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SYSTEMATIC REVIEW

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Clinical effect of probiotics in prevention or treatment of gastrointestinal disease in dogs: A systematic review

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Abstract

Background: Gastrointestinal diseases are prevalent in dogs, and probiotics could provide safe alternatives to conventional treatments.

Objective: To evaluate the clinical effects of probiotics when used in the prevention or treatment of gastrointestinal disease in dogs compared with no treatment, only symptomatic treatment, or conventional treatment.

Methods: A systematic review was preformed searching AGRICOLA, AGRIS, CAB Abstracts, Embase, Ovid MEDLINE, and Web of Science to identify articles published before April 1, 2017. Selection criteria were original research report, those published in peer reviewed journal, and study investigating in vivo use of probiotic for prevention or treatment of gastrointestinal disease in dogs. Studies were rated based on the level of evidence, and methodological quality was evaluated by the following variables: similarities between groups at baseline, risk of bias, and study group size.

Results: One hundred sixty-five studies were identified, of which 17 met the inclusion criteria—12 concerned acute gastrointestinal disease and 5 concerned chronic gastrointestinal disease. The level of evidence ranged between randomized controlled studies and crossover uncontrolled trials; estimated risk of bias was generally moderate to high; and sample sizes were small. Feces consistency was the most frequently evaluated clinical variable.

Conclusions and Clinical Importance: The current data point toward a very limited and possibly clinically unimportant effect for prevention or treatment of acute gastrointestinal disease. For chronic gastrointestinal disease, dietary intervention remains the major key in treatment, whereas probiotic supplement seems not to add significant improvement. However, studies were often underpowered, underscoring the need for future larger, preferably multicenter studies.

KEYWORDS

acute diarrhea, chronic diarrhea, stress diarrhea, synbiotic

Abbreviations: CCECAI, canine chronic enteropathy clinical activity index; cfu, colony forming unit; CIBDAI, canine inflammatory bowel disease activity index; EFSA, European Food Safety Authority; FS, fecal score; IBD, inflammatory bowel disease; LOE, level of evidence; RCT's, randomized controlled trials.

1 | INTRODUCTION

Gastrointestinal diseases are common in dogs[1,2] and a high percentage of dogs presenting with diarrhea receive probiotics as the only or as a supplemental treatment.[3] In addition, probiotics have received increasing scientific attention over the last decade.

The original observation of a possible positive role played by selected bacteria is attributed to Elie Metchnikoff, the Russian born Nobel Prize recipient. He suggested that "The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes."[4] Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host."[5,6] Probiotics can contain either a single strain or a combination of strains and might be combined with a prebiotic.

Prebiotics are defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of 1 or a limited number of bacteria in the colon."[7] Products containing both probiotic and prebiotic are termed synbiotic. Probiotics have primarily been used in human medicine in the treatment of gastrointestinal diseases.[6,8] Based on several systematic reviews and meta-analyses in human research, there is mounting evidence for a positive effect of probiotics in patients with antibiotic-associated-diarrhea,[9-17] irritable bowel syndrome,[15,18-22] and necrotizing enterocolitis.[23-27]

In human medicine, species and strains of the genus *Lactobacillus* and *Bifidobacterium* are the most commonly used probiotics, but strains belonging to the genus *Streptococcus*, *Bacillus*, *Propionibacterium*, *Escherichia*, and *Enterococcus* as well as the yeast *Saccharomyces* are also used as probiotics.[9-27] To date, the European Food Safety Authority (EFSA) has examined 6 bacterial strains (*Enterococcus faecium* NCIMB 10415 (4b1705), *E. faecium* NCIMB 10415 (4b1707), *Lactobacillus acidophilus* DSM 13241 25, *Bifidobacterium* sp. *Animalis*, *Bacillus subtilis* C3102 (4b1820), and *L. acidophilus* D2/CSL (4b1715)) for their safety and efficacy as probiotics or feed additives in dogs.[28-30] Currently, the 2 *E. faecium* strains and the *B. subtilis* strain are approved for use as feed additives in dogs.[31] Recently, *L. acidophilus* D2/CSL (4b1715) has also been evaluated to be safe for use in dogs and cats, with a potential to reduce the moisture of feces of dogs and cats receiving the additive at 5×10^9 colony forming unit (cfu)/kg feed according to EFSA.[30] The objective of the current systematic review was to evaluate the evidence concerning the clinical effects (development of vomitus or diarrhea, duration of diarrhea, feces consistency, defecation frequency, hospitalization duration, or case fatality) of probiotics when implemented in the prevention or treatment of gastrointestinal disease in dogs.

2 | METHODS

The reporting in this systematic review was according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[32] However, summary and synthesis for the meta-analysis data as well as additional analysis were not performed because of the low number of studies and large variations in probiotic strains investigated as well as reported outcomes. Methods of the analysis and inclusion criteria were specified in advance and documented in a protocol (dated March 20, 2017, not published, but available on request) before data extraction. The detailed protocol was developed and approved in relation to the initiation of a postgraduate master's thesis project finalizing the Master of Companion Animal Sciences specialization in Internal Medicine, University of Copenhagen, Denmark. The PICO method was used to formulate the research questions and guide the search strategy.[32] The research question was: "What is the evidence concerning clinical effects (propensity to develop diarrhea under stressful conditions, daily defecation frequency, stool consistency, duration of vomiting or diarrhea, hospitalization length and case fatality) of probiotics, when implemented in the prevention or treatment of signs of gastrointestinal disease in dogs?"

2.1 | Eligibility criteria

2.1.1 | Types of studies

Randomized clinical trials, cohort studies, and case reports published in peer-reviewed journals studying the use of probiotics for prevention or treatment of signs of gastrointestinal disease in dogs were used. If the abstract was in English, no language restriction was made and no publication date restriction was made.

2.1.2 | Types of participants

Dogs of any age, at risk of developing signs of gastrointestinal disease because of stress or parasitism or that had been diagnosed with any acute or chronic gastrointestinal disease, were selected. Both studies on kennel dogs and privately owned dogs were considered.

2.1.3 | Types of intervention

Trials comparing the clinical effects of in vivo use of probiotics or synbiotics as the only or as a supplemental intervention in the prevention or treatment of signs of gastrointestinal disease in dogs were considered. All probiotic species and strains as well as all probiotic doses were considered. No discrimination was made between vehicle(s) used in different studies included in the analysis.

2.1.4 | Types of comparison/control

Dogs with similar health/disease status not treated with probiotics or synbiotics were used as controls.

2.1.5 | Types of outcome

Primary outcome measures were the clinical effect of probiotics on signs of gastrointestinal disease evaluated by the following variables: attitude/activity, appetite, vomiting frequency, feces consistency, daily defecation frequency, weight change, hospitalization duration, case fatality, as well as number of dogs developing diarrhea because of

stress or parasitism. In addition, clinical indices of severity of canine chronic gastroenteritis such as the canine inflammatory bowel disease (IBD) activity index (CIBDAI)[33] and the canine chronic enteropathy clinical activity index (CCECAI)[34] were included.

2.2 | Information sources

Studies were identified by searching electronic databases from March 22 to April 1, 2017. This search was applied to Agricola (1970-present), Agris (1975-present), CAB Abstracts (1910-present), Embase (1974-present), MEDLINE (1946-present), and Web of Science (1945-present). No limits were applied to publication year or language, other than a requirement that the abstract should be in English.

2.3 | Search

We used the following search terms to search the electronic databases: Dog*; canine; gastro*; intestinal*; enteritis; hemorrhagic gastroen*; inflammatory bowel disease; IBD; Inflammatory bowel syndrome; IBS; dysbios*; small intestinal bacterial overgrowth; SIBO; protein losing enteropat*; PLE; helicobact*; colitis; parasite*; giardia*; viral; virus; probiotic*; synbiotic*; lactobacill*; bifidobacter*; eschericia; coli; saccharomyc*; yeast; fung*; streptococc*; bacill*; proprionibact*; VSL*. Web of Science was searched separately using the search terms: dog*; canine; probiotic* and synbiotic*.

Search strategy: MEDLINE (OVID)

1. dog*
2. canine
3. 1 or 2
4. gastro*
5. intestinal*
6. enteritis
7. hemorrhagic gastroen*
8. inflammatory bowel disease
9. IBD
10. inflammatory bowel syndrome
11. IBS
12. dysbios*
13. small intestinal bacterial overgrowth
14. SIBO
15. Protein losing enteropat*
16. PLE
17. helicobact*
18. colitis
19. parasite*
20. giardia*
21. viral*
22. virus*
23. 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22
24. 3 and 23
25. probiotic*
26. symbiotic*
27. 25 or 26
28. 3 and 23 and 27
29. lactobacill*
30. bifidobacter*
31. escherichia
32. coli
33. saccharomyc*
34. yeast
35. fung*
36. streptococc*
37. bacill*
38. proprionibact*
39. VSL*
40. 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39
41. 3 and 23 and 27 and 40

2.4 | Study selection

Eligibility assessment was performed by 1 author (A.P.J.) based on titles and abstracts in an unblinded standardized manner. By manually screening references of retrieved papers as well as papers reviewing the use of probiotics in dogs from 2010 and onward, eligible studies missed by the electronic search was identified. Subsequently, duplicates were manually removed. Studies fulfilling the inclusion criteria and those in which fulfillment of the criteria could not be determined from the abstract were retrieved as full texts. Papers in languages other than English were translated into Danish using Google Translate. If in doubt of eligibility, the abstract/paper was discussed with the coauthor (C.R.B.) and disagreements between reviewers were resolved by consensus.

2.5 | Data collection process

Based on the Cochrane Consumers and Communication Review Groups's data extraction template,[35] a data extraction sheet was developed. Ten randomly selected studies covering different study designs were used to test the developed sheet and make final adjustments. Because treatment and prognosis of acute and chronic gastrointestinal disease differ, studies were categorized to be either acute or chronic gastrointestinal disease. One reviewer (A.P.J.) extracted the data from included studies and the second author checked the extracted data. Disagreement was resolved by discussion between the 2 authors, leading to a consensus between the 2.

2.6 | Data Item

Information was extracted from each included trial on (1) the characteristics of dog populations (including whether they were kennel dogs or privately owned dogs, age, breed, indication for testing probiotic, and diagnosis); (2) the type of intervention (including type, dose, duration, and frequency of the probiotic; versus placebo or symptomatic

or other treatment); and (3) the type of clinical outcome measures (including the dogs attitude/level of activity, appetite, presence of vomiting, fecal consistency, presence of blood or mucus in the feces, defecation frequency, and weight loss). For dogs diagnosed with chronic gastrointestinal disease, clinical scoring indices (CIBDAI and CCECAI) were included, with the latter adding serum albumin levels, presence of ascites/peripheral edema, and owners' subjective assessment of pruritus to the above-mentioned clinical criteria.

2.7 | Risk of bias in individual studies

To ascertain the validity of eligible studies, both authors assessed all included trials independently. To obtain enough studies for the review, it was decided to include not only randomized studies, but also cohort and case-control studies as well as case series. To determine the strengths of the individual study designs of each study, eligible studies were graded for level of evidence (LOE) on a scale of I to IV and with a subclassification of LOE II to 1, 2, and 3 according to the pyramid of evidence described by Harris and Turner.[36] Studies categorized as LOE I included evidence obtained from a systematic review (or meta-analysis) of all relevant randomized controlled trials (RCTs). Studies categorized as LOE II included evidence obtained from at least 1 randomized controlled trial. Studies categorized as LOE III included (1) evidence obtained from pseudo-RCT (alternate allocation or some other method); (2) evidence obtained from comparative studies (including systematic reviews of such studies) with concurrent controls and allocation not randomized, cohort studies, case control studies, or interrupted time series with a control group; and (3) evidence obtained from comparative studies with historical controls, 2 or more single-arm studies or interrupted time series without a parallel control group. Finally, studies categorized as LOE IV included evidence obtained from case series, either post-test or pretest/post-test, and represented the lowest LOE.[36] The methodological qualities of the studies were individually evaluated by both authors, for the following 3 measures: similarities among groups at baseline, risk of bias, and size of study groups. Characterization of similarities between groups at baseline was evaluated to be good, fair, or poor based on the following criteria. It was considered good when the health/disease status of included animals in each group was evaluated and presented along with information on age, sex, and breed. Evaluation of health/disease status should include individual clinical examination of study subjects and a relevant thorough diagnostic workup (CBC and biochemistry, fecal examination, evaluation of severity of gastrointestinal signs, and, if indicated, characterization of disease severity by endoscopy). It was considered fair when the health/disease status of included animals in each group was assessed based on a clinical examination and evaluation of severity of signs of gastrointestinal disease and presented along with information on age, sex, and breed. It was considered poor when important information such as severity of signs of gastrointestinal disease, age, breed, or sex was lacking for each group. Risk of bias was evaluated using the Cochrane collaboration's tool for assessing risk of bias.[37] The studies were categorized as having high, moderate (unclear), or low risk of selection, performance, detection, attrition, reporting, and other bias. Finally, the overall combined risk of bias was defined using a predefined numerical system used in previous veterinary systematic reviews (Table S1A and S1B).[38-40] Sample size depends on the outcome variable, its prevalence, and the expected effect size between study groups. For a general assessment of the study size, groups were defined as good, moderate, small, and very small according to the criteria used in previous veterinary systematic reviews: >50 (good), 20-49 (moderate), 10-19 (small), and <10 (very small) animals per group.[38-40] If authors had conducted sample size calculations for specific variables, those calculations were included in the analysis. Furthermore, because results regarding clinical signs over a given study period often include repeated measures, it was evaluated whether adequate statistical analysis of repeated measures (eg, repeated measures analysis of variance or Friedmans test) was appropriately applied.

2.8 | Summary measures, synthesis of results, risk of bias across studies, and additional analyses

The primary outcome measure was the difference in severity of clinical signs of gastrointestinal disease between trial groups (number of feces samples with unacceptable consistency, defecation frequency, and CIBDAI of CCECAI). The evidence for or against efficacy of probiotic treatment on signs of acute or chronic gastrointestinal disease was graded as good, fair, or insufficient, according to the following criteria modified from Summers et al.[39] Evidence was graded according to multiple (good), at least 1 (fair), or no (insufficient) RCT's with a low or low-to-moderate estimated overall risk of bias. Evidence was also considered insufficient if (1) interpretation of results was hampered by low power of the studies or (2) results of outcome measures were conflicting. Evidence (good or fair) was considered to support probiotic treatment if efficacy was demonstrated. Alternatively, evidence (good or fair) was considered advising against probiotic treatment if lack of efficacy or adverse effects was demonstrated. Because of the heterogeneity in study designs, study populations, probiotic strains, and indications for intervention among the identified trials, calculations of relative risks or other statistical combinations of trials were not performed. Risks of bias across studies were evaluated based on a synthesis of the evaluation of the methodological quality of the individual studies, and additional analyses were not included.

2.9 | Ethical consideration

In 13 of the 17 included studies, study protocols were documented to have been approved by an ethical committee.

3 | RESULTS

3.1 | Study selection

After the literature search and selection according to the PRISMA flow diagram (Figure 1), 17 papers were included for further review: 12 studies related to the prevention or treatment of acute

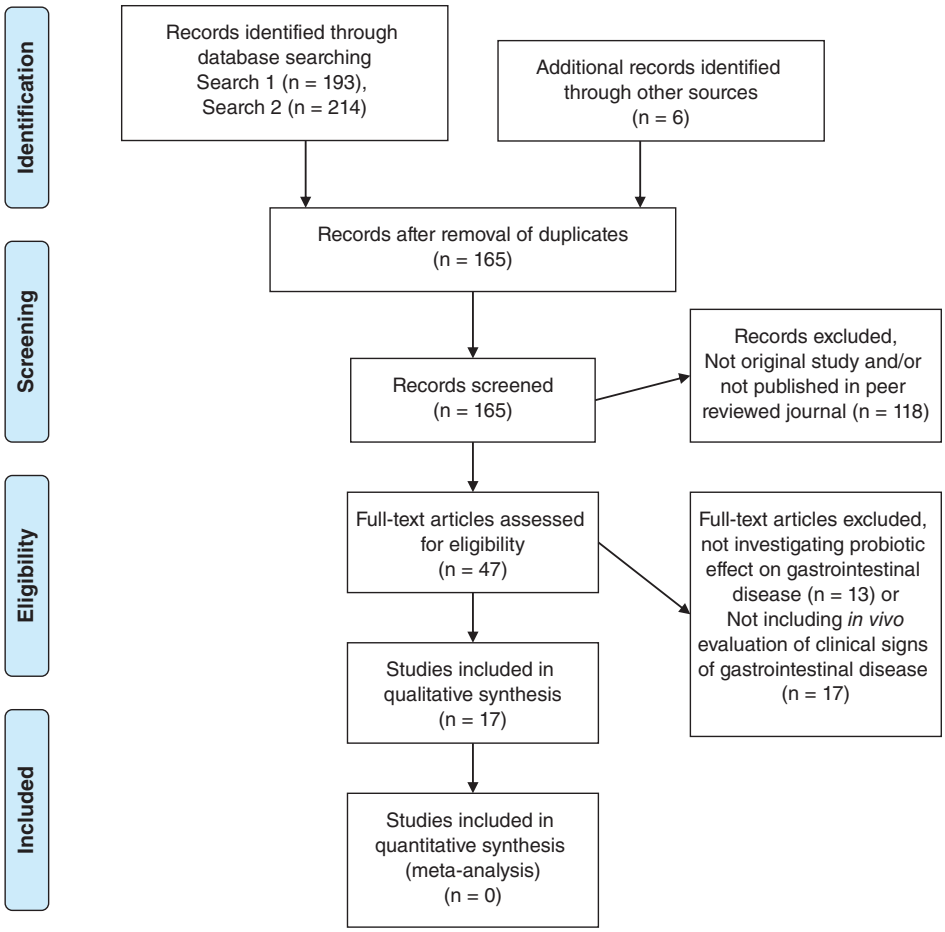

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection process. Papers were selected based on the following inclusion criteria: (1) original research report; (2) published in peer reviewed journal; (3) study relating to the use of probiotic evaluated for gastrointestinal disease in dogs; and (4) studies relating to in vivo use of probiotic for prevention or treatment of gastrointestinal signs in dogs. *Source*: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA statement. *PLoS Med.* 2009;6 (7):e1000097. doi:10.1371/journal.pmed.1000097

gastrointestinal disease (acute) and 5 studies concerned prevention or treatment of chronic gastrointestinal disease (chronic) (Tables 1 and 2).

3.2 | Study characteristics

Published studies investigating in vivo use of probiotics in prevention or treatment of gastrointestinal disease in dog were found to be sparse. Only 17 studies fulfilled inclusion criteria for the current systematic review.

Of the 17 studies included,[41-57] 7 (5 acute[41-45] and 2 chronic[53,54]) were categorized as RCTs. 1 (acute[46]) was categorized as LOE-III-1, pseudo-RCT because of the allocation by alternation, 7 (6 acute[47-52] and 1 chronic[55]) were classified as controlled clinical trials, 4 (3 acute[49,51,52] and 1 chronic[55]) of these claimed to be randomized, but randomization was not described, and finally, 2 (chronic[56,57]) were crossover uncontrolled trials (Tables 1 and 2).

The included studies involved study groups ranging between 6 and 399 dogs (Tables 1 and 2). For studies investigating prevention or treatment of signs of acute gastrointestinal disease, study participants were healthy racing sled dogs,[43] healthy dogs undergoing kenneling stress,[45,50,51] dogs with subclinical giardiasis,[41] dogs with lincomycin-induced diarrhea,[48] puppies diagnosed with hemorrhagic gastroenteritis,[46] adult dogs diagnosed with acute or intermittent gastroenteritis,[42,44,47,49] and dogs diagnosed with parvovirus diarrhea[52] (Table 1). For prevention or treatment of signs of chronic gastrointestinal disease, study participants were pet dogs diagnosed with idiopathic inflammatory bowel disease,[53] dogs with nonspecific dietary sensitivity[57] or food responsive diarrhea,[54,55] and dogs with tylosin-responsive diarrhea[56] (Table 2).

Specification of bacterial species included in the probiotic was missing in 1 of the 17 studies included,[47] whereas specification of bacterial strain(s) used was provided in 11 of 17 included studies. Only 1 probiotic species was used in 10 of the studies: 4 studies used *E. faecium*,[41,45,50,53] 2 studies used *L. acidophilus*,[46,57] 2 studies used *Bifidobacterium animalis*,[49,51] 1 study used *Lactobacillus rhamnosus*,[56] and 1 study used *Saccharomyces boulardii*.[48] The remaining 6 studies in which information about bacterial species tested was provided used a combination of 2 or more probiotic species.[42-44,52,53,55] The daily dose of probiotic was mentioned in 15/17 studies. Twelve of these used doses between 10^8 and 10^{10} cfu/d.[41-45,49-56] One study tested different doses of probiotic[52] (Table 1).

The most widely used primary outcome measure was fecal consistency/fecal score (FS). Comparison of FS between studies was hampered by application of different fecal scoring systems, and the definition of abnormal versus normal feces consistency varied among studies. For some studies, no specification of abnormal versus normal fecal consistency was made. In some studies, mean fecal consistency score was reported over time as repeated measures, whereas other

TABLE 1 Summary of studies included for systematic review of clinical effects of probiotics when used for prevention or treatment of acute gastrointestinal disease in dogs

Study	Level of evidence, study design, and methodological quality	Study population	Probiotic intervention and comparator	Clinical effects (mean ± SD or [95% CI])	Statistical comparison (*P* value, HR)
Simpson et al[41]	LOE II Randomized, placebo-controlled, crossover trial low to moderate estimated overall risk of bias Low number of poorly characterized dogs in each group	Adult kennel dogs with chronic naturally acquired giardiasis	Tx: *Enterococcus faecium* SF68 [NCIMB10415] probiotic powder ~5 × 10^8 cfu/d + controlled diet for 6 wk C: placebo powder (not specified) + controlled diet for 6 wk	Fecal score similar between groups All dogs remained subclinical	No statistical difference
Herstad et al[42]	LOE II Randomized double-blinded, placebo-controlled clinical trial Low estimated risk of bias Low number of fairly well-characterized dogs in each group	Hospitalized dogs diagnosed with acute gastroenteritis	Tx: ZooLac Propaste[a] C: Placebo (paste) Dosing until clinical signs resolved <10 kg BW: 1 mL TID 10-25 kg BW: 2 mL TID 25-50 kg BW: 3 mL TID	Days to last abnormal feces: Tx: 1.3 d [0.5-2.1] C: 2.2 d [1.3-3.1] Days to last abnormal feces or vomiting: Tx: 1.4 [0.5-2.4] C: 2.2 [1.4-3.1] Days to first normal feces: Tx: 2.9 [2.1-3.7] C: 3.4 [2.6-4.2] Duration of vomiting (d): Tx: 0.9 [0.5-1.3] C: 1.2 [0.2-2.2]	Significant difference between groups for days to last abnormal feces ($P < .04$) No statistical difference in days to last abnormal feces or vomiting ($P < .05$) or days to first normal feces ($P = .14$) or duration of vomiting ($P \geq .16$) Hazard ratio for abnormal feces after start of treatment: Tx versus C: 0.81 [0.57-1.09] and normal feces after start of treatment: Tx versus C: 0.90 [0.63-1.24] and vomiting after start of treatment: Tx versus C: 0.96 [0.63-1.48]
Gagné et al[43]	LOE II Randomized, blinded, placebo-controlled clinical trial, Low to moderate estimated risk of bias Very low number of poorly characterized dogs in each group	Healthy racing sled dogs Coincided with a presumed contagious diarrheal outbreak	Florentero synbiotic[b] Tx: 5 g Florentero SID + controlled diet for 6 wk C: 5 g placebo[c] SID + controlled diet for 6 wk	Total days of diarrhea per group: Tx: 6 C: 17	Significant difference in total days of diarrhea between groups ($P < .02$) and mean variation from baseline in week 5 ($P < .02$) Other weeks: NS
Gómez-Gallego et al[44]	LOE II Randomized, multicenter, double blinded, placebo-controlled clinical trial Low to moderate estimated risk of bias Low to moderate number of poorly characterized dogs in each group	Pet dogs (>6 m old) presenting with acute or intermittent mild to moderate non-hypo-proteinemic diarrhea	*Lactobacillus fermentum* VET 9A, *L. rhamnosus* VET 16A, and *L. plantarum* VET 14A (2×10^9 cfu/mL) Tx: 0.2 L sourmilk with 2×10^9 cfu/mL SID or 0.1 L sourmilk with 2×10^9 cfu/mL BID for 7 d C: Placebo (0.2 L water with titanium oxide) for 7 d	Change in mean feces consistency (1 = very hard feces, 5 = watery diarrhea) Day 7: Tx: −1.712 C: −1.279 Day 28: Difference Tx versus C: −0.362 Average difference day 1-28: Tx versus C: −0.271	No difference in change in mean feces consistency day 1-6 but statistical significant difference day 7 ($P = .04$) No difference in mean feces consistency day 28 ($P = .08$) but significant difference in average for day 1-28 ($P = .03$)

(Continues)

TABLE 1 (Continued)

Study	Level of evidence, study design, and methodological quality	Study population	Probiotic intervention and comparator	Clinical effects (mean ± SD or [95% CI])	Statistical comparison (*P* value, HR)
Rose et al[45]	LOE II Randomized, double-blinded, placebo-controlled clinical trial Low estimated risk of bias Good number of poorly characterized dogs in each group	Dogs entering an animal shelter	*Enterococcus faecium* NCIMB 10415 4b1707, ($2 * 10^9$ cfu/capsule) and Preplex (46.4 mg/capsule): Tx: 1 capsule SID C: placebo (1 × 180 mg maltodextrin) SID	Mean percentage of days scored as diarrhea during a dogs stay: Tx: 2.0% C: 3.2% Dogs with ≥1 day of diarrhea for the first 14 d: Tx: 18.8% C: 27.2% Dogs with ≥2 d of diarrhea for the first 14 d: Tx: 4.6% C: 8.0%	Significant difference in percentage of days scored as diarrhea during a dogs stay ($P = .008$) and dogs having ≥1 d of diarrhea for the first 14 d ($P = .02$) and dogs having ≥2 d of diarrhea for the first 14 d ($P = .03$)
De Camargo et al[46]	LOE III-1 Open label controlled clinical trial Moderate to high estimated risk of bias, moderate number of poorly characterized puppies in each group	Hospitalized puppies with hemorrhagic gastroenteritis (2-5 m) Symptomatic therapy: Ringers lactate with 10% potassium Metoclopramide Cimetidine Cephalexin	Enterolac (*Lactobacillus acidophilus DSM13241 and Baobab pulp, concentration not provided*) Tx: Symptomatic treatment + Enterolac <5 kg BW: ½ bag BID 5-10 kg BW: 1 bag BID >10 kg BW: 2 bags BID C: Symptomatic treatment	Hospitalization duration: Tx: 4.66 ± 2.65 d (1-15 d) C: 4.46 ± 2.68 d (1-10 d) Mortality rate: Tx: 37.5% C: 26%	No difference in hospitalization duration ($P = .7$) or in mortality rate ($P = .49$)
Kishan et al[47]	LOE III-2 Controlled clinical trial Moderate to high estimated risk of bias Very low number of poorly characterized dogs in each group	Canine enteritis caused by bacteria (*micrococci*, *staphylococci*, *streptococci*, *Klebsiella*, *Proteus*, *Clostridium*, *Salmonella or Shigella*)	Sporlac (lactobacillus spores; 150 million/1.8 g) Tx_1: Sporlac, 1.8 g BID for 3 d Tx_2: Chloranphenicol + streptomycin + Sporlac 1.8 g BID for 3 d C: Chloranphenicol + streptomycin for 3 d	Response to treatment Tx_1: 57.1% Tx_2: 71.4% C: 85.7%	No statistical evaluation of outcome
Aktas et al[48]	LOE III-2 Controlled trial Moderate to high estimated risk of bias Very low number of poorly characterized dogs in each group	Adult kennel dogs with lincomycin induced diarrhea (150 mg/kg/d IM until development of diarrhea (Tx_1 and C) or for 10 d (Tx_2) Controlled feeding	*Saccharomyces boulardii* Tx_1: 1000 mg *Saccharomyces boulardii*/d for 10 d when diarrhea developed Tx_2: 1000 mg *Saccharomyces boulardii*/d for 10 d together with lincomycin injections C: no probiotic	Percentage of dogs developing diarrhea: Tx_1: 87.5% Tx_2: 0% C: 70% Duration of diarrhea Tx_1: 2.9 ± 0.4 C: 6.5 ± 2.0	Percentage of dogs developing diarrhea was not statistically evaluated Significant difference in duration of diarrhea ($P < .05$)

(Continues)

TABLE 1 (Continued)

Study	Level of evidence, study design, and methodological quality	Study population	Probiotic intervention and comparator	Clinical effects (mean ± SD or [95% CI])	Statistical comparison (*P* value, HR)
Kelley et al[49]	LOE III-2 (Randomized) blinded placebo-controlled clinical trial Low to moderate estimated risk of bias Low number of poorly characterized dogs in each group	Young adult guide dogs diagnosed with acute idiopathic diarrhea	*Bifidobacterium animalis* AHC7[d] Tx: cocoa butter treats with 1×10^{10} cfu BID + controlled diet for 2 wk C: placebo (cocoa butter treats) + controlled diet for 2 wk	Days to resolution of diarrhea: Tx: 3.9 ± 2.3 C: 6.6 ± 2.7	Significant difference in days to resolution between groups ($P < .01$)
Bybee et al[50]	LOE III-2 Double blinded, placebo-controlled, crossover clinical trial Low to moderate estimated risk of bias Good number of poorly characterized dogs in each group	Dogs housed in an animal shelter (duration of stay 2-7 d, median duration 2-3 d)	Fortiflora *Enterococcus faecium* SF68-2.1 × 10^9 cfu/g 3 periods of 4 wk: Period 1: controlled diet only in both rooms Period 2: controlled diet and probiotic in room 1, controlled diet and placebo (palatability enhancer) in room 2 Period 3: controlled diet and placebo in room 1, controlled diet and probiotic in room 2	Percentage of dogs having ≥1 episode of diarrhea Tx: 9.8% C: 12.5% Number of dogs having diarrhea ≥2 d Tx: 1/102 C: 1/80	No difference in percentage of dogs having ≥1 episode of diarrhea ($P > .05$) or number of dogs having diarrhea ≥2 d ($P > .05$)
Kelley et al[51]	LOE III-2 (Randomized) multicenter, placebo-controlled clinical trial Moderate to high estimated risk of bias moderate number of poorly characterized dogs in each group	Healthy young dogs undergoing kenneling stress	*Bifidobacterium animalis* AHC7 Tx I: 1.5 × 10^7 cfu/d Tx II: 1.5 × 10^8 cfu/d Tx III: 1.5 × 10^9 cfu/d C: placebo 0 cfu/d Probiotic or placebo given SID for 5 wk together with controlled diet before and 3 wk after relocation	Fecal score (1-5) during relocation: Week 3: Tx I: 3.89 ± 0.07 Tx II: 3.87 ± 0.07 Tx III: 3.92 ± 0.07 C: 3.67 ± 0.07 Average fecal score week 1-3: Tx I: 3.87 ± 0.05 Tx II: 3.91 ± 0.05 Tx III: 3.94 ± 0.05 C: 3.75 ± 0.07 Mean number of unacceptable feces/3 wk of kenneling: LS means ± SEM Tx I: 1.5 ± 0.6 Tx II: 1.2 ± 0.7 Tx III: 1.2 ± 0.7 C: 3.1 ± 0.6 Percentage of dogs producing unacceptable feces during 3 wk of kenneling	No difference between probiotic treatment (Tx I-III) and control in fecal score week 1–2 Significant difference week 3 ($P < .05$) and average score, week 1-3 ($P < .03$) No difference in mean number of unacceptable feces during 3 wk of relocation ($P < .10$) or percentage of dogs producing unacceptable feces during 3 wk of relocation ($P < .15$)

(Continues)

TABLE 1 (Continued)

Study	Level of evidence, study design, and methodological quality	Study population	Probiotic intervention and comparator	Clinical effects (mean ± SD or [95% CI])	Statistical comparison (*P* value, HR)
				Tx I: 7.6 ± 7.0 Tx II: 5.95 ± 3.6 Tx III: 8.75 ± 3.5 C: 16.4 ± 3.3	
Arslan et al[52]	LOE III-2 (Randomized) open label, controlled, clinical trial Moderate estimated risk of bias Very low number of poorly characterized dogs in each group	Young dogs with parvoviral enteritis	VSL#3[e] Tx: supportive and symptomatic treatment and VSL#3 (450×10^9 cfu) SID for 1-3 wk C: supportive and symptomatic treatment for 1-3 wk	Clinical score (0: no clinical signs, 1: slightly advanced, 2: moderately advanced, 3: severe stages) Day 0 Tx: 2.80 ± 0.13 C: 2.60 ± 0.16 Day 3 Tx: 1.20 ± 0.23 C: 1.90 ± 0.28 Day 5 Tx: 0.25 ± 0.30 C: 0.85 ± 0.46	Significant difference in clinical score between group day 3 and 5 ($P < .05$)

Abbreviations: BID, twice/day; C, control; CCECAI, canine chronic enteropathy clinical activity index; CI, confidence interval; CIBDAI, canine inflammatory bowel disease activity index; cfu, colony forming units; FOS, fructo-oligosaccharides; HR, hazard ratio; IBD, inflammatory bowel disease; LOE, level of evidence; NS, not significant; (randomized), study claimed to be randomized, but procedure not described; SID, once/day; TID, 3 times/day; Tx, probiotic treatment.

[a]*Lactobacillus acidophilus*, 1.35×10^9 cfu/mL; *Pediococcus acidilactici*, 2.85×10^9 cfu/mL; *Bacillus subtilis*, 2.82×10^9 cfu/mL; *Bacillus licheniformis*, 2.85×10^9 cfu/mL; *Lactobacillus farciminis*, 2.85×10^9 cfu/mL (Chem Vet A/S, Denmark).

[b]*Enterococcus faecium* SF68 (5.67×10^8 cfu/g), *Bacillus coagulans*, 3.75×10^7 cfu/g), *Lactobacillus acidophilus*, 7.2×10^8 cfu/g), fructooligosaccharides (400 mg/g), mannanoligosaccharides (80 mg/g), Vitamin B_1 (2,5 mg/g), Vitamin B_2 (0,8 mg/g), Vitamin B_3 (19,2 mg/g), Vitamin B_6 (0,8 mg/g), brewer's yeast (80 mg/g), soy lecithin (30 mg/g), magnesium stearate (10 mg/g), microcrystalline cellulose (266 mg/g), mono-and diaglyceraldehyde (30 mg/g), and silica dioxide (7 mg/g), Candioli Pharma, Rome, Italy.

[c]Brewer's yeast (190 mg/g), soy lecithin (71 mg/g), magnesium stearate (24 mg/g), microcrystalline cellulose (629 mg/g), mono-and diaglyceraldehyde (71 mg/g), and silica dioxide (16 mg/g),), Candioli Pharma.

[d]Iams Prostora, Procter & Gamble Pet Care.

[e]VSL#3 strains; *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii spp. bulgaricus*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, and *Streptococcus salivarus* spp. *thermophilus* (VSL Pharmaceuticals Inc, Gaithersburg, Maryland).

TABLE 2 Summary of studies included for systematic review of clinical effects of probiotics when used for prevention or treatment of chronic gastrointestinal disease in dogs

Study	Level of evidence, study design, and methodological quality	Study population	Probiotic intervention and comparator	Clinical effects (mean ± SD or median (range) or [95% CI])	Statistical comparison (*P* value, HR)
Rossi et al[53]	LOE II Randomized, open label, controlled clinical trial Low to moderate estimated risk of bias Low number of well-characterized dogs in each group	Pet dogs diagnosed with idiopathic inflammatory bowel disease	SIVOY[a] Tx: 112-225 10^9/10 kg BW/day C: prednisolone 1 mg/kg/d + metronidazole 20 mg/kg BID	CIBDAI score: Baseline: Tx: 7 (5-10) C: 9 (7-13) Day 90: Tx: 0 (0-2) C: 0 (0-3) Median time to clinical remission (d) Tx: 10.6 (5-15) C: 4.8 (2.5-7)	Significant difference in CIBDAI score between baseline and day 90 (Tx and C) ($P < .001$) Significant difference in CIBDAI score between groups at baseline ($P < .001$) But not at day 90 (NS) Significant difference in median time to clinical remission ($P = .001$)
Schmitz et al[54]	LOE II Randomized, blinded controlled clinical study Low estimated risk of bias Very low number of poorly characterized dogs in each group	Dogs with food responsive diarrhea	Synbiotic *D*-C[b] *Enterococcus faecium* NCIMB 10415 E1707 (10^9) + FOS + gum Arabic Tx: 1 × 10^9 cfu/d + hydrolyzed diet for 6 wk C: placebo (maltodextrin) + hydrolyzed diet for 6 wk	CCECAI score: Baseline: Tx: 4 (1-6) C: 5 (2-7) 2 wk: Tx + C: 2 (0-4) 6 wk: Tx + C: 2 (0-3)	CCECAI score: No difference in CCECAI score at baseline (NS) Significant difference in CCECAI score from baseline to week 2 and week 6 (Tx and C), ($P < .001$) No difference in CCECAI score between groups at week 2 or week 6 ($P = .72$)
Sauter et al[55]	LOE III-2 (Randomized) double-blinded, placebo-controlled clinical trial Low to moderate estimated risk of bias Low poorly characterized dogs in each group	Dogs with food responsive diarrhea	*Lactobacillus acidophilus* NCC2628 (10^{10} cfu/g) *Lactobacillus acidophilus* NCC2766 (10^{10} cfu/g) *Lactobacillus johnsonii* NCC2767 (10^{10} cfu/g) Tx: 1 g SID for 4 wk C: placebo (growth medium and fermentation products) SID for 4 wk	CIBDAI score median (range) Day 0: Tx: 7 (5-8) C: 5 (2-8) Day 28: Tx: 0 (0-5) C: 1 (0-3) Ratio: CIBDAI after treatment/CIBDAI before treatment Tx: 0.1 (−0.005 to 0.2) C: 0.3 (0.09-0.4)	No significant difference
Westermarck et al[56]	LOE III-3 Crossover clinical trial Moderate to high estimated risk of bias Very low number of poorly characterized dogs in the group	Dogs with tylosin responsive diarrhea	*Lactobacillus rhamnosus* (5 × 10^9 CFU/capsule) Tx: 1 capsule BID for up to 1 mo to prevent diarrhea development following cessation of tylosin C: no treatment following cessation of tylosin	Relapse of diarrhea after cessation of tylosin treatment Tx: 100% median 7 d (range, 3-26 d) C: 100% median 7 d (range, 3–26 d) Fecal score: Tx: 4.5 C: 4.75	No significant difference

(Continues)

TABLE 2 (Continued)

Study	Level of evidence, study design, and methodological quality	Study population	Probiotic intervention and comparator	Clinical effects (mean ± SD or median (range) or [95% CI])	Statistical comparison (*P* value, HR)
Pascher et al[57]	LOE III-3 Crossover clinical trial Moderate estimated risk of bias Very low number of fairly well-characterized dogs	German Shorthair Pointers with nonspecific dietary sensitivity	*Lactobacillus acidophilus* DSM 13241 28 wk: C_1: 12 wk diet alone Tx: 12 wk: diet + probiotic (6×10^6 cfu/g dry food) C_2: 4 wk diet alone	Fecal score: Only figure provided Defecation frequency: Only figure provided	Significant difference in fecal score in favor of Tx versus C_1 ($P < .05$) Significant difference in defecation frequency in favor of Tx versus C_1 ($P < 0.01$)

Abbreviations: C, control; CCECAI, canine chronic enteropathy clinical activity index; CI, confidence interval; CIBDAI, canine inflammatory bowel disease activity index; cfu, colony forming units; FOS, fructo-oligosaccharides; HR, hazard ratio; IBD, inflammatory bowel disease; LOE, level of evidence; NS, not significant; (randomized), study claimed to be randomized, but procedure not described; SID, once/day; BID, twice/day; TID, 3 times/day; Tx, probiotic treatment.

[a]VSL#3 strains; *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii spp. bulgaricus*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, and *Streptococcus salivarus* spp. *thermophilus* (VSL Pharmaceuticals Inc).

[b]*Enterococcus faecium* NCIMB 10415 E1707 (1×10^9 cfu), FOS and gum Arabic (Probiotix International/Protexin, Somerset, United Kingdom).

studies reported days/time to first normal feces, number of days or percentage of dogs with diarrhea, or number of days to relapse of diarrhea. Four studies used clinical scoring as the primary outcome (Tables 1 and 2).

Secondary outcome measures included defecation frequency, clinical improvement, appetite evaluation, frequency of vomiting, weight loss, hospitalization duration, and case fatality. Often these variables were reported to be evaluated, but results were seldom presented (Tables 1 and 2).

3.3 | Risk of bias within studies

A summary of "risk of bias" for each study is listed in Tables 1 and 2 and Table S1A and S1B. More recent studies were more likely to be categorized as having a low/moderate estimated risk of bias. For studies concerning prevention or treatment of signs of acute gastrointestinal disease, the estimated risk of bias was categorized as low for 2 studies,[42,45] low/moderate for 5 studies,[41,43,44,49,50] moderate for 1 study,[46] and moderate/high for 4 studies.[47,48,51,52] Study group size was very small (<10 dogs per group) in 4 studies,[43,47,48,52] small in 4 studies,[41,42,44,49] moderate in 2 studies,[45,51] and good in 2 studies.[45,50] Similarity at baseline was poorly characterized in 11 studies,[41,43-52] and a fair characterization was found in 1 study.[42] For studies concerning prevention or treatment of signs of chronic gastrointestinal disease, the estimated risk of bias was categorized as low for 1 study,[54] low/moderate for 2 studies,[53,55] moderate for 1 study,[57] and moderate/high for 1 study.[56] Study group size was very small (<10 dogs per group) in 3 studies[54,56,57] and small (10 < n < 20 dogs per group) in 2 studies.[53,55] Similarity at baseline was poorly characterized in 3 studies,[54-56] a fair[57] characterization was found in 1 study, and in 1 study it was well characterized.[53] Sample size evaluation was done by power calculation in 1 RCT concerning clinical severity assessed by CCECAI[54] and 1 controlled trial, evaluating if probiotic could affect the risk of experiencing at least 1 episode of diarrhea in dogs diagnosed with nonspecific food sensitivity.[45] In the study using the CCECAI, calculation was based on a type I error of 0.05 and a type II error of 0.1, which corresponds to a power of 90%. The necessary number of animals in each group (treatment versus placebo) was estimated to be n = 11. In the study using the criteria of at least 1 episode of diarrhea in relation to prevention of kenneling diarrhea, power calculation estimated the sample size needed to be 582 dogs (Table S1A and S1B). Calculations were based on a change in incidence of diarrhea from 30% for the placebo-treated dogs to 20% for the probiotic-treated dogs with a significance level of .05 and power of 80%. In comparison, none of the studies on signs of chronic gastrointestinal disease had sample sizes ≥11. Eight studies on signs of acute gastrointestinal disease had sample size ≥11 and only 1 had sample size ≥582[45] (Table S1B). The dropout rate varied between 0 and 33% for studies on signs of acute gastrointestinal disease and 0 and 73% for signs of chronic gastrointestinal disease. One study did not include statistical analyses.[47] Statistical analyses accounted for repeated measures in 5 studies,[41,44,48,52,54] 1 study included dog as a random effect to account for repeated measures,[53] whereas the rest of the studies converted FSs into categories such as number of days with diarrhea, number of days to last abnormal feces without taking the effect of the individual dog into account in the statistical analysis.

3.4 | Results of individual studies and synthesis of results

For an overview of results of individual studies, please refer to Tables 1 and 2 and Table S1A and S1B. For studies evaluating effect on prevention of kenneling diarrhea, 1 study found no difference in number of dogs having ≥1 or ≥2 episodes of diarrhea,[50] 1 study found a small but significant improvement in FS during week 3 of kenneling,[51] and 1 study, representing the largest number of participants, found a slight reduction in mean percentage of days scored as diarrhea and number of dogs experiencing ≥1 or ≥2 days of diarrhea with probiotic treatment.[45] For studies evaluating the effect of probiotic in dogs with acute gastrointestinal disease (acute or intermittent hemorrhagic gastroenteritis including parvovirus diarrhea), 1 study did not make a statistical evaluation,[47] 1 study found that probiotic treatment reduced the number of days to resolution of diarrhea,[49] while 1 study found a slight reduction in number of days to last abnormal feces, but no difference in days to last abnormal feces or vomiting, number of days to first normal feces or duration of vomiting.[49] One study found a significant difference in change in mean feces consistency on day 7 and on average throughout the study period in favor of probiotics, but not on days 1-6 or 28 of the study.[44] One study used a clinical scoring system evaluating body temperature, degree of dehydration, heart and respiratory rates, capillary refill time, colors of the mucosal membranes, sizes of the submandibular lymph nodes, appetite, frequency of vomiting, diarrhea, dullness, and fecal consistency.[52] The clinical score was significantly improved in favor of the group treated with probiotic on days 3 and 5 after start of treatment (Table 1). A significant difference in days of diarrhea in favor of probiotic was found in healthy sled dogs,[43] but this finding might have been confounded by a contagious outbreak in the kennel, probiotic significantly reduced duration of diarrhea in lincomycin-induced diarrhea,[48] whereas no effect was observed in chronically Giardia sp. infected dogs,[41] both groups remained subclinical. Regarding the studies on treatment of dogs with chronic diarrhea, there was an overall improvement over time for trial subjects, but no additional effect of probiotic treatment was identified based on CECCAI[54] and CIBDAI scoring[53,55] or time to relapse of diarrhea.[56] One study found a significant improvement in FS and defecation frequency in dogs with nonspecific dietary sensitivity after 12 weeks of probiotic supplementation compared to a prior 12 week feeding with only a control diet.[57] However, there was no difference between scores and defecation frequency when comparing the probiotic period with the 4 week period after the probiotic period, where dogs were only controlled feed.[57]

Other clinical variables evaluated separately were feces frequency, vomiting, appetite, weight loss, hospitalization deration, and case fatality (Table S2). Vomiting was evaluated in 1 study,[42] with no statistically significant difference observed between study groups. One study reported a significant improvement in appetite and a decreasing frequency of vomiting, when evaluated together.[53] This study also evaluated weight loss, and found weight loss to be less for the probiotic-treated group. This difference was statistically significant after 7 days of treatment, but not at the follow-up visit 28 days after treatment. Two studies evaluated case fatality.[46,52] Both studies evaluated treatment for parvovirus related gastrointestinal clinical signs. Both studies found no significant difference in mortality between treatment groups. One study evaluated hospitalization and found no significant difference with probiotic treatment.[46]

Only 1 study in this analysis compared different probiotic dosages,[51] but still the difference in dosages were relatively narrow 10^7, 10^8, and 10^9 cfu/d. In this study, evaluating prevention of signs of stress-related diarrhea during relocation, an overall statistically significant difference in favor of probiotic was seen for mean FS and for frequency of abnormal feces during the first 3 weeks after relocation, but no difference was observed between probiotic dosages. Only when weeks were evaluated separately, dogs supplemented with 10^8 and 10^9 cfu/d had significantly fewer abnormal feces during the first week after relocation. Furthermore, even though the difference in FSs between control group and treatment groups were statistically significant, the clinical relevance is questionable. FS was evaluated on a scale from 1 to 5 of which 1 was categorized as liquid and 5 as extremely dry. In the control group, FS was estimated to 3.75 ± 0.04 compared to 3.87 ± 0.05 for 10^7 cfu/d, 3.91 ± 0.05 for 10^8 cfu/d and 3.94 ± 0.05 for 10^9 cfu/d.

3.5 | Risk of bias across studies

When assessing the risk of bias that could affect the cumulative evidence, selective reporting bias was an issue for several studies, especially relating to acute gastrointestinal disease. Four studies on acute gastrointestinal disease[41,43,45,49] and 4 studies on chronic gastrointestinal disease[53-55,57] were assessed to have low risk of selection bias; 3 studies on acute gastrointestinal disease[42,46,47] and 1 study on chronic gastrointestinal disease[56] were assessed to have moderate risk of selection bias whereas 4 studies on acute gastrointestinal disease[44,48,50,51] were assessed to have high risk of bias (Table S1A and S1B). Poor characterization of study participants and lacking information about dropouts were the main causes for being evaluated to be at risk of bias. Furthermore, 12 of the studies reported industry involvement (Table S1A and S1B); with such a high degree of industry involvement, there is a risk of publication bias as the incentive to publish studies showing no effect of probiotics could be low.

3.6 | Additional analyses

Because of the large heterogeneity in included studies, with regard to indication, study designs, and choice of probiotic treatment, further analyses were not performed.

4 | DISCUSSION

4.1 | Summary of evidence

The primary aim of the current systematic review was to identify and assess the available evidence related to the effect of probiotics in

preventing or treating signs of gastrointestinal disease in vivo. Overall, for the prevention or treatment of acute gastrointestinal disease, the evidence is not sufficiently robust to determine the effectiveness of probiotics in reducing clinical signs (FS, defecation frequency, and days with diarrhea). Some studies showed a slightly reduced number of days with diarrhea or improved FS in relation to kenneling and acute diarrhea, but the clinical relevance remains questionable. One study that evaluated prevention of diarrhea in kennel dogs showed an improvement in feces consistency[45] and this study was evaluated to have a low estimated risk of bias and a high number of dogs in each group. However, the probiotic product used in that study also contained a prebiotic and the effect proven could therefore not be exclusively linked to the probiotic. Most studies relating to signs of acute gastrointestinal disease were evaluated to have a moderate to high estimated risk of bias, with a very small or small number of dogs and poorly reported baseline characteristics of study groups. For the prevention or treatment of clinical signs of chronic gastrointestinal disease, the evidence was primarily against an effect of probiotic supplementation on clinical variables and results indicated that dietary intervention is more important than probiotic supplementation, but all of these studies included a very small or small number of dogs, significantly adversely affecting statistical of power. One of the studies compared probiotic (VSL#3) as a single treatment with a combination treatment with prednisone and metronidazole in dogs with IBD. Both groups improved significantly and the proportion of dogs achieving remission was similar but it took longer to achieve remission with probiotics compared with conventional treatment.[53] There were no indications that probiotics affected hospitalization or case fatality in relation to parvovirus diarrhea,[46,52] but again because of the moderate to high estimated risk of bias in these studies, no conclusion could be made.

4.2 | Limitations

Sample size calculation depends on the initial frequency of disease/clinical signs and the expected improvement in frequency of a specific variable after treatment. As mentioned before, abnormal feces consistency (diarrhea) was the most widely used clinical variable evaluated in the included studies. Expected frequency of diarrhea was higher in studies evaluating treatment of diarrhea compared with studies evaluating prevention of diarrhea. Considering a type I error of 0.05 and type II error of 0.2, the sample sizes needed were estimated to be between 11 and 100, with a frequency of diarrhea decreasing from 100% to between 50 and 90% when probiotics were used.[58] When estimating sample sizes in studies preventing diarrhea, a significantly higher sample size would be needed than was used in these studies. Sample sizes needed were estimated to be between 81 and 199 dogs based on the assumptions that frequencies of diarrhea were estimated to be present in 20%-40% of the dogs in the untreated group and that probiotic treatment would decrease this frequency by up to 50%.[58]

In the 12 studies concerning treatment of diarrhea, sample sizes were ≥11 in only 5 studies and of these 5 studies, only 1 study had sample sizes ≥30. A dropout between 14 and 33% of initially included dogs was seen in 3 of the 5 studies with sample sizes ≥11, including the study with sample sizes >30. Sample sizes were ≥81 in only 1 of the 5 studies on preventative effects. Additionally, a reduction in frequency of diarrhea by 50% when implementing probiotics as preventive treatment could be too optimistic. In human studies evaluating the use of probiotics to reduce diarrhea frequency, a reduction of up to 25% has most commonly been reported.[13,14,16,19,25] It is thus likely that review conclusions are biased because of the studies being underpowered.

Effects of probiotics are strain specific, and documented effects of a product containing probiotics depend on the strain used. In this study, comparison between studies were difficult considering the diverse use of probiotic species and for some studies the lack of specification of strain used. Comparison between studies could be further confounded considering that the vehicle used for a certain probiotic product could also influence the probiotic effect.

Considering the optimal dose of probiotics, the official definition of a probiotic includes the administration of an "adequate amount" in order to obtain a health benefit. Any further specification of "an adequate" dose has not been made.[6,59] The effective dose of probiotic is influenced by multiple variables, including health endpoint, the specific probiotic used, delivery vehicle, and route of administration. One review has examined dose recommendations for probiotics used in humans.[60] The study concluded that no homogeneous picture could be observed, when dose and efficacy of probiotics were evaluated. Most studies in humans use doses of 10^8-10^{11} cfu/d.[9-27] Negative or positive effects of probiotics outside this relatively narrow dose range are not available. Products available on the Danish market containing probiotics recommend the same dose range and most studies in the current review use doses of 10^8-10^{10} cfu/d. The lack of diversity in doses makes dosage recommendation for canine gastrointestinal diseases impossible.

For all probiotics, it is important for a successful treatment that there is consistency in regard to label information of the probiotic strain(s) and the dose included in the probiotic product. Unfortunately, a study that evaluated 25 commercially available products used for animals in Canada documented poor quality control of probiotic products for the veterinary market.[61] Twenty-one of 25 products had label information regarding specific bacterial names. Information was primarily listed for genus and species, whereas none of the labels contained information about bacterial strain. Fifteen of 25 products had label information regarding viable growth and only 4 of these 15 products actually had a viable growth equal to or higher than label information claimed. In the current review, the included probiotic products could not be tested for consistency with claim and it is possible that products evaluated did not meet expected specifications.

Finally, the current review focused on clinical effects of probiotics and no other possible health effects. To the pet and the caregiver, an increased defecation frequency is stressful. It increases the need for walks both day and night as well as risk of fecal soiling indoors. Furthermore, passing of normal feces is often a criterion for discharging a

dog hospitalized with acute diarrhea. Several studies have investigated other possible health benefits of probiotic treatment. This includes immune and microbiome modulation as well as possible modulation of virus or parasite shedding. In 1 study, fecal IgA and canine distemper virus vaccine-specific circulating IgG and IgA were higher in the group receiving probiotic (*E. faecium*).[62] In another study, lymphocyte proliferation and rabies titer were significantly higher in sled dogs given probiotic (*E. faecium*) 4 weeks after the first vaccination.[63] Another study showed an increase in the T-cell markers: FoxP3+ and TGF-β+ in dogs treated with probiotic (VSL#3) as well as what was considered a more balanced effect on the microbiome compared with conventional treatment for IBD.[53]

5 | CONCLUSION

Based on the current review of 17 studies evaluating clinical effect of probiotic supplementation to prevent or treat clinical signs of gastrointestinal disease, the evidence points toward a limited and possibly clinically unimportant effect for prevention or treatment of acute gastrointestinal disease. For chronic gastrointestinal disease, dietary intervention remains the major key in treatment, whereas probiotic supplement seems not to add significant improvement. However, this conclusion is based on a limited number of studies, with a wide methodological diversity, and mainly low sample sizes. There is a high risk that most of the studies evaluated in the current review were severely underpowered especially taking into consideration that baseline characteristics of study groups were generally very poorly documented.

To achieve better evidence for or against the use of probiotics in gastrointestinal disease in dogs, there is a need for much larger randomized controlled studies, preferably multicenter, based on rigid protocols focusing on securing and reporting procedures and baseline characteristics in much more detail for future evaluations.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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.

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SUPPORTING INFORMATION

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

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