e.g. bacterin vs. bacterial extract), diagnostic method for seroconversion, type of disease exposure (natural vs. deliberate) in experimental trials. For observational studies, the criteria used to associate morbidity with Lyme infection is another important source of heterogeneity. If adequate data are available, subgroup analyses will be performed to explore the above listed potential sources of heterogeneity. Subgroup analyses for commercially available vaccines will also be performed, if possible, to provide clinically relevant information.

Adverse effects will be summarized in a narrative synthesis.

### **Strategy for presentation of results**

Tables will summarize study and population details, as well as information about interventions and, if applicable, the disease challenge. Studies will be grouped by study design (observational or experimental). A table with study outcomes will be grouped by vaccine type and subtype. For observational studies, information about adjustment for confounders will also be included. A risk of bias assessment table will be provided for each study by outcome, since there are likely to be multiple outcomes per study.

Using the GRADE approach, a summary of findings table will be prepared (Guyatt et al. 2011, Guyatt et al. 2013). The summary of findings table will include summary effect sizes (summary odds ratios) for each outcome.

## Discussion

This systematic review will provide synthesis of current evidence regarding the efficacy of the canine Lyme vaccine in North America. Results can be used to guide future trials and to inform veterinary practitioners of the current state of evidence for Lyme vaccines.

## Competing interests

The authors have no competing interests to declare.

## Authors' contributions

NAV will serve as review leader, and therefore responsible for coordinating the review, and performing primary and secondary screening, data extraction and risk of bias assessment. NAV is responsible for preparing all drafts of the protocol and the final manuscript, with input from Dr. Andrew S. Peregrine for content matters. The development of search strategies was performed by NAV and MCM with input from AMV. JMS will act as methodological expert, serving as a consultant for conducting the review, and will also perform statistical analyses. JMS will also participate as a reviewer in relevance screening, data extraction and risk of bias assessment. MCM will participate as a reviewer, and perform primary and secondary screening, and participate in data extraction and risk of bias assessment. All authors read and approved this final manuscript.

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Appendix I: Draft literature search strategy

Example of search strategy carried out in PubMed 1984 onwards. Date of search 07/10/16.

#	Searches	Results
1	dog* or canine* or canines.mp. [mp=all fields]	218403
2	vaccine or vaccin* or bacteri* or immunization or immunisation or immunisations or immunity or immun*.mp. [mp=all fields]	1773747
3	lyme or borreliosis or borrel* or burgdorferi.mp. [mp=all fields]	18292
4	1 and 2.mp. [mp=all fields]	19618
5	1 and 3.mp. [mp=all fields]	662
	2 and 3.mp. [mp=all fields]	8694
6	1 and 2 and 3.mp. [mp=all fields]	406