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REVIEW

Immunology

Probiotics as an adjunct in the treatment of canine atopic dermatitis: a systematic review and meta-analysis of *in vivo* studies in dogs

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ABSTRACT. Probiotics have been suggested as a treatment for canine atopic dermatitis, a form of dermatopathy common in dogs. This systematic review and meta-analysis aimed to evaluate the effect of probiotics as an adjuvant in treating canine atopic dermatitis *in vivo* studies with dogs. The study was conducted following the *PRISMA* guidelines. Only clinical studies in dogs with atopic dermatitis that received intervention with probiotics were selected, being just original articles in English from the last 5 years (2019–2023). A total of 293 articles were obtained, and after the inclusion criteria, only 5 articles were eligible and included in the systematic review and meta-analysis. The 5 studies used different probiotics at concentrations of 10^8 to 10^{10} *CFU*/mL/g, 3 studies with oral administration for 12 weeks, and 2 studies with topical use for 4 weeks. The meta-analysis results show that probiotics did not present significant effects against atopic dermatitis by evaluating the *CADESI-4* scale (P=0.08) and the *PVAS* scale (P=0.85). Furthermore, regarding the method of administration of probiotics (oral or topical), the meta-analysis showed that there were also no significant results when the disease index was evaluated using *CADESI-4* (P=0.07) or *PVAS* (P=0.92). We concluded that, even without significant effects, all trials showed a reduction in *CADESI-4* and *PVAS* scores, reflecting a reduction in the severity of atopic dermatitis in dogs that used probiotics as treatment adjuvants.

KEYWORDS: atopic dermatitis, *Canis lupus familiaris*, dermatopathology, probiotics, skin disease

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INTRODUCTION

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host [9]. These beneficial microorganisms have been increasingly used in recent decades due to their contribution to the health and well-being of humans and animals. Some studies report the effectiveness of the effects of probiotics in the treatment of allergic pathologies and skin inflammations, especially atopic dermatitis. Among these contributions, probiotics have been suggested as a treatment for canine atopic dermatitis (AD), an allergic and inflammatory dermatopathy, genetically predisposed and common in dogs [27, 28, 45].

The individual's microbiota is essential for the balance of the immune system and, therefore, the appearance of skin diseases and the alteration of the immune response is related to the imbalance of the microbiota of the intestine and skin. This correlation is demonstrated as the "gut-skin axis" and involves interrelationships in which the local microbiome affects the immune functioning of distant sites [7] Probiotics can balance the microbiome through several mechanisms, among them, acting on the intestinal immune barrier by restoring intestinal permeability, reducing the production and release of pro-inflammatory cytokines [23].

Although the specific mechanisms of the immunomodulatory effect of probiotics in AD have not been fully clarified, studies report

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a close correlation between the intestinal microbiota and several factors associated with the pathophysiology of AD, such as immunity and inflammation [22]. This has been proven in clinical trials using different probiotic strains, which reported beneficial effects in the prevention and treatment of AD [36, 45]. In the experiments by Kim *et al*. [17, 18], both carried out with mice using the probiotic *Lactobacillus rhamnosus*, clinical improvement of symptoms and prevention of the development of AD were obtained through the inhibition of inflammatory cytokines.

However, with multifactorial etiology, the pathogenesis of canine AD is quite complex and results from an interaction between environmental and genetic factors [3], mediated by the immune system [25]. It has characteristic clinical aspects and is commonly associated with the production of immunoglobulin E (IgE) specific to allergens [12]. There is no cure for atopic dermatitis and clinical management is based on controlling the condition [24].

Methods for improving and controlling the clinical signs of canine AD include avoiding contact with allergens, using essential fatty acids, administering steroidal anti-inflammatory drugs, antipruritic medications and allergen-specific immunotherapy. The use of anti-inflammatory and antipruritic medications, including topical/systemic glucocorticoids, cyclosporine and oclacitinib, are the main therapeutic options for canine AD. However, there are limitations in treatment, since prolonged use or high doses of commonly used medications have been associated with some adverse effects [38], therefore, there is a need for alternative treatments.

In this context, probiotics have been used as alternative treatments for canine AD; however, there are still few clinical studies in dogs and the results and efficacy are not well known by the medical and scientific community of veterinary medicine. Thus, the objective of this study is to perform a systematic review and meta-analysis to evaluate the effect of probiotics as an adjuvant in the treatment of canine atopic dermatitis in *in vivo* studies with dogs.

MATERIALS AND METHODS

Protocol

This systematic review and meta-analysis were conducted in accordance with the updated *PRISMA* guidelines−Preferred Reporting Items for Systematic Reviews and Meta-Analyses (page) [31].

Selection criteria

Only original articles of clinical studies in dogs that evaluated the effect of probiotics as an adjuvant in the treatment of canine atopic dermatitis, published between 2019 to 2023, and in English.

The selection of articles was structured according to the acronym PICOS (Table 1) where: Population (P)−Dogs with atopic dermatitis; Intervention (I)−Oral or topical use of probiotics; Control (C)−Placebo or Baseline; Results (O)−clinical signs of atopic dermatitis; Study design (S)−any clinical trials.

Inclusion criteria

Studies were considered eligible if they met the following inclusion criteria: (1) described a clinical trial; (2) included studies with dogs; (3) the animal model is diagnosed with atopic dermatitis; (4) the clinical intervention was the oral or topical use of probiotics with species and dosage described; (5) evaluation of clinical signs of atopic dermatitis; (6) the assessment scales *CADESI*-4 (Canine Atopic Dermatitis Extend & Severity Index) [29] or *PVAS* (Pruritus Visual Analog Scale) [37] were reported.

Exclusion criteria

Studies with the following characteristics were excluded: (1) unexplained disease; (2) unspecified probiotic dosage; (3) studies with dogs without atopic dermatitis; (4) studies using another animal model (e.g., rats) or human model; (5) experimental *in vitro* studies; (6) evaluation of only clinical signs without use of *CADESI*-4 or *PVAS* scales; (7) studies without results presented; (8) relevant results not reported (9) studies that did not use probiotics in atopic dermatitis; (10) records that are not original research articles (e.g. reviews, editorials, letters, comments, book chapters, theses, and dissertations).

Literature review strategies

The systematic review included clinical studies in dogs that evaluated the effect of probiotics as an adjuvant in the treatment of canine atopic dermatitis, published in the last 5 years, from 2019 to 2023. The search was performed in the following databases: Scopus, PubMed, Science Direct. The search strategies and descriptors used were the following: Probiotic OR Lactobacillus OR Bifidobacterium AND “Atopic dermatitis” OR pruritus OR “skin disease” AND dog OR “canis lupus”. Publications were limited to English, and their references were reviewed to identify additional studies.

Data extraction and risk of bias assessment

The studies identified through the search strategy were independently assessed by two authors regarding the inclusion and exclusion criteria. The initial selection phase consisted of analyzing the titles and abstracts and, finally, reading the studies in full. The data extracted from the selected studies were the following: authors and year of publication, study design, study population and sample size, probiotic intervention with concentration and dose, route of administration and duration of the intervention, complementary treatments, control group and, main results of the clinical signs of atopic dermatitis measured by *CADESI*-4 or *PVAS* scales.

The quality of the eligible studies was assessed based on the criteria of the Cochrane tool−”Risk of Bias” [6], specific for determining bias in intervention studies in animal models. The assessment includes the following: generation of random numbers; allocation

Table 1. Clinical studies in dogs included in the systematic review on the use of probiotics as an adjuvant in the treatment of canine atopic dermatitis and published between 2019 and 2023

Study	Study design	Population	Intervention	Concentration	Via	Duration	Complementary treatment	Control	Results
Lee *et al*. [21]	Single-center open study	11 dogs with AD. Probiotics group (n=7) and Control group (n=4)	*Bifidobacterium longum*	5 × 10^10 *CFU*/ 2 g	Oral	1 × daily/12 weeks	Oclacitinib treatment (1 × or 2 × daily)	Placebo powder, 2 g/ oral/ 1 × daily/ 12 weeks (n=4 adult dogs with AD)	Oral administration of B. longum was effective in improving skin lesions and could be considered as a supportive therapy for canine AD. In the *CADESI-4* there was a significant decrease score in the probiotics group compared to the baseline (T0=38 and T4=32) but there was no significant between-group difference at each time point (e.g. T4−control group 20 and probiotic group 32). In the *PVAS* there was no significant decrease score in the probiotics group compared to the baseline (T=3 and T4=1.8) and although the scores in both groups consistently decreased until the end of the experiment (e.g. T4 −control group 2.8 and probiotic group 1.8), there were no significant within-group differences at all time-points.
Kawano *et al*. [15]	—	15 dogs with AD	*Lactobacillus paracasei* M-1 and kestose (prebiotic)	2 × 10^10 *CFU*/ 1 g and 400 mg/ tablet (kestose)	Oral	1 × daily/ 12 weeks	Prednisolone treatment	Only compared to the baseline score	The synbiotic activity of L. paracasei M-1 and trisaccharide kestose can improve CAD. In the *CADLI* there were a significant reduction in the socre (T0=28.0, T30=20.0, T60=20.0 and T90=12.0). PVAS score also significantly decreased (T0=6.0, T30=3.0, T60=3.0 and T90 2.0). Also the prednisone use reduction was observed over 90 days (T0=112.0 mg and T90=80.0 mg) in the 15 dogs.
Kim *et al*. [20]	—	6 dogs with AD	Synbio-glucan spray, containing *Lactobacillus plantarum, Bifidobacterium longum, and Pediococcus pentosaceus*	—	Topic	Spray on skin lesion areas, 3 × daily/ 4 weeks	One dog used Lokivetmab to control severity	Only compared to the baseline score	Synbio-glucan functional spray was efficacious and safe for the treatment of AD in dogs. The patients exhibited remission of skin lesions approximately one month after the treatment with the Synbio-glucan functional spray. The scores before and 30 days after treatment were significantly decreased on the *CADESI-4* (T0 ± 83 e T30 ± 38) and *PVAS* (T0 ± 8 e T30 ± 3). No side effects were seen in patients related to the use of the functional spray.
Yamazaki *et al*. [48]	—	21 dogs with AD. Probiotic group (n=11) and Placebo group (n=10)	*Enterococcus faecium* SF68	1 × 10^8 *CFU*/ 1 g	Oral	2 × daily/ 12 weeks	Oclacitinib treatment	Placebo hydrolysed palatability-enhancer 1 g/ oral/ 2 × daily/ 12 weeks (n=10 adult dogs with AD)	Supplementation with *SF68* probiotic was associated with no difference in oclacitinib dose reduction versus placebo. Both groups experienced a reduction, but nothing statistically significant, in *CADESI-4* and *PVAS* scores. In the *CADESI-4*, the scores at T0 were (placebo group 10.6 and *SF68* group 10) and at T3 were (placebo group 6 and SF68 group 5.7). In the *PVAS* there was no significant change from baseline in either group (T0−placebo group 2.1 and *SF68* group 2.6) at any time point (T3−placebo group 1.3 and *SF68* group 2.2).
Santoro *et al*. [39]	Preliminary, open-label, uncontrolled study	10 dogs with AD	Water-based spray solution, containing *Lactobacillus reuteri* and *Lactobacillus rhamnosus,* tamarind extract and polyphenols	—	Topic	Spray on skin lesion areas, 1 × daily/ 4 weeks	None	Only compared to the baseline score	Significant and rapid decrease in the clinical signs associated with CAD after use of the spray. In the *CADESI-4*, a significant reduction in the total score was seen at each time point: on D0=16.5, D14=8.5, D28=9.5 and D42=6.5. In the PVAS, a significant reduction in the total score was seen at each time point: D0=4.9, D14=5.2, D28=4.1 and D42=2.5. A significant change in the skin hydration and pH of the inguinal, axillary and interdigital areas was not seen.

concealment; random housing of animals; blinding of intervention and outcome evaluators; incomplete outcome; selectivity of reports; and other sources of risk. For each item, the risk was assessed as low, high or uncertain.

Statistical analysis and meta-analysis

The Review Manager 5.4 software was used for statistical analysis and meta-analysis. For the results of continuous variables, the Standard Mean Difference (SMD) was used to describe the effect size of the variables and the 95% confidence intervals (CI). The I^2 test was used to analyze the heterogeneity between the results. In the case of low heterogeneity ($I^2 \leq 50\%$), a fixed effects model analysis was performed, while in the presence of heterogeneity ($I^2 \geq 50\%$) a random effects model was assumed. The value of $P<0.05$ was considered significant for all statistical tests.

RESULTS AND DISCUSSION

Systematic review

Using the search strategy and descriptors: Probiotic OR Lactobacillus OR Bifidobacterium AND "Atopic dermatitis" OR pruritus OR "skin disease" AND dog OR "canis lupus"; 202,926 articles were found, of which 94,027 articles were obtained after applying the original research article filters. After applying the time interval filter between 2019 and 2023, 93,734 articles were excluded, and 293 articles were obtained. Of these, applying the inclusion and exclusion criteria, 287 articles were excluded, and 6 articles were obtained. Furthermore, 1 article was excluded because it did not corroborate the established criteria (puppies without atopic dermatitis). In total, 200,923 records were excluded, and 5 articles were finally selected for inclusion in the review. The authors independently assessed the 5 articles based on the level of evidence and the 5 studies were included in the systematic review and meta-analysis (Fig. 1).

The 5 studies included in the systematic review, which supported the use of probiotics as an adjuvant in the treatment of canine atopic dermatitis, were analyzed. In all included studies, the selected patients were adult dogs diagnosed with canine atopic dermatitis (AD), both in the probiotic group and in the control group. The minimum number of participants in the studies was n=6 [20] and the

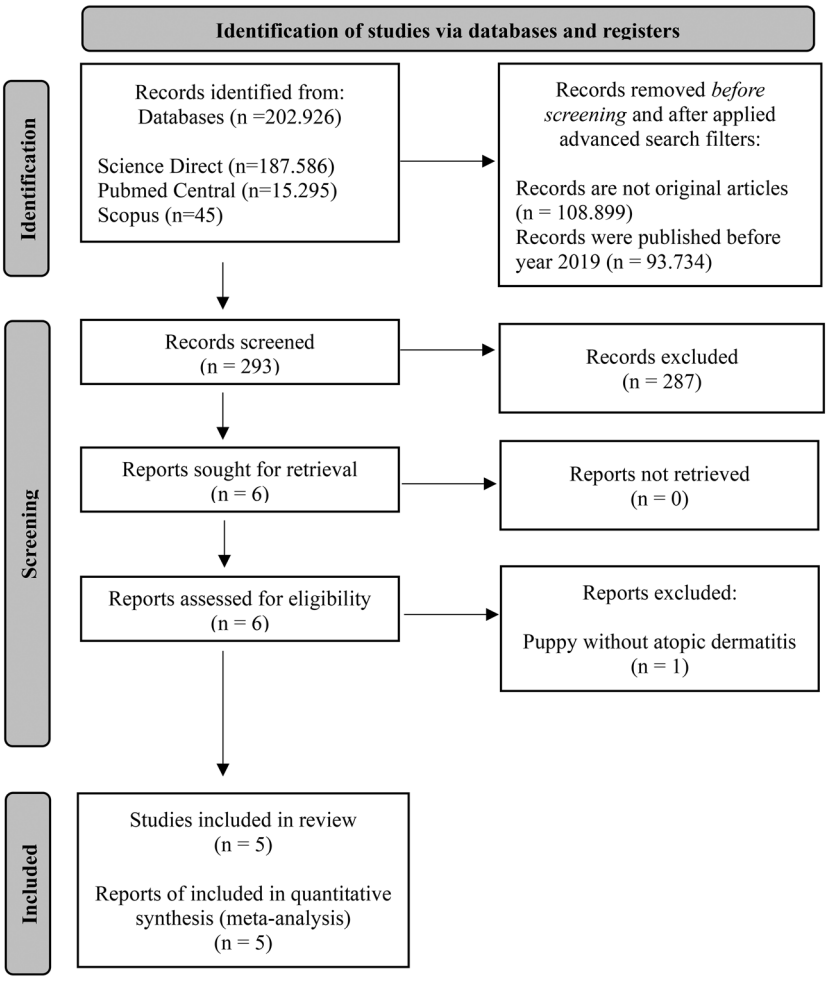

Fig. 1. *PRISMA* flow diagram for identification and selection of studies on the use of probiotics as an adjuvant in the treatment of canine atopic dermatitis. *PRISMA*, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

maximum was n=21 animals [47]. Among this, it was observed that the sample mean of the treatment group that received probiotics was n=10. Regarding therapeutic intervention with probiotics, the use of several strains was observed, which were *Bifidobacterium longum, Lactobacillus paracasei, Pediococcus pentosaceus*, *Enterococcus faecium, Lactobacillus reuteri* and *Lactobacillus rhamnosus*. All strains included in the studies participating in this review are strains approved by the Brazilian Health Regulatory Agency (ANVISA) for use as probiotics [4].

The dose recommended in the studies was a concentration of 10^8 to 10^{10} *CFU* per milliliter or gram, corroborating the information described in ANVISA, which states that the minimum viable quantity for probiotics should be 10^8 to 10^{10} *CFU*, this being the daily suggestion for consumption of the ready-made probiotic product, and in addition, it should be ingested daily to ensure a continuous effect [4].

Two studies used the probiotic topically [20, 39] and the others administered it orally. The duration of treatment was 12 weeks, except in the studies of topical use [20, 39] which were 30 and 28 days, respectively. Furthermore, in relation to these studies using the topical administration route, they do not specify the probiotic concentration contained in the product used, which makes it difficult to evaluate and compare the results and reduces reliability.

Still about intervention, Kim *et al.* [20] and Santoro *et al.* [39] used more than one probiotic strain in the formulations of the topical administration route spray*: L. plantarum, B. longum, Pediococcus and L. reuteri; L. rhamnosus*, respectively, in addition to containing secondary and bioactive metabolites in the formulations, which makes it difficult to analyze the action and efficacy of the specific strain. In studies in which the probiotic was administered orally [15, 21, 47], only one species of strain was used in the clinical trial, *Bifidobacterium longum, Lactobacillus paracasei* M-1 and *Enterococcus faecium* SF68, respectively. However, in the study by Kawano *et al.* [15], the administration of the probiotic *Lactobacillus paracasei* M-1 was associated with the prebiotic FOS 1-kestose together.

Regarding the probiotic species used in the studies included in the review, the literature states that *Bifidobacterium longum* promotes immune modulation by reducing pro-inflammatory cytokines [19, 40, 48], improves insulin sensitivity and lipid profile [22, 44], and helps prevent colorectal cancer by inhibiting tumor cells [41].

Lactobacillus plantarum benefits muscle health and bone structure [34], reduces obesity in mice fed a high-fat diet [5], acts as an immune modulator and potential vaccine vector [43], and protects against diet-induced liver complications [10].

Pediococcus acidilactici helps control infections and metabolic disorders [1, 14], reduces hyperpigmentation, and improves skin hydration [33]. *Pediococcus pentosaceus*, in turn, contributes to intestinal fermentation and anti-inflammatory properties [8].

Lactobacillus reuteri helps with childhood constipation, improving the frequency and consistency of bowel movements [35], restores the intestinal microbiota and strengthens the immune system [30], and reduces lung inflammation [42].

Enterococcus faecium modulates the intestinal microbiota, promoting the population of beneficial bacteria while reducing microorganisms associated with obesity [32] and reducing the frequency and duration of childhood diarrhea [11, 16].

Lactobacillus rhamnosus improves growth, immunity, and intestinal microbiota, also elevates intestinal IgA levels, strengthening immunity [26] and reduces inflammation caused by *E. coli* in neonates [46].

Finally, the last probiotic species used in the studies included in the review is *Lactobacillus paracasei*, which improves canine atopic dermatitis, reducing lesions and the need for prednisolone [15]. It also has antimicrobial properties against *E. coli* O157:H7 [2] and modulates the immune response via macrophages [13].

About the design of the studies, the studies by Kawano *et al.* [15], Kim *et al.* [20] and Yamazaki *et al.* [47] do not describe the study design, which allows for a margin of uncertainty regarding the results obtained. However, the studies by Lee *et al.* [21] and Santoro *et al.* [39] describe it as a central open study and a preliminary uncontrolled open study, respectively.

The studies by Lee *et al.* [21] using *Bifidobacterium longum* and Yamazaki *et al.* [47] using *Enterococcus faecium* SF68 present a probiotic treatment group and a placebo control group. However, the studies by Santoro *et al.* [39] using water-based spray solution, containing *Lactobacillus reuteri* and *Lactobacillus rhamnosus*, tamarind extract and polyphenols; Kim *et al.* [20] using Synbio-glucan spray, *containing Lactobacillus plantarum, Bifidobacterium longum*, and *Pediococcus pentosaceus*; Kawano *et al.* [15] using *Lactobacillus paracasei* M-1 and kestose (prebiotic) do not have a placebo control group, they considered the control as the initial time (baseline) of the animal itself before starting the intervention, with the evaluation of the response of the proposed intervention being carried out and compared only with the single group included in the study, that is, all participants received the study medication and are evaluated, a fact that may consider a high risk of bias due to the lack of blinding of participants and evaluators [6]. We observed that in all studies, except the study by Santoro *et al.* [39], dogs received drug treatments for canine AD and continued during clinical trials. Among the medications used, Oclacitinib, Prednisolone and Lokivetmab stand out, which are commonly used in the treatment of canine AD [38]. Additionally, the studies do not present an intervention group that received the proposed treatment with probiotics alone without any additional medication, which would be important to evaluate the effect of the probiotic alone, since interference in the action of the probiotic and its immunological mediation may have occurred due to the concomitant use of other anti-inflammatory and antipruritic medications.

Regarding the results obtained in the clinical trials included in this review, both demonstrated a decrease in the *CADESI*-4 and *PVAS* scale scores, but there was no significant difference. However, it was observed in all studies that the use of probiotics managed to reduce, even if not statistically significant, the *CADESI-4* and *PVAS* scores, which may represent a slight clinical improvement for the animal. The reduction in the *CADESI-4* and *PVAS* scores in canine AD with the use of probiotics as an adjuvant treatment corroborates a previous, double-blind, randomized study with 41 dogs, by Ohshima-Terada *et al.* [28] in which the probiotic strain *Lactobacillus paracasei K71* was administered at a dose of 5 mg/kg once a day for 12 weeks, and a control group with placebo. The clinical trial demonstrated an improvement in the *CADESI-4*, *PVAS* and pruritus scores in both groups at 12 weeks compared to the initial week. The *CADESI-4* and *PVAS* scores in the probiotic group (CADESI=58.6 and *PVAS*=2.3) were slightly lower than

those in the control group (*CADESI*=62.4 and *PVAS*=2.5), but without statistical significance. Regarding the degree of improvement in *CADESI-4* from the initial week to week 12, the value for the probiotic group (45.8%) was higher than that of the control group (38.1%), but not significant (*P*=0.96). Concomitantly, the *PVAS* score of the probiotic group (*PVAS*=2.3) was lower than that of the control group (*PVAS*=2.5), but not statistically significant (*P*=0.28). Based on the results obtained, the study concludes that there was no significant effect in favor of the use of the probiotic; however, its use was considered relevant due to the reduction in the *CADESI-4* and *PVAS* evaluation scores, with consequent improvement in the clinical condition of the animals. Considering the analysis of the 5 studies included in this systematic review, which are the only studies selected from the last 5 years (2019–2023) with clinical trials in dogs to evaluate the use of probiotics as an adjuvant in the treatment of canine AD, the need and importance of denser studies, with a greater number of animals, longer duration and standardization of participants (medications used, age, general clinical and dermatological status) and standardization of the proposed treatment/intervention in terms of specific strain, concentration of the probiotic strain, route of administration and type of evaluation, being possible a safer evaluation of the effect of probiotics as an adjuvant in the treatment of canine AD.

Risk of bias analysis of the studies

The risk of bias for each study is shown in Table 2. All studies described that the generation of animal groups was random but did not report the method of random sequence generation and, therefore, were assessed as having an unclear risk of bias. In addition, the studies did not provide sufficient information on the concealment of group allocation or on the blinding of professionals and outcome assessors; all reported the measures that were used to shelter the strain, except in the study by Kawano *et al.* [15].

Meta-analysis

The meta-analysis performed among the 5 studies indicated that there was no significant difference between probiotic treatments as an adjuvant in the treatment of canine atopic dermatitis, *P* values >0.05 (Figs. 2–5).

The meta-analysis study was based on the use of the *CADESI-4* and *PVAS* evaluation methods to assess the effectiveness of the use of probiotics in canine atopic dermatitis, comparing the use of probiotics vs. placebo or baseline in both oral and topical administration routes.

Figures 2, 3, 4 and 5 present the meta-analysis in relation to the effectiveness of the use of probiotics in reducing the severity of canine atopic dermatitis. Figures 2 and 3 assess the efficiency of the probiotic group and placebo group. In studies that do not have a control group, the *CADESI-4* and *PVAS* scores before treatment at the initial time −T0 (baseline) were compared with the final time score−TF of the treatment, of the animal itself (n) of the study. Figures 4 and 5 assess the use of probiotics and placebo by comparing the oral administration route and topical administration route. In studies that do not have a control group, the *CADESI-4* and *PVAS* scores before treatment at the initial time−T0 (baseline) were compared with the final time score−TF of the treatment, of the animal itself (n) of the study.

Table 2. Risk of bias of studies included in the systematic review on the use of probiotics as an adjuvant in the treatment of canine atopic dermatitis and published between 2019 and 2023

Studies	Random sequence generation	Allocation concealment	Blinding of participants and professionals	Blinding of outcome assessors	Incomplete outcomes	Selective outcome reporting	Other sources of bias
Kawano *et al*. [15]	Uncertain	Uncertain	High	High	Uncertain	Uncertain	Uncertain
Kim *et al*. [20]	Uncertain	Uncertain	High	High	Uncertain	Uncertain	Uncertain
Lee *et al*. [21]	Uncertain	Uncertain	Low	Uncertain	Uncertain	Uncertain	Uncertain
Santoro *et al*. [39]	Uncertain	Uncertain	High	High	Uncertain	Uncertain	Uncertain
Yamazaki *et al*. [48]	Uncertain	Uncertain	Low	Low	Low	Uncertain	Uncertain

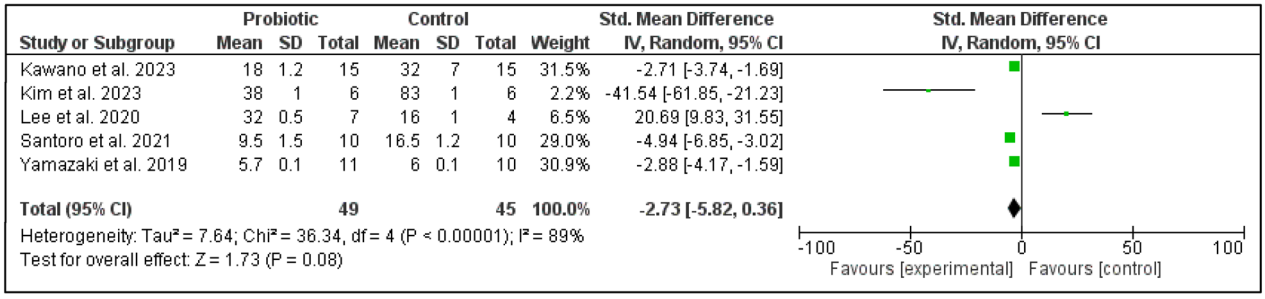

Fig. 2. Effect of probiotic *vs.* placebo on reducing the severity of canine atopic dermatitis measured by the *CADESI-4* scale. *CADESI-4*, canine atopic dermatitis extent and severity index; DF, degrees of freedom; I^2, squared statistic; CI, confidence interval.

Effect of probiotics vs. placebo in reducing the severity of canine atopic dermatitis measured by the CADESI-4 and PVAS scales: The random-effects model was used to investigate the efficiency of probiotics in reducing atopic dermatitis, assessed using the *CADESI-4* or *PVAS* methods. The results of the meta-analysis show that probiotics did not have significant effects against atopic dermatitis, both

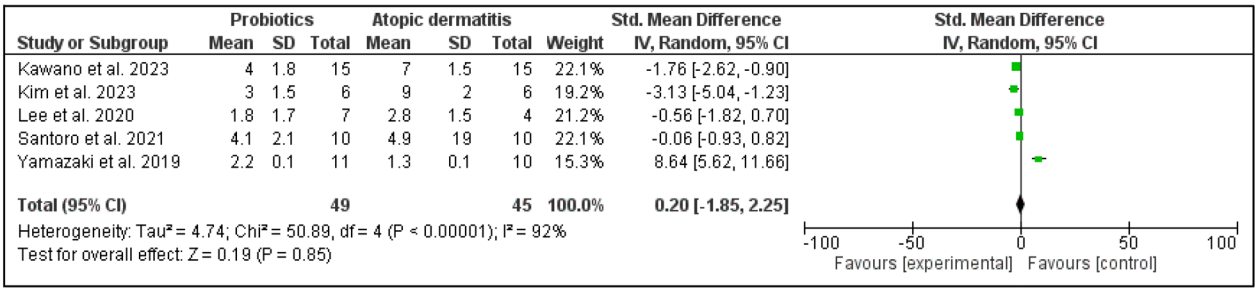

Fig. 3. Effect of probiotic *vs.* placebo on reducing the severity of canine atopic dermatitis measured by the *PVAS* scale. *PVAS* scale, pruritus visual analog scale; DF, degrees of freedom; I^2, squared statistic; CI, confidence interval.

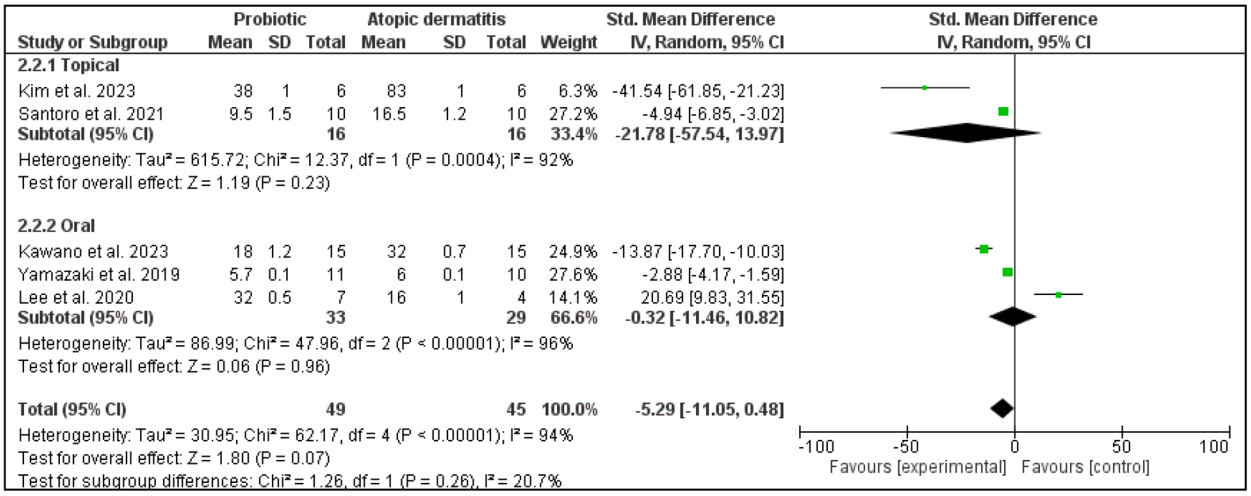

Fig. 4. Effect of probiotic *vs.* placebo administered orally or topically on reducing the severity of canine atopic dermatitis measured by the *CADESI-4* method. *CADESI-4*, canine atopic dermatitis extent and severity index; DF, degrees of freedom; I^2, squared statistic; CI, confidence interval.

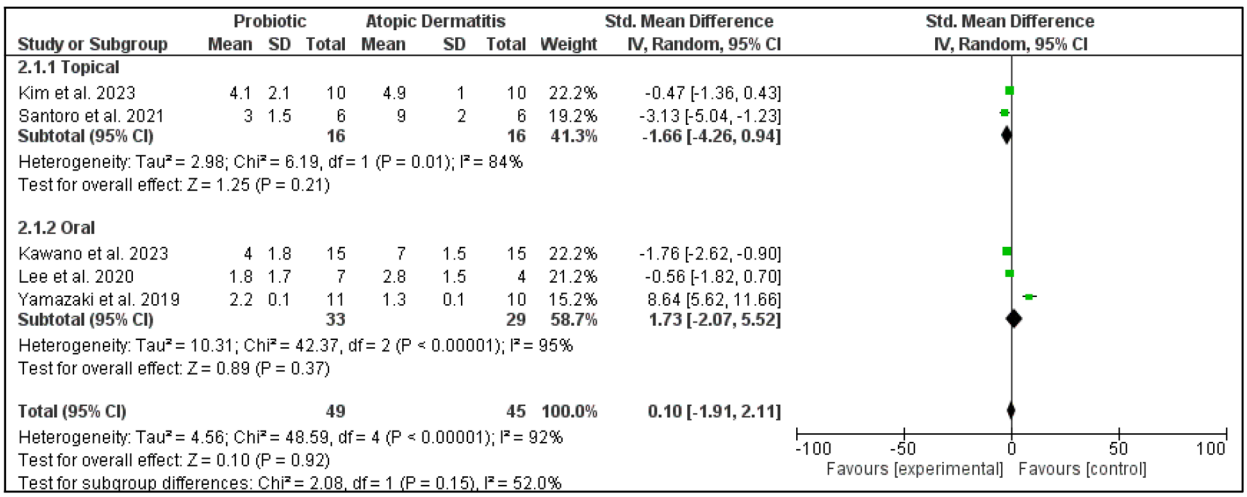

Fig. 5. Effect of probiotic *vs.* placebo administered orally or topically on reducing the severity of canine atopic dermatitis measured by the *PVAS* method. *PVAS* scale, pruritus visual analog scale; DF, degrees of freedom; I^2, squared statistic; CI, confidence interval.

assessed by *CADESI-4* (SMD: −2.73, 95% CI: −5.02–0.36, I^2: 89%; *P*=0.08) (Fig. 2), and assessed by *PVAS* (SMD: −0.20, 95% CI: −1.85–2.25 I^2: 92%; *P*=0.85) (Fig. 3).

Effect of probiotic vs. placebo administered orally or topically in reducing the severity of canine atopic dermatitis measured by the CADESI-4 scale and PVAS: The random-effects model applied by the meta-analysis to evaluate the efficiency of probiotics, when administered orally or topically, in reducing atopic dermatitis also showed that there were no significant results when the disease index was evaluated through the *CADESI-4* method (SMD: −5.29, 95% CI: −11.05–0.48, I^2: 94%; *P*=0.07) (Fig. 4) and *PVAS* (SMD: 0.10, 95% CI: −1.91–2.11, I^2: 92%; *P*=0.92) (Fig. 5).

The results obtained in the meta-analysis demonstrated that there was no significant difference in the use of probiotics in reducing the *CADESI-4* and *PVAS* scales, when compared between the probiotic group vs. placebo or baseline, both in the oral and topical administration routes. The heterogeneity of the studies (Figs. 2–5), however, does not allow us to determine the true magnitude of the action of probiotics in reducing the severity of canine AD as measured by *CADESI-4* and *PVAS*. This heterogeneity is due to the different interventions in each study, resulting from the use of different strains, doses of probiotics, use of other medications during the study, different durations of treatments, and the subjectivity of the *CADESI-4* and *PVAS* scales for assessing the severity of canine AD. Given the heterogeneity of the characteristics of the populations between the different studies, in addition to the non-standardization of the gender of the probiotic strain and recommended dosage, the analysis by subgroups does not show potentially real results.

The studies by Kim *et al.* [20] and Santoro *et al.* [39] that referred to the use of topical solution presented high heterogeneity, attributed to several methodological factors. Among them, the lack of information on the concentration of probiotics used and the use of different vehicles added to the formulation stand out, with the study by Santoro *et al.* [39] using a water-based spray solution containing a mixture of Lactobacillus (*L. reuteri* and *L. rhamnosus*) with the culture media and metabolites, tamarind extract and polyphenols, and the study by Kim *et al.* [20] using Synbio-glucan (b-glucan, oat and *L. plantarum*, *B. longum* and *Pediococcus pentosaceus*) (30 mL), distilled water (51.4 mL), glycerin (2 mL), solubilizer (0.6 mL), and 40% alcohol (16 mL). In other words, both have components added to the formulated solution other than the probiotic, which may affect the probiotic used or the clinical results. In addition, neither study compared experimental groups with a control group and conducted the study exclusively on a single group. These methodological aspects compromise the reproducibility and reliability of the findings, resulting in an undetermined or high risk of bias (Table 2). Specifically in the criteria related to the blinding of participants, professionals, and outcome evaluators, the risk of bias was classified as high, evidencing the low methodological robustness of the studies analyzed.

The studies included in the meta-analysis individually demonstrated a decrease in the *CADESI-4* and *PVAS* scale scores, but with scores too low to determine a statistically significant difference (*P*>0.05). However, it was observed in all studies that the use of probiotics managed to reduce, even if not statistically significant, the *CADESI-4* and *PVAS* scores, which may represent a slight clinical improvement for the animal.

The limitations of this systematic review include the use of different strains of probiotics and administration routes, which may lead to different individual results and conclusions in each study. In addition, there were several important differences between the participants in the clinical trials: they continued to use medications previously prescribed for AD control, no dietary control was established, a difference in the administration route (oral or topical), use or not of other medications not described during the clinical trial, in some, there was a restriction on the use of antibiotics and use of specific medications such as Oclacitinib, corticosteroids and other requirements that were not present in all clinical trials. In the statistical analysis of the meta-analysis, heterogeneity was found between the populations of the different studies. This constituted a limitation when analyzing the results, as it made it impossible to determine the reduction in the *CADESI-4* score expected in patients with canine AD after probiotic administration.

In conclusion, canine atopic dermatitis is a chronic dermatopathy and symptom control is necessary for the quality of life of animals and guardians. However, currently, medications used have long-term deleterious effects, making it necessary to seek alternative treatments for this condition. In addition, according to previous research in the literature, to our knowledge, this is the first systematic review and meta-analysis on the use of probiotics as an adjuvant in the treatment of canine atopic dermatitis.

The studies selected and included in this systematic review and meta-analysis demonstrated that the administration of probiotics, either orally or topically, did not present a significant effect when compared between the probiotic group and the placebo or baseline control group in reducing the severity of canine AD. However, it was observed in all studies that the use of probiotics managed to reduce, even if not statistically significant, the *CADESI-4* and *PVAS* scores, which may represent a slight clinical improvement for the animal.

Despite the fact that the inclusion of only five studies may limit the ability to derive clinically meaningful and reliable outcomes, it is important to underscore the considerable scarcity of *in vivo* investigations that apply appropriate qualitative parameters for outcome assessment. Moreover, the heterogeneity among the selected studies, particularly regarding the bacterial strains employed, dosage regimens, and methods of administration, poses a challenge to the derivation of consistent findings, especially with respect to the meta-analytical component of this review. These limitations underscore the need for caution among veterinary practitioners when interpreting and disseminating uncertain evidence about the use of probiotics in the context of canine atopic dermatitis (CAD). In this regard, the present review serves as a relevant informational resource to assist veterinary professionals in critically assessing the available literature and grounding their therapeutic approaches in studies characterized by methodological rigor and low risk of bias. Given that the recent evidence analyzed herein failed to confirm the efficacy of probiotics in the treatment of CAD, it becomes evident that further large-scale, standardized clinical trials are required to establish more definitive conclusions.

It is important to carry out more homogeneous, dense studies, with a greater number of animals, longer duration and standardization of participants (medications used, age, general clinical and dermatological status) and standardization of the proposed treatment/intervention in terms of specific strain, concentration of the probiotic strain, route of administration and type of evaluation, to

investigate a possible significant effect of the use of probiotics as an adjuvant in the treatment of canine AD.

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