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SYSTEMATIC REVIEW

Efficacy of *Borrelia burgdorferi* vaccine in dogs in North America: A systematic review and meta-analysis

Nadine A. Vogt[1] | Jan M. Sargeant[1,2] | Melissa C. MacKinnon[1] | Ali M. Versluis[3]

[1]Department of Population Medicine, University of Guelph, Ontario, Canada

[2]Centre for Public Health and Zoonoses, University of Guelph, Guelph, Ontario, Canada

[3]Research and Scholarship Team, McLaughlin Library, University of Guelph, Guelph, Ontario, Canada

Correspondence
Nadine A. Vogt, Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada.
Email: nvogt@uoguelph.ca

Funding information
University of Guelph; The Ontario Veterinary College

Background: Lyme borreliosis, a tick-borne disease, is endemic to some parts of North America and is an emerging disease in other parts of the world. Vaccination is an increasingly common, although controversial, method used in the prevention of Lyme disease in dogs; the reported efficacies of *Borrelia burgdorferi* vaccines in dogs are highly variable, ranging from 50% to 100%.

Objectives: To determine the efficacy of vaccines for prevention of Lyme disease in dogs in North America.

Methods: Experimental and observational study designs were eligible for inclusion. The outcome of interest was the reduction of incidence of clinical illness after exposure to *B. burgdorferi*. Electronic databases searched were MEDLINE, Web of Science, and Centre for Agricultural Biosciences Abstracts. Clinical signs were extracted as dichotomous outcomes: lameness, anorexia, pyrexia, depression, and lymphadenopathy. Study quality was assessed using tools from the Cochrane collaboration.

Results: In total, 3 observational studies and 13 challenge trials were included. None of the challenge trials assessed lymphadenopathy, but for each of the remaining 4 clinical signs, a meta-analysis was performed. Compared to unvaccinated dogs, vaccinated dogs had a reduced odds of developing lameness, depression, pyrexia, and anorexia (odds ratio: 0.15-0.23).

Conclusions and Clinical Importance: Based on the quantitative synthesis of results from challenge studies, vaccinated dogs are less likely to develop clinical signs after exposure to *B. burgdorferi* compared to unvaccinated dogs. These results should be interpreted with caution, however, as several shortcomings related to quality and study design were identified. Future studies should focus on larger sample sizes in field conditions.

KEYWORDS

dog, evidence-based medicine, Lyme disease, vaccine efficacy

1 | INTRODUCTION

Lyme disease is the most common tick-transmitted disease worldwide and is known to infect humans as well as other domestic animals including horses, cats, and dogs.[1] The agents responsible for Lyme disease are a diverse group of spirochete bacteria within the *Borrelia* genus. Although a new species of *Borrelia* was recently identified as the cause of several human cases of Lyme disease in the upper Midwestern United States, the predominant genospecies responsible for Lyme disease in North America is *Borrelia burgdorferi* sensu stricto (henceforth referred to as *B. burgdorferi*).[2] *Borrelia burgdorferi* is spread primarily via deer ticks, *Ixodes scapularis*.[3]

The prevalence of ticks and the associated proportion of Lyme-infective ticks are highly variable across geographic locations, and endemic foci (hotspots) have been identified.[4] Dogs within these hot spots can have seroprevalence of up to 100% *B. burgdorferi*, whereas in locations only a few kilometers away, the seroprevalence can be

Abbreviations: CAB, Centre for Agricultural Biosciences; MeSH, medical subject headings; OR, odds ratio.

drastically lower, at less than 5%.[5] In contrast with *B. burgdorferi* infection in humans, 95% of dogs infected with *B. burgdorferi* do not develop clinical illness.[6] Clinical signs of *B. burgdorferi* infection in dogs can include polyarthritis, pyrexia, lethargy, anorexia, and lymphadenopathy. An association between *B. burgdorferi* infection and fatal glomerulonephropathy has been suggested, but a causal link has not been established.[7,8]

The most commonly used methods for prevention of *B. burgdorferi* infection in dogs include tick avoidance, prevention of tick infestation using acaricides, and vaccination. The primary limitation of many acaricides is that they might not offer complete protection if they target only the adult stage of ticks; meanwhile, nymphs (immature ticks) are also capable of transmitting *B. burgdorferi*.[9] Another potential drawback with acaricides is that most require monthly reapplication/administration, which could reduce owner compliance and thus product efficacy. Vaccination offers an alternative approach to prevention of disease, but efficacy is unclear with efficacies ranging from 50% to 100%.[10–13]

Lyme disease is a concern for pet owners and veterinarians in North America. Our primary objective was to investigate the efficacy of vaccines for the prevention of Lyme disease in dogs in North America. This article was prepared in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA statement.[14]

2 | METHODS

2.1 | Protocol and registration

The intended search strategy, eligibility criteria, study selection, data collection process, assessment of risk of bias, and the approach used for synthesis were included in the protocol published in advance, which is available online from SYREAF (systematic reviews for animals and food) and the University of Guelph Atrium, at: http://hdl.handle.net/10214/10049.

2.2 | Eligibility criteria

A summary of inclusion and exclusion criteria can be found in Table 1. The population of interest included pet dogs in Canada and the United States. Mexico was excluded because it is uncertain whether Lyme disease is endemic in this region.[15,16] For the intervention, studies that reported the use of a canine *B. burgdorferi* vaccine were eligible. Vaccines used outside North America were not eligible, because additional species of *Borrelia* other than *B. burgdorferi* are responsible for Lyme disease on other continents; vaccines used to control Lyme disease in those regions might not be relevant to the North American population of dogs. Since the 1st published report of Lyme disease in dogs appeared in 1984, only studies published in 1984 or later were eligible for inclusion.[17] Eligible study designs included primary research studies using experimental (natural or deliberate disease challenge) and analytical observational study designs. The latter type of study design was considered eligible, as these studies approximate real-life exposure to Lyme disease.

Eligible studies had to include outcomes assessing at least 1 of 2 measures of vaccine efficacy:

- reduction of incidence of clinical illness after exposure to *B. burgdorferi* (critical outcome) and
- reduction of incidence of seroconversion after exposure to *B. burgdorferi* (noncritical outcome).

Critical outcomes represented clinical outcomes that are relevant to practitioners and pet owners. Critical outcomes were modified slightly from the protocol, so that all dogs that were exposed to *B. burgdorferi* were assessed for clinical signs, rather than only dogs confirmed with *B. burgdorferi* infection. Without a reliable disease model for Lyme disease in the dog and no gold standard test for determining infection status, it was determined that infection status could not reliably be determined; thus, exposure to *B. burgdorferi* was considered sufficient.[18] Although the protocol stated that noncritical outcomes would be assessed for both types of study designs (ie, experimental and observational), they were not assessed for

TABLE 1 Eligibility criteria for a systematic review of the efficacy of the canine *Borrelia burgdorferi* vaccine in North America

	Inclusion criteria	Exclusion criteria
Study type	Experimental studies (natural or deliberate disease challenge)	Ecological studies
	Analytical observational studies	Descriptive studies
	English or French language	Reviews
	Studies published in 1984 or later	
Population	Pet dogs in the United States and Canada	Pet dogs in Mexico and outside North America
Intervention	Vaccines that protect against *B. burgdorferi*	Vaccines that do not protect against *B. burgdorferi*
	Monovalent and multivalent vaccines	
	Commercially and noncommercially available vaccines	
Comparator group	Concurrent placebo or control group	No concurrent placebo or control group
Outcomes	Assessed at least one of the following measures of vaccine efficacy:	
	(1) Critical outcomes: Incidence of clinical illness after exposure[a]	Did not assess vaccine efficacy (either clinical illness or seroconversion) in dogs
	(2) Noncritical outcomes: Incidence of seroconversion[b] after exposure[a]	No exposure to ticks

[a] Natural or deliberate exposure to ticks capable of carrying *B. burgdorferi* or needle inoculation with *B. burgdorferi*.
[b] Noncritical outcomes were defined as the "incidence of infection given exposure" in our protocol.

experimental study designs for the above reasons and because there was considerable diversity in the methods used to determine the infection status, which would prevent combination of these data. In order to combine these data in a meta-analysis, there need to be a basis for preferring one method over the other; also, in the absence of a gold standard, this cannot be accomplished. With limited options for determining infection status under field conditions, seroconversion was assessed as a noncritical outcome in observational studies.[19,20] Although adverse effects were not strictly considered an outcome measure, they were recorded.

2.3 | Information sources

Electronic searches of the MEDLINE (via PubMed) (1984-2016), Centre for Agricultural Biosciences (CAB) Abstracts (via CAB Direct) (1984-2016), and Web of Science Core Collection (1984-2016) databases were performed. These databases were selected for their coverage of a wide variety of journals in the life sciences, biomedical sciences, and veterinary sciences. The search strategy was adapted for each search resource, accounting for differences in syntax, indexing, and functionality. The search was performed on November 29, 2016.

The gray literature was also searched. The 1st 500 abstracts sorted on relevance in Google Scholar were accessed. Relevant theses and dissertations were identified using ProQuest Theses and Dissertations Database and Thesis Canada Portal. Vaccine companies, including Boehringer Ingelheim, Zoetis, Merial, Merck, United Biomedical, and Neotech, were contacted to obtain any relevant unpublished literature (both American and Canadian companies were contacted, when applicable). In addition, reference list checking and citations searches were performed for all eligible studies.

2.4 | Search

The search strategy comprised 3 concepts: canine, vaccine, and borreliosis. The search strategy used to identify relevant articles in MEDLINE was as follows:

- ((dog OR canine) AND (vaccine OR bacterin OR immunization OR immunity) AND (lyme OR borrelia OR borreliosis OR burgdorferi)) AND ("1984/01/01"[PDat]: "2016/12/31"[PDat])

A search using Medical Subject Headings (MeSH) was performed within each database in addition to the above standard search, if possible. In MEDLINE, the subject heading search was as follows:

- "dogs"[MeSH] AND ("vaccines"[MeSH] OR "immunization"[MeSH] OR "immunity"[MeSH]) AND ("Borrelia"[MeSH] OR "Lyme disease"[MeSH]) AND ("1984/01/01"[PDAT]: 2016/12/31"[PDAT])

No language restrictions were placed on the search. All searches performed and their results are presented in Supplemental Information. Search results were uploaded to EndNote bibliographic management software (Thomson Reuters, Philadelphia, Pennsylvania).

2.5 | Study selection

Search results were loaded into EndNote, and duplicates were removed. The search results were then loaded into an online systematic review program (Distiller SR, Ottawa, ON, Canada), and additional duplicates were removed using Distiller SR. Primary reviewers were veterinarians with postgraduate training in epidemiology and the methodology of systematic reviews. Before screening and data extraction, primary reviewers received training to ensure consistency. Screening and data extraction were performed independently by 2 reviewers using pretested forms. Level 1 screening was performed on titles and abstracts using 3 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*)?"

References were excluded if both reviewers answered "no" to at least one of the questions. Vaccine interventions describing specific species of *Borrelia* other than *burgdorferi* were excluded at Level 1 (Question 3). References that passed Level 1 screening moved forward to Level 2 screening using the full text. Two independent reviewers used the above questions (Questions 1-3) again to assess relevance, before answering additional questions for Level 2 screening

4. Was this study performed in Canada or the United States or was the primary author affiliation from Canada or the United States?
5. What kind of study design was used?
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 (ie, reduction of incidence of clinical illness after exposure or reduction of incidence of seroconversion after exposure)?

Question 5 was modified from "is the study design eligible?" in the protocol to "what kind of study design was used?" in order to streamline the data extraction process, so that references could be diverted to the appropriate form at this stage and thus minimize conflicts during data extraction. Articles for which the answers to questions 4, 6, and 7 were "yes" moved forward to data extraction. An additional revision to the protocol was made: conference proceedings were excluded during Level 2 screening because they represent a lower quality of evidence and might have posed issues with data extraction and risk of bias assessment because of a lack of detail in short documents. At all stages of screening, disagreements between reviewers were resolved by consensus, and no arbitrator was needed.

2.6 | Data collection process

Conflicts during data extraction were resolved by consensus. Authors of eligible studies were contacted for additional unpublished work, and responses received within 1 month were included.

2.7 | Data items

For each study, study characteristics extracted were geographic location (country, province/state), season, and year of publication. The month and year of study initiation and conclusion were extracted, rather than extracting study duration (as stated in the protocol). Population information extracted included mean age of dogs and, for observational studies, any comorbidities or associated treatments used to restrict the study population. For interventions, the type and subtype of vaccine, as well as the dose, frequency, and method of administration were recorded. For the comparator group(s), the type of intervention was extracted (ie, placebo vs unvaccinated). For studies with multiple intervention groups, dogs that received the same type and dose of active vaccine ingredient were grouped in the same intervention group, regardless of the type and dosage of adjuvant used. Comparator group information was extracted such that it was clear whether there was a single or multiple control groups. If applicable, the type of disease challenge was recorded (ie, ticks vs needle inoculation). Dogs were considered to be in separate treatment groups if they differed by the type of challenge used, even if they received the same intervention. In addition, dogs were considered to be in separate treatment groups if a different vaccine dose was used, which was a modification to the protocol.

Commercial availability of the vaccine was extracted only for observational studies, instead of all studies, as stated in the protocol. It was determined that commercial availability was not relevant for challenge trials because a vaccine that was commercially available at the time of the trial might have different active ingredients compared to the currently commercially available version of the vaccine. The most useful measure of commercial availability in challenge trials would be to link previous studies with currently commercially available vaccines; however, this information was unavailable and potentially could not be verified because of proprietary rights of vaccine companies.

A summary of critical and noncritical outcomes extracted can be found in Table 2. Seroconversion, a noncritical outcome, was extracted as a dichotomous outcome in observational studies. The method used to determine seroconversion was also extracted. For studies with natural disease exposure (ie, observational studies and randomized controlled trials), the criteria used to attribute clinical signs with *B. burgdorferi* infection and not another cause were extracted, when reported.

Critical outcomes included measures of morbidity: lameness, anorexia, pyrexia, depression, and lymphadenopathy. These clinical signs were each considered separately. Outcome data were not extracted for studies that assessed all clinical signs combined into a single outcome. All critical outcomes were extracted as dichotomous measures. For example, dogs with at least 1 incident case of lameness during the study period were considered a positive case for lameness in that study. None of the studies reported an effect size; therefore, raw data were extracted, including the number of animals in each intervention group, as well as the number of animals with and without the particular clinical sign in each group.

TABLE 2 Outcomes extracted for a systematic review of the efficacy of the canine *Borrelia burgdorferi* vaccine in North America

	Critical outcomes[a] (incidence of clinical illness after exposure)	Noncritical outcomes (incidence of seroconversion[b] after exposure)
Experimental studies	Lameness Anorexia Pyrexia Depression Lymphadenopathy	Not assessed because of lack of gold standard for determination of infection status
Analytical observational studies	Lameness Anorexia Pyrexia Depression Lymphadenopathy	Seroconversion

[a] Each clinical sign was considered separately. Outcome data were not extracted for studies that assessed all clinical signs and combined into a single outcome.
[b] Noncritical outcomes were defined as the "incidence of infection given exposure" in our protocol.

2.8 | Risk of bias in individual studies

Risk of bias assessment was performed at the outcome level (if multiple outcomes) or study level (if one outcome) by 2 reviewers independently, using pretested forms. Risk of bias in observational studies was assessed using Cochrane's tool for the Risk of Bias in Non-randomized Studies of Interventions.[21] Domains assessed were confounding, selection, measurement of interventions, departures from intended interventions, missing data, measurement of outcomes, and selective reporting of results. The judgment outcome options included a low, moderate, serious, and critical risk of bias. The form used to assess risk of bias in observational studies including specific criteria for judgment can be found in Supplemental Information.

Risk of bias in experimental studies was assessed using the Cochrane Collaboration's Risk of Bias tool.[22] Domains assessed were selection, performance, detection, attrition, reporting, and other bias. The judgment outcome options included low, high, and unclear risk of bias. The risk of bias regarding randomization was modified from the Cochrane tool because of the anticipated level of reporting in the veterinary literature; the risk of bias was low as long as the authors stated that randomization was used to allocate animals to intervention groups, even if the study did not report further details regarding the method of randomization. If the authors did not state whether or not randomization was used, the risk of bias was unclear. The risk of bias for this domain was high if authors stated that animals were assigned to groups in a manner which did not include a formal random process. For trials with multiple intervention groups, the risk of bias assessment was performed at the trial level, as the methods did not vary among different intervention groups.

2.9 | Summary measures

Raw data were extracted, as none of the studies reported effect sizes.

2.10 | Synthesis of results

Observational studies and experimental studies were analyzed separately. For critical outcomes, a separate random effects meta-analysis was performed for each clinical sign independently. All meta-analyses were performed at the trial level using R Statistical Software and the "metafor" package.[23,24] A meta-analysis was only performed if at least 3 trials were eligible. Effect sizes were calculated as odds ratios (ORs) using raw data and the "escalc" command. A multilevel model was built using the "rma.mv" command and the restricted maximum likelihood method, including trial as a random effect to account for multiple trials within a publication. For studies that used the same control group for multiple treatment groups, these treatment groups were kept separate only if there was an a priori difference identified for potential subsequent subgroup analyses (ie, different vaccine doses or different challenge). To account for the use of the same control group in these multiple treatment arm studies, an approximate adjustment was made,[25] an additional random effect was added to account for within-study clustering, and calculation of the covariance among sampling errors was performed before model building to account for correlation between different OR estimates using the same control group.[26] Trials in which there were zero cases of clinical signs in both intervention and comparator groups were included in the meta-analysis by using a continuity correction factor of 0.5. To obtain a measure of heterogeneity, I^2 was manually computed in R.[27] See Supplemental Information for R code.

2.11 | Risk of bias across studies

Funnel plots were used to evaluate publication bias provided that at least 10 trials were included in a meta-analysis.

2.12 | Additional analyses

None of the intended subgroup analyses were performed because of low heterogeneity.

3 | RESULTS

3.1 | Study selection

Results for the total number of articles screened, assessed for eligibility, included in the review, and excluded from the review, along with reasons for exclusion at Level 2, are presented in Figure 1. Complete search strategies with the number of articles retrieved can be found in Supplemental Information. In total, 1000 electronic records remained after duplicates were removed. Two records containing unpublished work were identified from a vaccine company. A total of 22 records were identified through our gray literature searches; after removal of duplicates, 16 records remained. Citation searches and reference list checking did not yield any new records. No further unpublished work was identified by contacting authors of eligible studies. Thus, a total of 1018 records from both electronic and gray literature searches remained after removal of duplicate records. All articles identified as relevant for full-text screening were in English, thus no records were excluded based on language. Reasons for exclusions of articles at Level 2 are presented in Figure 1, and citation information is available in Supplemental Information. In total, 16 manuscripts describing 20 separate trials (containing independent control groups) were eligible for inclusion. Among the 16 eligible manuscripts, 3 were observational studies (3 trials)[12,20,28] and the remaining 13 experimental studies (17 trials)[1,11,13,29–38] were challenge studies. No eligible randomized controlled trials were identified.

3.2 | Study characteristics

The results for the characteristics of the 16 articles are presented in Table 3 (observational) and Table 4 (challenge studies). Among observational studies, 1 cohort study was identified and the remaining 2 studies were cross-sectional. None of the challenge studies reported the month or year of study initiation or termination. Most challenge studies induced disease using an infected tick challenge and tested puppies younger than 6 months of age. Various doses of bacterins and recombinant vaccines were used among challenge studies (Table 4).

3.3 | Risk of bias within studies

Results for the risk of bias among experimental studies are presented in Figure 2. Although the risk of bias assessment was performed at the outcome level for each trial (eg, lameness outcome), the results are presented at the publication level for ease of interpretation, because the results did not differ between outcomes and trials. None of the challenge trials indicated that methods were used to ensure allocation concealment or blinding of personnel. Although many experimental studies were classified as a "low" risk of bias for random sequence generation because they stated that dogs were randomly assigned to an intervention group, only 1 study specified the actual method used to randomly assign dogs to an intervention group.[38]

For all 3 observational studies, the risk of bias owing to confounding was judged to be "critical," because none of the studies accounted for the use of tick preventives. Selection bias in both cross-sectional observational studies was judged as "serious" because all dogs included in the study were selected for inclusion because they presented for heartworm testing. For the cohort observational study, there was no information on which to base a judgment for the selection of dogs into the study or the measurement of outcomes (it is unknown whether dog owners or veterinarians assessed dogs for clinical signs). In addition, the cohort study did not provide information regarding inclusion or exclusion criteria for control dogs; therefore, no judgment could be made in the domain of classification of interventions. Bias in the classification of interventions, bias because of deviation from intended interventions, bias in the measurement of outcomes, and bias because of selective reporting were all judged to be "low" for both cross-sectional observational studies. Bias because of missing data was judged as "moderate" for both cross-sectional observational studies as there was no information about which dogs were excluded from the analysis. Bias because of deviations from intended interventions, missing data, and selective reporting were judged as "low" for the cohort observational study.

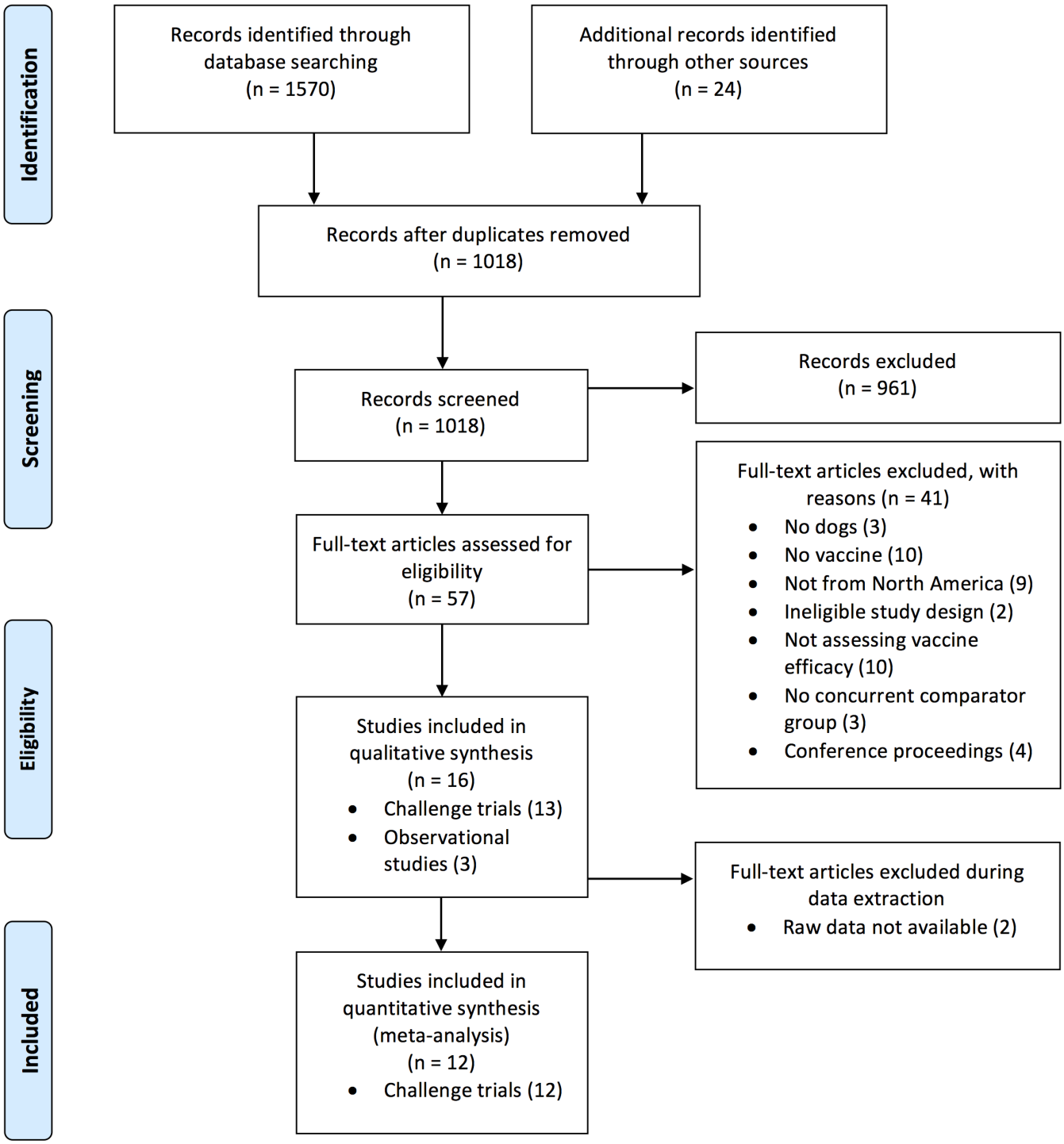

FIGURE 1 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart showing the selection of studies eligible for a systematic review of the efficacy of the *Borrelia burgdorferi* vaccine in dogs

3.4 | Results of individual studies

A summary of study results for included observational studies is presented in Table 3. Only 1 observational study (cohort study design) assessed clinical signs, but because these were grouped together, data for individual clinical signs could not be extracted. This observational study did not assess seroconversion after vaccination; therefore, raw data could not be extracted for that noncritical outcome. The other 2 observational studies (cross-sectional study design) provided raw data for seroconversion between vaccinated and unvaccinated dogs.

A summary of study results for 17 included challenge trials from 13 publications is presented in Table 4. At least 1 critical outcome was assessed in 16 of the 17 trials. Sixteen challenge trials assessed lameness, 7 evaluated anorexia, 5 assessed pyrexia, 6 assessed depression, and none assessed lymphadenopathy.

According to 4 studies that included information about adverse events, serious adverse events were uncommon. Three of these studies noted mild swelling and reddening at the injection site.[1,30,32] One of these studies reported that 8 of 10 dogs had a slight swelling at the injection site at the 1st vaccination and 3 of the 10 dogs had a similar

TABLE 3 Characteristics of analytical observational studies included in a systematic review of the efficacy of the canine *Borrelia burgdorferi* vaccine in North America

Study	State/Province	Year(s) of study	Study design	N (study population)	Mean age (study population)	Vaccine description	Control description	Methods used to evaluate serological response	Commercially available (brand name, vaccine company)	Number of *Borrelia* seropositive dogs in each group (cases/total number of dogs)
Levy et al. (1993)[28]	Connecticut and New York state	July 1990 to February 1992	Cohort	6467 (1969 vaccinated, 4498 controls)	NR	Whole-cell inactivated bacterins (IM, 2 doses 3 wk apart, boostered annually, dose NR)	NR	N/A[a]	Yes (LymeVax, Zoetis)	N/A[a]
Levy (2002)[20]	Connecticut	April to August 2001	Cross-sectional	202 (163 vaccinated, 39 controls)	8.5 y	Whole-cell inactivated bacterin (2 doses before 6 mo of age, boostered annually, dose and route admin NR)	Not vaccinated for Lyme ever	C_6 ELISA (SNAP 3Dx, IDEXX Laboratories)	Yes (LymeVax, Zoetis)	Intervention (8/163) Control (25/39)
Levy et al. (2005)[12]	Connecticut	April to August 2001	Cross-sectional	79 (60 vaccinated, 19 controls)	Vaccinated dogs: 2.96 y Control dogs: 3.16 y	Recombinant subunit OspA vaccine (1 mL, SC, 2 doses before 6 mo of age, boostered annually)	Not vaccinated for Lyme ever	C_6 ELISA (SNAP 3Dx, IDEXX Laboratories)	Yes (Recombitek Lyme, Merial)	Intervention (15/60) Control (12/19)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; NR, not reported.
[a] Could not extract raw data because serological response was not assessed after vaccination. Could not extract critical outcomes because clinical signs were grouped together.

reaction at the 2nd vaccination.[32] One observational study reported that 38 of 1969 dogs (1.9%) had minor reactions, and that 1 dog had a reaction after the 2nd vaccine dose; all reactions were reported to have resolved without complications by 72 hours after vaccination.[28] No further details were provided as to the type of clinical signs observed during these minor reactions.[28]

3.5 | Synthesis of results

Only 2 observational studies provided sufficient raw data for the non-critical outcome; therefore, we did not perform a meta-analysis among observational studies, because at least 3 studies were required to perform a meta-analysis. The results of random effects meta-analyses of critical outcomes among challenge trials are presented in Figures 3–6. All 4 meta-analyses had multiple trials within one or more publications, thus a random effect for trial was included in the multilevel model. Only the lameness outcome included trials that used a single control group for multiple treatment comparisons; thus this model also included a random effect for estimates within studies. The calculated ORs, summary ORs, and associated confidence intervals for all critical outcomes are presented in Figures 3–6. The calculated ORs for critical outcomes in individual studies were all consistently equal to or less than 1. Confidence intervals for calculated ORs of individual studies were overlapping, and frequently spanned across the null OR of 1. Summary ORs of critical outcomes ranged from 0.15 to 0.23, and the associated confidence intervals were less than 1 for all critical outcomes except for anorexia. The pooled results suggest that vaccinated dogs were consistently less likely to develop clinical signs, and the effect was significant for lameness, depression, and pyrexia, but not for anorexia. There was low heterogeneity for all meta-analyses, with I^2 at approximately 7% or less for anorexia, pyrexia, and depression, and 23% for lameness.

3.6 | Risk of bias across studies

A funnel plot was generated for the lameness outcome only. Publication bias could not be assessed for the remaining critical outcomes because there were 7 or fewer trials in each respective meta-analysis. Based on the funnel plot for the lameness outcome, publication bias was possible because the lower left corner of the funnel contained no studies (Figure 7). However, it should be noted that sample sizes were small, with more than half of trials having 10 dogs or less per treatment group.

3.7 | Additional analysis

Subgroup analyses were not performed because of low heterogeneity.

4 | DISCUSSION

4.1 | Summary of evidence

There was consistency in the direction of the estimated summary ORs for lameness, anorexia, pyrexia, and depression outcomes, with all estimates less than 1. Although the confidence intervals were wide

TABLE 4 Characteristics of challenge studies included in a systematic review of the efficacy of the canine *Borrelia burgdorferi* vaccine in North America

Study	State/ Province	Age (study population)	Vaccine description	Control description	Type of challenge	Use of immunosuppression	Critical outcomes evaluated and (number of cases/total number of dogs) in intervention (int) and control (ctrl) groups
Chu et al. (1992)a[29]	NR	All dogs <18 mo old	Lot 89 *B. burgdorferi* inactivated bacterin with adjuvant (1 mL, IM, 2 doses 3 wk apart)	Unvaccinated	Needle inoculation of *B. burgdorferi*	Dexamethasone (2 mg) given on days 1, 2, 4, 6 after challenge	Lameness (int: 0/10, ctrl: 6/8) Anorexia (int: 0/10, ctrl: 3/8) Pyrexia (int: 4/10, ctrl: 7/8) Depression (int: 0/10, ctrl: 2/8)
Chu et al. (1992)b[29]	NR	All dogs <18 mo old	Lot 89 *B. burgdorferi* inactivated bacterin with adjuvant (1 mL, IM, 2 doses 3 wk apart)	Unvaccinated	Needle inoculation of *B. burgdorferi*	None	Lameness (int: 0/10, ctrl: 2/6) Anorexia (int: 0/10, ctrl: 1/6) Pyrexia (int: 3/10, ctrl: 4/6) Depression (int: 0/10, ctrl: 1/6)
Chu et al. (1992)c[29]	NR	10- to 13-wk-old puppies	Lot 90 *B. burgdorferi* inactivated bacterin with adjuvant. Fractional dose of lot 89 (1 mL, IM, 2 doses 3 wk apart)	Unvaccinated	Needle inoculation of *B. burgdorferi*	Dexamethasone (2 mg) given on days 1, 2, 4, 6 after challenge	Lameness (int: 1/10, ctrl: 7/10) Anorexia (int: 0/10, ctrl: 4/10) Pyrexia (int: 7/10, ctrl: 9/10) Depression (int: 0/10, ctrl: 2/10)
Chang et al. (1995)a[1]	New York	6-wk-old puppies	Recombinant OspA with adjuvant (100 μg, IM, 2 doses 3 wk apart)	Placebo with various adjuvants and an unvaccinated group	Exposure to ticks	None	Lameness (int: 0/8, ctrl: 0/8) Anorexia (int: 0/8, ctrl: 0/8)
Chang et al. (1995)b[1]	New York	6-wk-old puppies	Recombinant OspA with adjuvant (10 μg, SC, 2 doses 3 wk apart)	Unvaccinated	Exposure to ticks	None	Lameness (int: 0/4, ctrl: 0/2) Anorexia (int: 0/4, ctrl: 0/2)
Chang et al. (1995)c[1]	New York	6-wk-old puppies	Recombinant OspA without adjuvant (100 μg, IM, 2 doses 3 wk apart)	Unvaccinated	Exposure to ticks	None	Lameness (int: 0/8, ctrl: 1/3) Anorexia (int: 0/8, ctrl: 0/3)
Coughlin et al. (1995)[30]	NR	12-wk-old puppies	Recombinant OspA, recombinant OspB with adjuvant (25 μg each rOspA and rOspB, SC, 2 doses 4 wk apart)	Unvaccinated	Exposure to ticks	None	Lameness (int: 0/11, ctrl: 1/11) Pyrexia (int: 0/11, ctrl: 6/11) Depression (int: 0/11, ctrl: 6/11)
Straubinger et al. (1995)[31]	New York	6-wk-old puppies	Treatment groups 1-4: recombinant OspA with adjuvant (100 μg, IM, 2 doses 3 wk apart) Treatment group 5: recombinant OspA with adjuvant (10 μg, SC, 2 doses 3 wk apart)	Unvaccinated	Exposure to ticks	None	Lameness groups 1-4 (int: 0/18) Lameness group 5 (int: 0/4) Lameness control group (26/46)
Ma et al. (1996)[32]	NR	12-wk-old puppies	Recombinant OspA and recombinant OspB with adjuvant (25 μg each rOspA and rOspB, SC, 2 doses 4 wk apart, then 13 mo later)	Unvaccinated	Exposure to ticks	None	Lameness (int: 0/10, ctrl: 5/10)
Conlon et al. (2000)[13]	NR	10- to 12-wk-old puppies	Treatment group 1: recombinant OspA (1 mL, SC, 2 doses 3 wk apart) Treatment group 2: recombinant OspA, lot was older than 4 y (1 mL, SC, 2 doses 3 wk)	Unvaccinated	Exposure to ticks	None	Unable to extract raw data

(*Continues*)

TABLE 4 (Continued)

Study	State/ Province	Age (study population)	Vaccine description	Control description	Type of challenge	Use of immunosuppression	Critical outcomes evaluated and (number of cases/total number of dogs) in intervention (int) and control (ctrl) groups
Straubinger et al. (2002)[11]	New York	8- to 10-wk-old puppies	Treatment group 1: recombinant OspA without adjuvant (SC, 2 doses 3 wk apart, dose NR) Treatment group 2: multiantigenic *B. burgdorferi* strain (strain N40) (1 mL, SC, 2 doses 3 wk apart) Treatment group 3: multiantigenic *B. burgdorferi* strain (strain N40) (1 mL, SC, 2 doses 3 wk apart)	Placebo (PBS sham)	Group 1: exposure to ticks Group 2: exposure to ticks Group 3: Needle inoculation with *B. burgdorferi*	None	Lameness group 1 (int: 0/2) Lameness group 2 (int: 0/6) Lameness group 3 (int: 0/2) Lameness control group (0/2)
Wikle et al. (2006)[33]	NR	9- to 12-wk-old puppies	Recombinant OspA (1 mL, SC, 2 doses 3 wk apart)	Placebo	Exposure to ticks	None	Lameness (int: 0/20, ctrl: 2/11) Anorexia (int: 0/20, ctrl: 0/11) Pyrexia (int: 0/20, ctrl: 0/11) Depression (int: 0/20, ctrl: 0/11)
LaFleur et al. (2009)[34]	NR	8-wk-old puppies	Bivalent *B. burgdorferi* bacterin (S-1-10 and 50772 strains) with an antibiotic and an antimycotic agent (1 mL, SC, 2 doses 3 wk apart)	Placebo	Exposure to ticks	Dexamethasone (0.4 mg/lb IM for 5 d) was administered only to dogs which did not develop joint abnormalities within 13 wk after challenge	Lameness (int: 2/15, ctrl: 4/15)
LaFleur et al. (2010)[35]	NR	8-wk-old puppies	Bivalent *B. burgdorferi* bacterin (S-1-10 and 50772 strains) with an antibiotic and an antimycotic agent (1 mL, SC, 2 doses 3 wk apart)	Placebo	Exposure to ticks	Dexamethasone (0.4 mg/lb IM for 5 d) beginning at week 19 after challenge	Lameness (int: 0/15, ctrl: 2/15)
Ball (2015)[36]	NR	8- to 9-wk-old puppies	Chimeric recombinant (recombinant OspA and chimeric protein composed of antigenic material from 7 types of OspC) (1 mL, SC, 2 doses 3 wk apart)	Placebo	Exposure to ticks	None	Lameness (int: 0/16, ctrl: 3/16)
LaFleur et al. (2015)[37]	NR	8-wk-old puppies	*B. burgdorferi* bacterin (50772 strain, OspA and OspB negative) with antibiotic and antimycotic agent (1 mL, SC, 2 doses 3 wk apart)	Placebo	Exposure to ticks	Dexamethasone (0.4 mg/lb IM for 5 d) beginning at week 19 after challenge	Lameness (int: 1/10, ctrl: 3/10)
Grosenbaugh et al. (2016)[38]	NR	7-8 mo	Recombinant OspA without adjuvant (1 mL, SC, 2 doses 3 wk apart)	Unvaccinated	Exposure to ticks	None	Lameness (int: 0/15, ctrl: 0/15) Depression (int: 0/15, ctrl: 0/15)

Abbreviation: NR, not reported.
Each row in the table corresponds to 1 trial. Separate trials from the same publication are indicated by letters a, b and c. Trials with multiple treatment groups are presented in the same row. Study authors reported that 2 dogs were lame in the treatment group but did not attribute the lameness to infection with *B. burgdorferi* because spirochetes were not recovered and serologic evidence of *B. burgdorferi* infection or exposure not detected. We include these dogs as positives here because the odds of lameness unrelated to *B. burgdorferi* infection should be equal in both treatment and control groups.

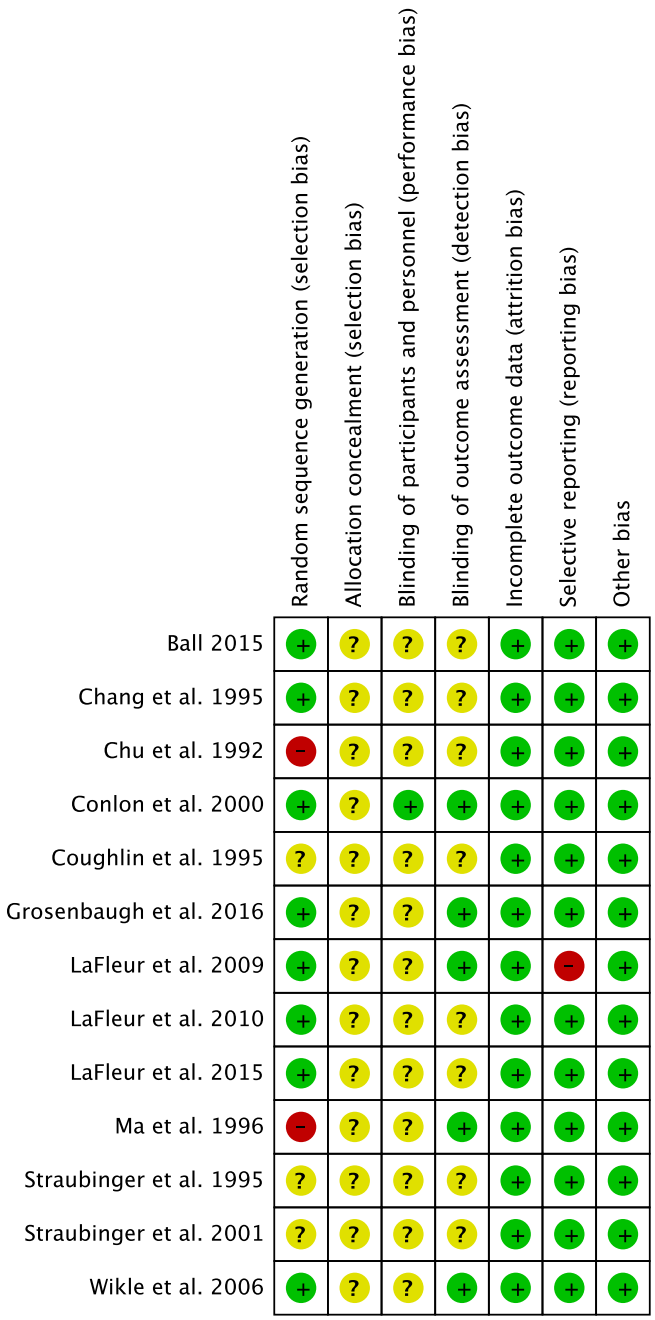

FIGURE 2 Risk of bias assessment for challenge trials included in a systematic review of the efficacy of the canine *Borrelia burgdorferi* vaccine. + indicates low risk of bias, – indicates high risk of bias, and ? indicates unclear risk of bias

for these individual studies, all intervals for the summary estimates were less than the null value of 1, except for the anorexia outcome. There was low heterogeneity for all critical outcomes (suggesting relatively consistent results across studies). Overall, the consistency in directionality between different clinical outcomes, despite wide confidence intervals, suggests that vaccination reduces the odds of clinical signs in vaccinated dogs compared to control dogs.

However, several issues relating to the assumption that all study dogs were exposed to *B. burgdorferi* were identified among these challenge studies. Most challenge studies tested only a subsample of ticks to determine the proportion of ticks infected with *B. burgdorferi* and then extrapolated this proportion to the population of ticks used to infect the study population of dogs (data not shown). The proportion of ticks infected with *B. burgdorferi* was often as low as 35%-40% in many of the challenge studies and ranged from 24% to 100%. Researchers tried to overcome this difficulty by ensuring that several ticks fed on each dog, but this effort does not guarantee that all dogs in each group were exposed to *B. burgdorferi*. Although the resulting misclassification of dogs with respect to *B. burgdorferi* exposure status would be non-differential between vaccinated and control dogs, differences in exposure might be magnified by small samples sizes, and the low frequency of clinical signs in dogs infected with *B. burgdorferi*. Conclusions drawn from our meta-analyses depend on the assumption that dogs in both the vaccinated and comparator groups were equally exposed to *B. burgdorferi* and had an equal likelihood of developing clinical signs under the null hypothesis. In future studies, this important assumption could be verified by testing all ticks to demonstrate that each dog was exposed to *B. burgdorferi*.

No overall assessment of risk of bias for each observational study was performed. Because of our modification from the Cochrane tool, our risk of bias assessment was lenient for the category of random allocation; we assessed the risk as "low" if the authors stated that random allocation was performed; however, further information about the method of random allocation is required to adequately assess whether this step was performed well or not. Although none of the challenge studies reported allocation concealment, this is unlikely to be a source of bias for experimental studies such as challenge trials, as animals are unowned.

In observational studies, researchers cannot control for confounding by indication in cross-sectional studies; they cannot be certain that infection status is not associated with vaccination status if dogs are not screened for *B. burgdorferi* before vaccination. To avoid this issue, future cross-sectional observational studies should ensure that dogs classified as vaccinated were negative for *B. burgdorferi* before vaccination.

Outcomes were divided into critical and noncritical outcomes to focus the review on outcomes that are perhaps considered to be more important for owners and practicing veterinarians (ie, clinical outcomes). However, because the proportion of dogs that demonstrate overt clinical illness with *B. burgdorferi* infection is small (5%),[6] a vaccine which prevents infection might be of value because it can aid in controlling the spread of *B. burgdorferi* by reducing the number of nonclinical, infected dogs. Although it is unknown whether dogs represent an important reservoir of *B. burgdorferi*, there is evidence that infected dogs can transmit the microorganism to uninfected ticks.[39] In this sense, what we have judged as noncritical outcomes (ie, reduction of incidence of seroconversion after exposure) should not necessarily be considered unimportant.

The information provided in this review will be useful for both practicing veterinarians and researchers. Not only will this review help to guide clinical decision-making, but it also provides valuable information regarding improvements in study design for researchers and has revealed important gaps in the literature. To date, limited research has been performed in field conditions (with natural disease exposure), and we were unable to perform a meta-analysis for observational studies because of an insufficient number of studies. Although we were able to perform several meta-analyses with challenge

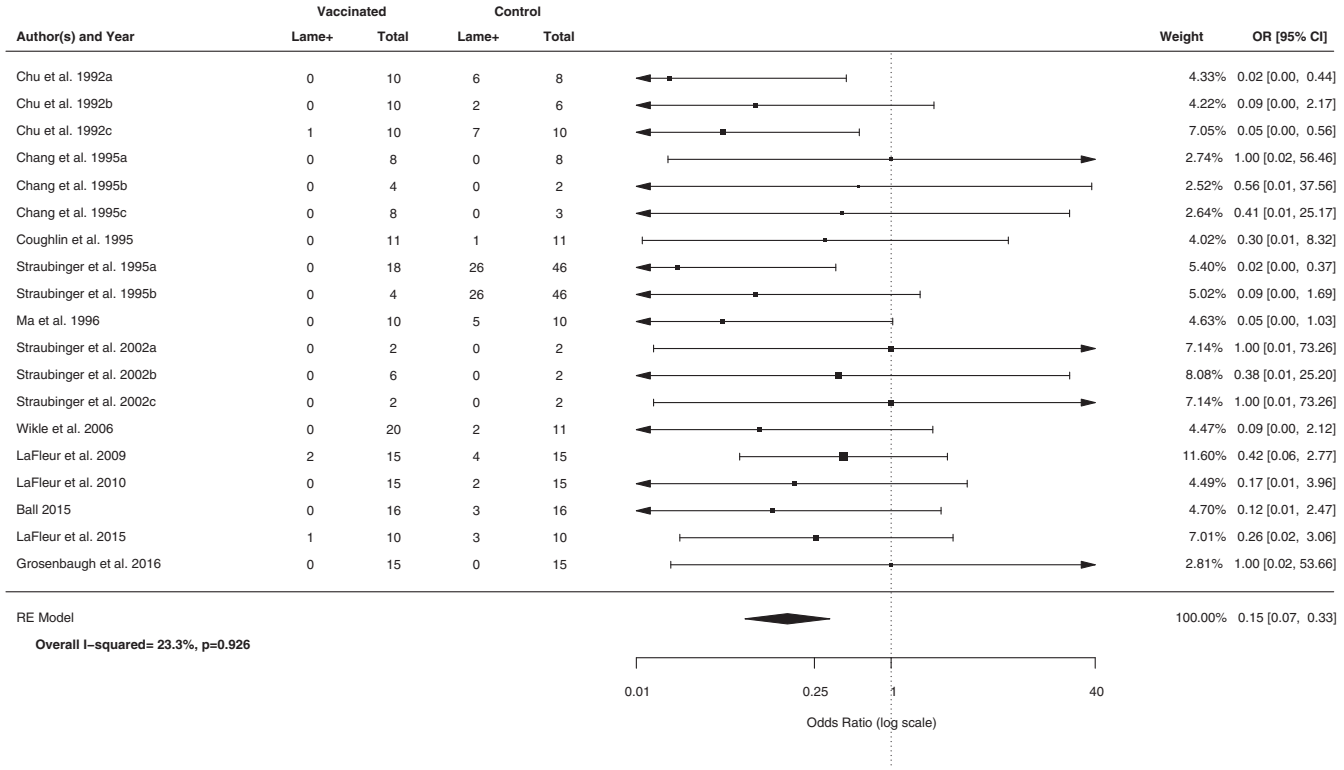

FIGURE 3 Forest plot of the odds ratio and 95% confidence interval (CI) for incident cases of lameness in dogs (Lame+) vaccinated for Lyme disease compared to control dogs in experimental studies with a deliberate disease challenge. Horizontal solid lines represent 95% CIs. The vertical dotted line symbolizes the null value. A multilevel random effects model was used

studies, it is possible that tick exposures in these studies are not representative of natural exposures.

4.2 | Limitations

Incomplete retrieval of identified research is an unlikely source of bias for this systematic review, as we were able to retrieve all relevant articles identified in the electronic databases. However, we cannot rule out the existence of unpublished studies performed by vaccine companies, which would have been eligible for inclusion. Because of proprietary rights of vaccine companies, these records might not be publicly available. It is possible that we did not retrieve all relevant publications through database searches, given that our search terms were not exhaustive. In addition, we were unable to assess for

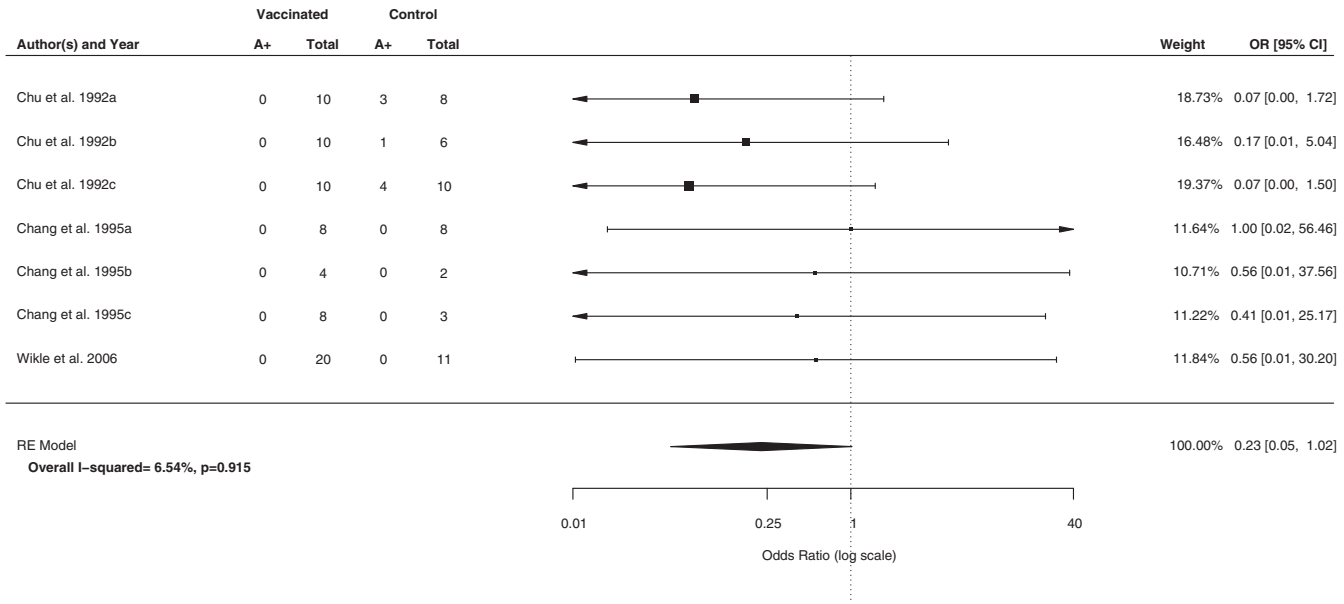

FIGURE 4 Forest plot of the odds ratio and 95% confidence interval (CI) for incident cases of anorexia in dogs (A+) vaccinated for Lyme disease compared to control dogs in experimental studies. Horizontal solid lines represent 95% CIs. The vertical dotted line symbolizes the null value. A multilevel random effects model was used

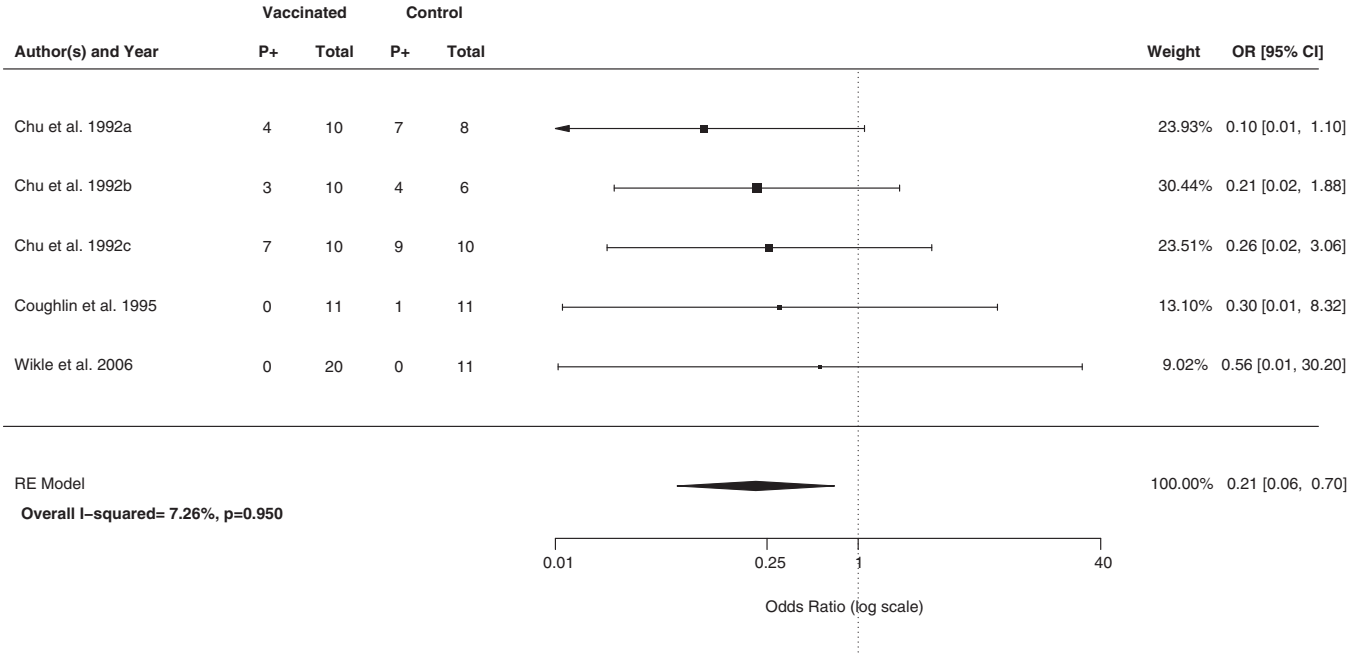

FIGURE 5 Forest plot of the odds ratio and 95% confidence interval (CI) for incident cases of pyrexia in dogs (P+) vaccinated for Lyme disease compared to control dogs in experimental studies. Horizontal solid lines represent 95% CIs. The vertical dotted line symbolizes the null value. A multilevel random effects model was used

publication bias in 3 of 4 meta-analyses because of a limited number of studies included in each meta-analysis.

In our definition of critical outcomes, 5 major clinical signs were identified as separate outcomes; this definition was chosen to provide more specific information regarding clinical signs. This resulted in the exclusion of studies that grouped all clinical signs together. However, only 1 study (observational) grouped all clinical signs together, and this had no impact on our ability to perform a meta-analysis for critical outcomes in observational studies, because this was also the only study to assess clinical signs among observational studies.

In this review, critical outcomes were extracted as a dichotomous outcome (ie, presence vs absence), thus clinical signs in different dogs were considered equivalent, which is an oversimplification and results in a loss of detail on the severity and duration of clinical signs. Although incorporating the severity and duration of clinical signs would have been ideal, the reporting of details surrounding clinical

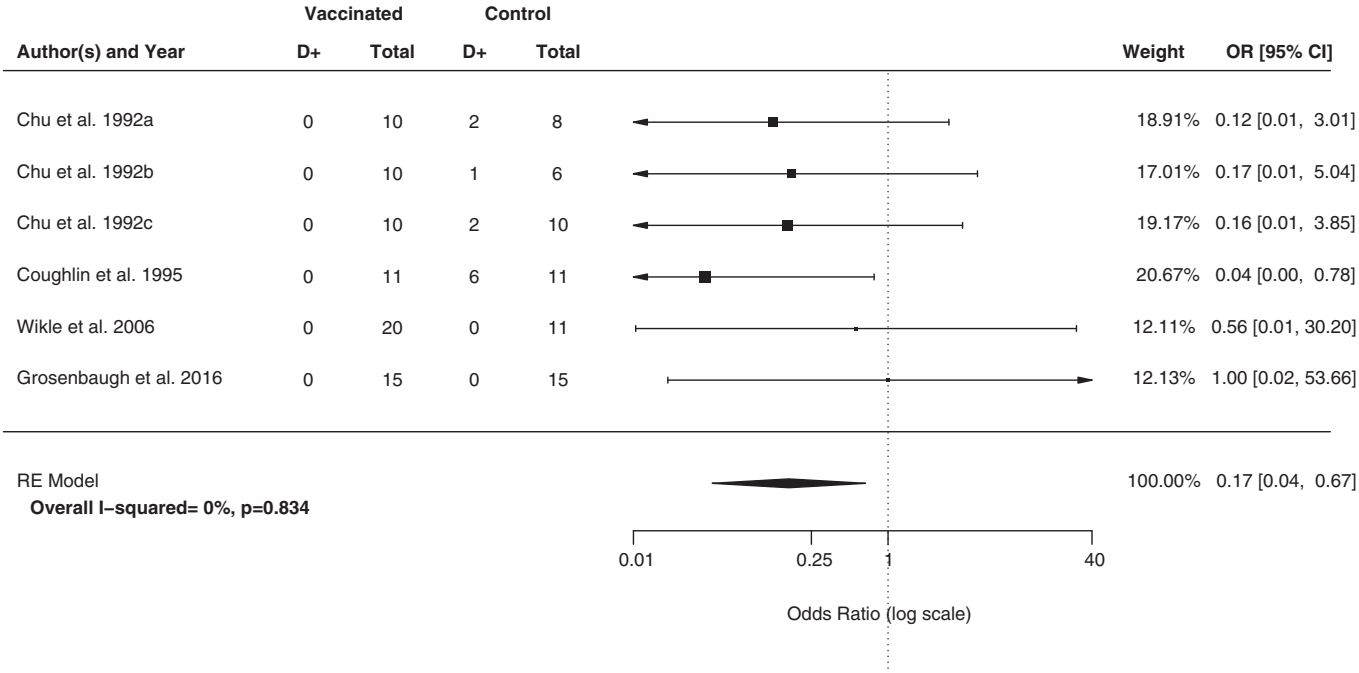

FIGURE 6 Forest plot of the odds ratio and 95% confidence interval (CI) for incident cases of depression in dogs (D+) vaccinated for Lyme disease compared to control dogs in experimental studies. Horizontal solid lines represent 95% CIs. The vertical dotted line symbolizes the null value. A multilevel random effects model was used

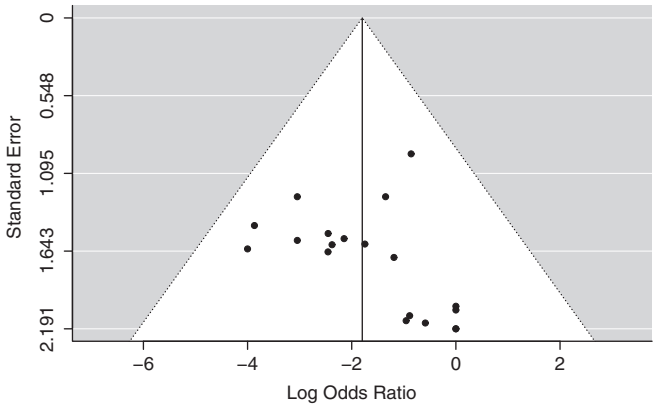

FIGURE 7 Funnel plot of the meta-analysis of published studies for the lameness outcome in experimental studies. Each plotted point represents the standard error and log odds ratio for unique cases of lameness in dogs vaccinated for Lyme disease compared to control dogs. The white triangle represents the region where 95% of the data points would lie in the absence of a publication bias

signs in the literature might have been a limiting factor. Many of the studies reported the number of episodes of a given clinical sign, but no definition was provided for what was considered an episode; it would be an error to assume all episodes are equivalent between different studies and then combine them in a meta-analysis. An additional limitation in our study is related to the lack of ranking or priority of importance of clinical signs, thus all were considered equivalent.

Finally, several changes were made to the protocol during the performance of the review. Most of these changes were related to outcomes extracted from experimental studies, after it was determined that no single gold standard for determining *B. burgdorferi* infection status in dogs exists.[18] Unfortunately, we were unable to perform planned subgroup analyses of commercially available vaccines, which would have provided the most clinically relevant information to practicing veterinarians. As previously discussed, linking current commercial availability with commercial availability at the time of publication was deemed necessary and would likely not be possible because of proprietary rights of vaccine companies.

5 | CONCLUSIONS

This review suggests that dogs vaccinated for Lyme disease have a lower odds of developing clinical signs than unvaccinated dogs, based on experimental studies with deliberate disease exposure. However, there were a number of limitations with regard to these studies and included small sample sizes, potential bias related to random sequence generation and blinding, and an unverified assumption of exposure of all dogs to infected ticks. The authors acknowledge that there are many challenges associated with studying Lyme disease in dogs, from a lack of reproducible disease model and gold standard method to determine infection status, to the low frequency of clinical signs in infected dogs.[6,18] Ideally, a meta-analysis would have been performed to assess vaccine efficacy in field conditions; however, we were unable to perform a meta-analysis for observational studies because only 2 of 3 eligible studies provided raw data for noncritical outcomes (seroconversion). No experimental field trials were identified by our study, highlighting a major gap in the literature on this topic. In addition to improvements in study design, future studies should focus on larger sample sizes in field conditions to provide the most relevant information for clinical practice.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antibiotics.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Nadine A. Vogt https://orcid.org/0000-0003-0156-4206
Jan M. Sargeant https://orcid.org/0000-0003-2420-1766

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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