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Efficacy of antimicrobial and nutraceutical treatment for canine acute diarrhoea: A systematic review and meta-analysis for European Network for Optimization of Antimicrobial Therapy (ENOVAT) guidelines

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ABSTRACT

Systemic antimicrobial treatments are commonly prescribed to dogs with acute diarrhoea, while nutraceuticals (prebiotics, probiotics, and synbiotics) are frequently administered as an alternative treatment. The aim of this systematic review and meta-analysis was to assess the effectiveness of antimicrobials and nutraceutical preparations for treatment of canine acute diarrhoea (CAD). The results of this study will be used to create evidence-based treatment guidelines. PICOs (population, intervention, comparator, and outcome) were generated by a multidisciplinary expert panel taking into account opinions from stakeholders (general practitioners and dog owners). The Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology was used to evaluate the certainty of the evidence. The systematic search yielded six randomised controlled trials (RCT) for antimicrobial treatment and six RCTs for nutraceutical treatment meeting the eligibility criteria. Categories of disease severity (mild, moderate, and severe) were created based on the presence of systemic signs and response to fluid therapy. Outcomes included duration of diarrhoea, duration of hospitalization, progression of disease, mortality, and adverse effects. High certainty evidence showed that antimicrobial treatment did not have a clinically relevant effect on any outcome in dogs with mild or moderate disease. Certainty of evidence was low for dogs with severe disease. Nutraceutical products did not show a clinically significant effect in shortening the duration of diarrhoea (based on very low to moderate certainty evidence). No adverse effects were reported in any of the studies.

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Introduction

Acute diarrhoea occurs commonly in the general dog population, and one of the most frequent presenting clinical signs in practice (Hubbard et al., 2007; Robinson et al., 2015). Canine acute diarrhoea (CAD) is generally mild and self-limiting, and most cases are treated as outpatients (Singleton et al., 2019). The pathophysiology is poorly understood, and the aetiology is likely to be multi-factorial. One study found that lifestyle factors such as dietary indiscretion, periods of kennelling, diet change, and home-cooked diets conferred greater risk than specific enteropathogens in the development of CAD (Stavisky et al., 2011).

Acute diarrhoea is one of the most common reasons for systemic (oral or injectable) antimicrobial treatment in dogs (De Briyne et al., 2013; Lutz et al., 2020), and in particular haemorrhagic diarrhoea is a driver of antimicrobial prescription (Singleton et al., 2019; Lehner et al., 2020). However, many guidelines for antimicrobial use and some authors have recommended that antimicrobials should not be used for dogs with haemorrhagic or non-haemorrhagic diarrhoea in the absence of signs of sepsis (Marks et al., 2011; Allerton et al., 2021; Unterer et al., 2021). Gastrointestinal nutraceuticals, such as probiotics, prebiotics, and synbiotics, have been recommended for dogs with acute diarrhoea (Singleton et al., 2019).

The primary aim of this systematic review and meta-analysis was to assess the effectiveness of antimicrobials and nutraceutical preparations for treatment of CAD. The results will inform the CAD Antimicrobial Use Guidelines of the European Network for Optimization of Antimicrobial Therapy (ENOVAT) and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Veterinary Microbiology (ESGVM). The ENOVAT guidelines initiative encourages policymakers to use the results of this study in the development of regional or national antimicrobial treatment guidelines for CAD.

Materials and methods

The protocol for this systematic review and meta-analysis was registered with syreaf.org (SYREAF, 2023), and adhered to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) statement (Page et al., 2021). There were no deviations from the protocol. The PRISMA checklist is presented in Supplementary file 1.

Population, intervention, comparator, outcome (PICO) question generation and importance of outcomes

The guideline group consisted of 18 panel members, of which 12 represented the veterinary clinical fields of gastroenterology, internal medicine, infectious diseases, general medicine and/or nutrition (LRJ, KS, MW, SU, CRB, SW, CP, FA, TB, KA, CA, EL). The remaining members represented the disciplines of veterinary microbiology (LG), veterinary pharmacology (AF), veterinary epidemiology (MB, DS), guideline methodology in human health (FF), and veterinary public health (UW). The work was chaired by an oversight committee (LRJ, DS) and a methodology taskforce (KS, MW, CP, MB) was established as a subset of the group. The PICO-questions and clinical subgroups (Fig. 1) were drafted by the oversight committee and methodology taskforce and distributed to the remaining panel members as an e-Delphi questionnaire that were iterated until agreement was reached. The acceptability of definitions and importance of outcomes among guideline end-users was assessed by structured interviews with 41 general veterinary practitioners and 33 dog owners (with experience of acute diarrhoea in their own dog) from across Europe and Israel. Questionnaires and interview protocols are presented in Supplementary file 2.

Clinical thresholds

A contextualised approach was used in the interpretation of the results, which meant that the confidence in the estimate of effects were assessed in relation to clinical thresholds, rather than in the confidence in the point estimates (Hultcrantz et al., 2017). Clinical thresholds for continuous outcomes were determined based on the results from the aforementioned questionnaires and structured interviews. Small and large thresholds for dichotomous outcomes were established by surveying the opinion of 12 panel members and 35 veterinarians and calculating the 25th percentile. When setting thresholds for treatment effect a conservative approach was used to enhance acceptability. The moderate thresholds were calculated as the value in between small and large. Surveys are presented in Supplementary file 2 and individual results are in Supplementary file 3. Clinical thresholds are summarised in Table 1.

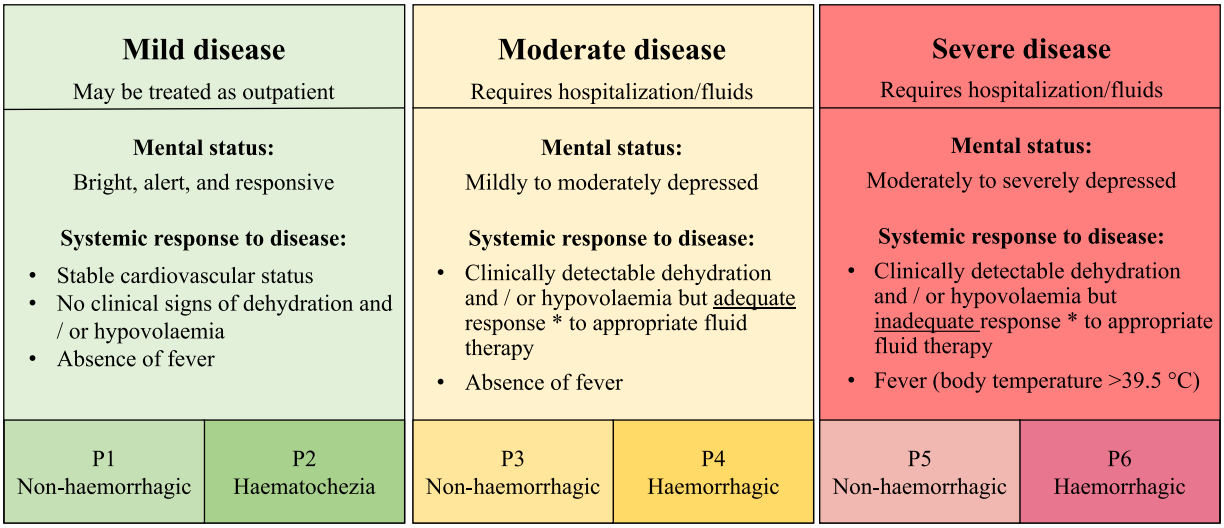

Fig. 1. Subgroup categorization of acute diarrhoea (P1-P6) by systemic clinical signs independent of volume or frequency of diarrhoea.

Table 1
Decision thresholds for each critical outcome. Duration of diarrhoea and duration of hospitalization is expressed in days. All other outcomes are number of events per 100 dogs.

Outcome (subgroup)	Trivial / small	Small / moderate	Moderate / large
Duration of diarrhoea (P1-P6)	1	2	3
Duration of hospitalization (P3-P6)	1	2	3
Mortality (P3-P6)	3	7	10
Progression of disease (P1-P2)	30	35	40
Progression of disease (P3-P6)	10	20	30

P1, Subgroup of dogs with mild disease and acute non-haemorrhagic diarrhoea; P2, Subgroup of dogs with mild disease and haematochezia; P3, Subgroup of dogs with moderate disease and acute non-haemorrhagic diarrhoea; P4, Subgroup of dogs with moderate disease and acute haemorrhagic diarrhoea; P5, Subgroup of dogs with severe disease and acute non-haemorrhagic diarrhoea; P6, Subgroup of dogs with severe disease and acute non-haemorrhagic diarrhoea

PICOs and subgroups

The guidelines group phrased six PICO questions: three concerning use of antimicrobials and three concerning use of nutraceuticals:

PICO 1: In dogs with acute diarrhoea, does antimicrobial treatment compared to no antimicrobial treatment have an effect?

PICO 2: In dogs with acute diarrhoea, does metronidazole treatment have a superior effect compared to beta-lactam treatment?

PICO 3: In dogs with acute diarrhoea, does long duration (≥7 days) of antimicrobial treatment have a superior effect compared to short duration (<7 days) of treatment?

PICO 4–6: In dogs with acute diarrhoea, does treatment with probiotics (PICO 4), synbiotics (PICO 5), or prebiotics (PICO 6) compared to no treatment shorten the duration of diarrhoea?

The population of dogs with acute diarrhoea was categorised into six subgroups (P1-P6) according to systemic disease severity, response to fluid therapy, and whether the diarrhoea was haemorrhagic or non-haemorrhagic (Fig. 1). Subgroups will hereafter be referred to as P1-P6. Selected outcomes were duration of diarrhoea, disease progression, days of hospitalization, mortality, and adverse effects of treatment. Prioritization of outcomes varied between different clinical subgroups as per published study protocol.

Search strategy and eligibility criteria

Databases that maximise veterinary journal coverage (Grindlay et al., 2012) were searched, including Cambridge Agricultural and Biological Abstracts (CAB Abstracts) from 1946, Web of Science Core Collection from 1956, and MEDLINE from 1950 until 31 August, 2021 as per published search strategy in the study protocol. As per the recommendation of Shojania et al. (2007) the search was updated on 15 November, 2022. No language or geographical restrictions were applied. Results were exported to EndNote (EndNote X9, Clarivate Analytics) and duplicates were removed. Randomised controlled trials (RCTs) of dogs with acute diarrhoea (<7 days duration) irrespective of aetiology and setting that included the desired outcomes were eligible for inclusion. Trials that included treatments of animals that involved multiple interventions in one treatment arm or comparators were excluded. For PICO 3, studies with other comparators were included if information about treatment duration was available. Pharmaceutical manufacturers of amoxicillin-clavulanic acid and metronidazole were contacted to obtain unpublished studies. Reference lists of included studies and narrative reviews about CAD were also searched.

Study selection and data collection

Three reviewers (CP, KS, MW) independently screened titles and abstracts in an online tool for systematic reviews (Rayyan, 2023; Ouzzani et al., 2016), as well as evaluating full texts, against the eligibility criteria mentioned above. Information about country, year, publication type, study design, population and baseline characteristics, intervention (substance, dosage, frequency) and comparator, assessment methodology, and exclusion and inclusion criteria, were extracted independently by the same reviewers into a data management tool (Excel, Microsoft). Outcome data on duration of diarrhoea, mortality, progression of disease, duration of hospitalization, and adverse effects (clinical and non-clinical) were also extracted from all studies. Study authors were contacted by email when data were missing. Disagreements between reviewers were resolved through discussion. The chairs of the drafting group (LRJ and DS) and methodological task force representative (MB) were available for consultation and discussion throughout the process.

Data synthesis

To estimate the pooled effect size measure across studies, a direct pairwise meta-analysis was performed. RevMan 5.3 (The Nordic Cochrane Center, The Cochrane Collaboration) was used to generate the meta-analyses visualised by forest plots. For continuous outcomes studies were pooled using mean difference (MD) with a 95% confidence interval (CI) using a random effects model. Dichotomous outcomes were analysed using the Peto odds ratio with a 95% CI using a fixed effects model due to few events. These statistical approaches allowed quantification of the differences between the measured parameters in the intervention and placebo groups and assessment of their significance. Statistical heterogeneity among the included studies was evaluated using the I^2 statistic and by visual inspection to identify outliers (Guyatt et al., 2023). I^2 values exceeding 60% were indicative of substantial heterogeneity and investigated further (Guyatt et al., 2011). Forest plots were used to visually present the results of individual studies alongside the overall pooled estimate. The robustness of results was evaluated by excluding outlying studies from each forest plot in a sensitivity analysis. For data that could not be pooled, a descriptive analysis was provided.

Where direct evidence could not be synthesised, a network meta-analysis (NMA) approach was employed. For treatments not directly compared in available RCTs, NMA can provide indirect estimates using a common comparator, and can combine direct and indirect evidence for all available treatment comparisons. The netmeta package in R was used to conduct a random-effects NMA using the frequentist approach. We assessed incoherence (i.e., disagreement between direct and indirect evidence) using the loop-specific approach and the node-splitting method.

Quality assessment

Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology was used to evaluate the quality of the evidence (Guyatt et al., 2008). Quality of evidence will hereafter be referred to as "certainty of evidence" in accordance with GRADE terminology (Guyatt et al., 2008). The RevMan files were exported to GRADEpro software (GRADE, 2023) to produce summary of findings tables that included anticipated absolute effects for dichotomous outcomes (Supplementary file 4). An interpretation of certainty ratings and a description of the domains that were used to evaluate the evidence is presented in Table 2. The GRADE domains were assessed by KS, MW, and LRJ for all individual subgroups (P1-P6) and outcomes. A contextualised approach was used to assess imprecision and inconsistency domains (Schünemann et al., 2022; Guyatt et al., 2023). Absolute effects were used for all outcomes to assess imprecision. The MD was used for continuous outcomes and for dichotomous outcomes, risk differences (RD) were calculated by applying the relative effects to a measure of baseline risk. We used the median from all studies in which participants received the comparator to calculate the baseline risk, with each study weighed equally. In instances, such as mortality, where the number of

Table 2
Certainty definitions and short descriptions of the five domains used to grade the evidence using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.

Definition	Description
Certainty[a]	
Very low	The true effect is probably markedly different from the estimated effect
Low	The true effect might be markedly different from the estimated effect
Moderate	The authors believe that the true effect is probably close to the estimated effect
High	The authors have a lot of confidence that the true effect is similar to the estimated effect
Domains[b]	
Risk of bias	Bias occurs when the results of a study do not represent the truth because of inherent limitations in design or conduct of a study. The body of evidence is rated at the outcome level rather than the study level.
Inconsistency	When several studies show consistent effects the certainty of evidence is higher. Statistical criteria for heterogeneity (I^2 and chi-squared test), similarity of point estimates and the overlap of CI are evaluated in the rating process.
Indirectness	Evidence is most certain when studies directly compare the interventions of interest in the population of interest, and report the outcome(s) critical for decision-making. Certainty can be rated down if the dogs studied are different from those that the recommendation refers to. Indirectness can also occur when the interventions studied are different than the real outcomes.
Imprecision[c]	This domain was recently updated and relies on thresholds and CI of absolute effects as a primary criterion for imprecision rating (i.e., CI approach). Certainty is downgraded when confidence intervals cross predefined clinical thresholds (trivial, small, moderate, large) that are of importance to stakeholders. If the relative effect is large the optimal information size is also considered.
Other considerations	Publication bias can contribute to further downgrading, but the consideration of large effects, plausible confounding, and dose response gradients can occasionally lead to upgrading of the certainty of evidence.

CI, confidence intervals
[a] Cited from Guyatt et al. (2008)
[b] Adapted from Guyatt et al. (2008)
[c] Adapted from Schünemann et al. (2022);

events was too low (wide confidence interval around the relative effect), we calculated the 95% CI around the absolute RD using the total number of dogs and events for that outcome. The effect estimate was considered precise if the 95% CI did not cross any thresholds. In contrast, the effect estimate was considered imprecise if the 95% CI was crossing one or more thresholds, because decision-making could be affected should the true effect lie in one or the other end of the 95% CI. The severity of imprecision was determined based on how many thresholds the 95% CI crosses.

The Cochrane risk-of-bias tool for randomised trials (RoB 2.0) was used to assess risk of bias (CP, KS, MW, MB). One author (MW) abstained from assessing risk of bias for studies they had written or co-authored (Werner et al., 2020, Unterer et al., 2011). One study, written in German (Israiloff, 2009), was assessed by native German speakers alone (MW, SU).

Results

PICO 1. In dogs with acute diarrhoea, does antimicrobial treatment compared to no antimicrobial treatment have an effect?

Included studies

The search yielded 1068 reports after duplicates were removed, of which six RCTs addressed the question (Fig. 2). Reasons for exclusion of potentially eligible reports that were selected for full text screening are presented in Supplementary file 4. Five of the studies (Unterer et al., 2011, Shmalberg et al., 2019, Werner et al., 2020, Langlois et al., 2020, Rudinsky et al., 2022) were published in peer reviewed journals and one study was part of a doctoral thesis (Israiloff, 2009). The six studies included a total of 232 privately owned dogs and there were no baseline differences regarding age, breed, sex, and severity of disease between the intervention and comparator group in any of the studies. One study included dogs representing the P1 subgroup only (Werner et al., 2020), one study included dogs in the P1 and P2 subgroups (Rudinsky et al., 2022), and two studies included dogs in both P1 and P3 subgroups (Shmalberg et al., 2019; Langlois et al., 2020). The dogs representing the P3 subgroup were treated as outpatients and were not hospitalised overnight but did receive intravenous or subcutaneous fluids for a few hours. Two studies included dogs representing the P4 subgroup which were hospitalised in a university-clinic setting (Israiloff, 2009; Unterer et al., 2011). No RCTs were found for dogs representing the P5 or P6 subgroups. Further details of study characteristics are presented in Table 3.

Effect of antimicrobial treatment versus no antimicrobial treatment

Duration of diarrhoea was reported in all six studies ($n = 232$ dogs) and was included in a pairwise meta-analysis as a continuous outcome (Fig. 2). The pooled MD for all subgroups (−0.28 days; 95% CI −0.77, 0.21; $I^2 = 55\%$) was in marginal favour of the intervention (antimicrobial treatment), but of trivial clinical effect as predefined by the panel and end-users. Subgroups of hospitalised (P4; MD −0.38 days; 95% CI −0.81, 0.04; $I^2 = 0$) and not hospitalised (P1-P3; MD −0.07 days; 95% CI −1.19, 1.05; $I^2 = 72\%$) dogs showed no clinically relevant difference between groups (Fig. 2). Heterogeneity in the not hospitalised group ($I^2 = 72\%$) was reduced when Rudinsky et al. (2022), which used a more conservative definition of time to remission, was excluded ($I^2 = 40\%$); however, the point estimate remained nearly the same. Results were thus not likely to be affected by heterogeneity.

Conversely, pooled duration of hospitalization (MD 0.37 days; 95% CI 0.04, 0.69; $I^2 = 0$) was in favour of the placebo (Fig. 3), but this was also a trivial effect. Mortality only occurred in the hospitalised population (Israiloff, 2009; Unterer et al., 2011). Pairwise meta-analysis (Fig. 4) was in favour of the placebo group (OR 1.43; 95% CI 0.24, 8.54; $I^2 = 0$). The anticipated absolute effects showed an increased risk of mortality of dogs treated with antimicrobials with eight more per 1000 dogs (95% CI, from 29 fewer to 47 more; Supplementary file 4). Disease progression was only reported in one study (Unterer et al., 2011). It occurred in two dogs from the placebo group that were excluded before outcomes were measured in the original trial. One of the dogs improved during the first days of hospitalization but had worsened clinical signs on day four, the other dog developed leucopenia (unpublished data provided by the author). Data relating to the two dogs have been included in the analysis (Fig. 5) with the aim of performing an intention to treat analysis RD (0.13; 95% CI 0.01, 2.14). The anticipated absolute effects showed a reduced risk of progression of disease in dogs that were treated with antimicrobials with 20 fewer per 1000 dogs (95% CI from 70 fewer to 30 more). There were no adverse effects reported in any of the studies. Antimicrobial treatment did not have a clinically relevant effect in comparison to no treatment for any of the outcomes.

Certainty of evidence

Risk of bias (Supplementary Figs. S1-S2; Supplementary file 4) was low in most of the six studies except for the allocation concealment domain, which was adequately reported in only one study (Israiloff, 2009). After contacting authors for additional information, no allocation concealment was performed in two of the studies, resulting in a high risk of bias (Unterer et al., 2011; Werner et al., 2020), and no information was received from the authors of the three remaining studies. The

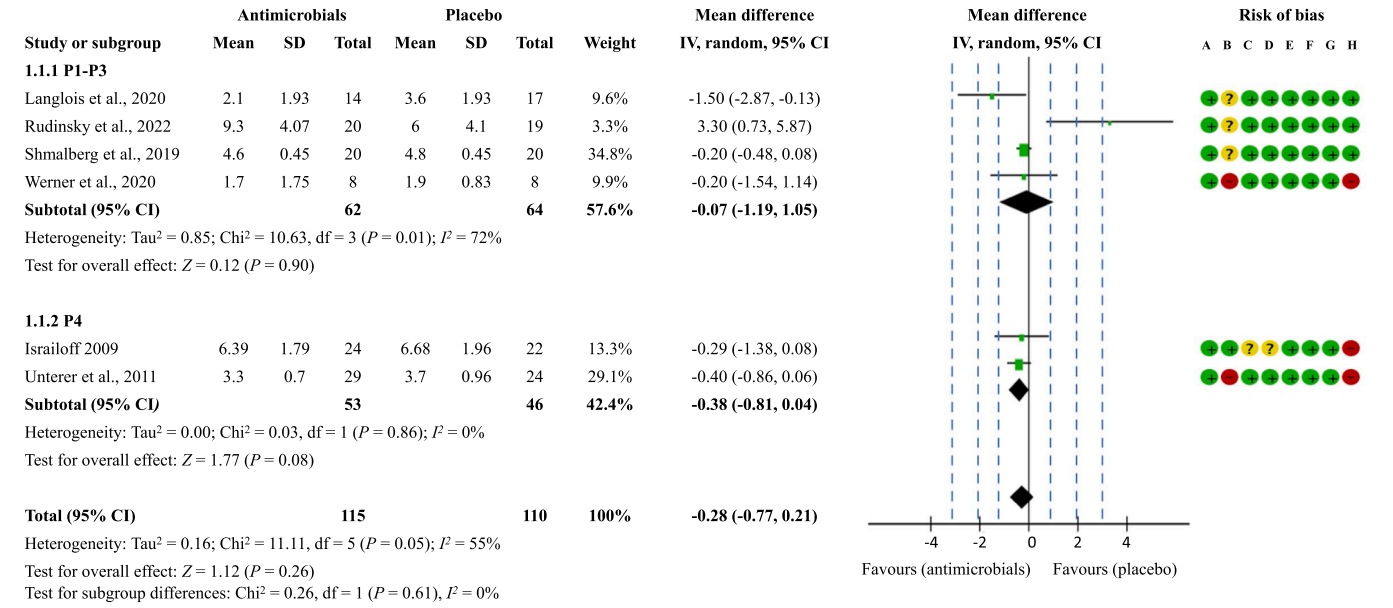

Fig. 2. Forest plot of duration of diarrhoea (days) in dogs with acute diarrhoea treated with an antimicrobial or a placebo for individual trials and overall (black diamond). Studies are subgrouped by dogs of population P1-P3 (dogs with mild disease and dogs with moderate disease and non-haemorrhagic diarrhoea) and population 4 (dogs with moderate disease with haemorrhagic diarrhoea). Effects of trials are presented as mean differences (95% confidence interval, CI; represented as whiskers). IV, inverse variance; SD, standard deviation. Dashed vertical lines represent clinical thresholds (<1 day, trivial effect; 1–2 days, small effect; 2–3 days, moderate effect; >3 days, large effect). P1, Subgroup of dogs with mild disease and acute non-haemorrhagic diarrhoea. P2, Subgroup of dogs with mild disease and haematochezia. P3, Subgroup of dogs with moderate disease and acute non-haemorrhagic diarrhoea. P4, Subgroup of dogs with moderate disease and acute haemorrhagic diarrhoea. Risk of bias analysis can be found on the right: A, random sequence generation (selection bias); B, allocation concealment (selection bias); C, masking of participants and personal (performance bias); D, masking of outcome assessment (detection bias); E, incomplete outcome data (attrition bias); F, selective reporting (reporting bias); G, other bias; H, overall. Green dots represent low risk, yellow dots unknown risk, and red dots high risk.

overall selection bias was not downgraded as there were no baseline differences between groups in any of the studies and randomization was adequate. Outcome was assessed by dog owners who were masked to group allocation (and by veterinarians for hospitalised dogs) by predefined scoring systems in all studies, none of which had been validated in dogs (Table 3). The review group did not consider the differences in scoring systems to be of substantial clinical importance and the evidence was thus not downgraded. Absolute effects were used to evaluate imprecision. The overall certainty of evidence was high in subgroups P1-P4 and low in P5-P6 (Table 4). Summary of finding tables for all outcomes are presented in Supplementary file 4. Sensitivity analysis using alternative statistical models and effect measures (random vs. fixed; Mantel-Haenszel vs. Peto), and the exclusion of studies with substantial weight, did not show a change in results.

PICO 2. In dogs with acute diarrhoea, does metronidazole treatment have a superior effect compared to beta-lactam treatment?

The search (Fig. 6) did not yield any studies comparing efficacy between the different antimicrobials. We consequently included the six trials that were selected for PICO 1 in a network meta-analysis to make an indirect comparison between metronidazole and beta-lactams. A network graph is presented in Fig. 7. Beta-lactams (amoxicillin-clavulanic acid) were marginally more efficient (MD −0.29 days; 95% CI −2.24, 1.65) in shortening the duration of diarrhoea in comparison to metronidazole but was not of clinical relevance (Table 5). Certainty of evidence was very low in subgroups P1-P6 (Table 4). Metronidazole does not have a superior effect in comparison to beta-lactams based on the included trials.

PICO 3: In dogs with acute diarrhoea, does long duration (≥7 days) of antimicrobial treatment have a superior effect compared to short duration (<7 days) of treatment?

The systematic search (Fig. 2) did not identify any studies comparing the effect of short vs. long duration of antimicrobial treatment. There were, however, eight trials that included duration of antimicrobial treatment and outcome from which data has been summarised narratively (Table 6). Dogs were treated for 7 days ($n = 75$ dogs), 10 days ($n = 20$ dogs), 7–10 days ($n = 20$ dogs), or 6 days ($n = 15$ dogs). Diarrhoea resolved in most dogs before antimicrobial treatment was terminated and diarrhoea did not exceed 7 days (mean or median) except in one study (Rudinsky et al., 2022). In most studies, resolution of diarrhoea occurred after 2–5 days, which is a similar duration to dogs that received no antimicrobials (PICO 1).

PICO 4–6: In dogs with acute diarrhoea, does treatment with probiotics (PICO 4), synbiotics (PICO 5), or prebiotics (PICO 6) compared to no treatment shorten the duration of diarrhoea?

Included studies

Four trials that addressed PICO 4 were identified in the systematic search (Fig. 8), and single trials were found for each of PICO 5 and PICO 6. All the included studies were prospective, randomised, and controlled and included a total of 293 (intervention group, $n = 144$; comparator group, $n = 149$) privately owned dogs that were presented to veterinary clinics and hospitals for idiopathic acute diarrhoea. A placebo that was indistinguishable from the intervention was given to the control group in all trials. There were no baseline differences between intervention and comparator groups in any of the studies. Three studies included only dogs from the P1 subgroup ($n = 189$; Herstad et al. 2010, Gomez-Gallego et al., 2016, Nixon et al. 2019), one study included dogs from subgroup P1 and P2 ($n = 40$; Rudinsky et al., 2022), and one study included dogs from both P1 ($n = 15$) and P3 subgroups ($n = 25$; Shmalberg et al., 2019). Dogs from subgroups P3 were treated as outpatients and were not hospitalised overnight but received intravenous or subcutaneous fluids for a few hours. One study included dogs from subgroups P4 ($n = 25$) who were all hospitalised (Ziese et al., 2018). All probiotic products contained *Lactobacillus* species, but most preparations included additional bacterial species (Table 7).

Table 3
Study characteristics for PICO 1 (*In dogs with acute diarrhoea, does antimicrobial treatment compared to no antimicrobial treatment have an effect?*). Prospective randomised double masked controlled trials. All dogs had acute diarrhoea of unknown aetiology. Faecal flotation and haematology / serum biochemistry were used in all studies to exclude parasitism and other systemic causes of disease.

Study (author, year)	Subpopulation	Intervention			Comparator		Duration (days)	Clinical outcome assessment	
		n (dogs)	Substance	Dose, route	*n* (dogs)	Placebo		Scoring method	Assessment criteria
Shmalberg et al., 2019	P1 and P3	20	Metronidazole	11.2–24.0 mg/kg twice daily, PO	20	Sucrose	10	Waltham faecal scoring	Faecal consistency on a scale of 1–5. Time to remission was defined as days to the first normal faecal score (≤3)
Langlois et al., 2020	P1 and P3	14	Metronidazole	10–15 mg/kg twice daily, PO	17	Microcrystalline cellulose	7	Bristol faecal chart	Faecal consistency on a scale 1–7. Time to remission was defined as days to two normal sequential faecal scores (≤4)
Rudinsky et al., 2022	P1 and P2	20	Metronidazole	5–10 mg/kg twice daily, PO	19	Tablet (ingredients not stated)	7–10	Waltham faecal scoring (including photography for study investigators)	Faecal consistency on a scale of 1–5 and wellness survey. Time to remission was defined as three consecutive days with a normal faecal score (≤3)
Werner et al., 2020	P1	8	Amoxicillin-clavulanic acid	12.5–25 mg/kg twice daily, PO	8	Lactose	7	Canine acute diarrhoea severity index (CADS)	Activity, appetite, vomiting, faecal consistency (scale 0–3), frequency of defecation. Time to remission was defined as days to the first normal CADS-Index (≤3)
Unterer et al., 2011	P4	30	Amoxicillin-clavulanic acid	7 mg/kg daily, SC *or* 12.5 mg/kg twice daily, PO	30	Not stated	7	Canine haemorrhagic gastroenteritis (HGE) activity index	Appetite, vomiting, faecal consistency (scale 0–3), frequency of defecation, dehydration. Time to remission was defined as days to the first normal canine HGE activity index (0)
Israiloff, 2009	P4	24	Amoxicillin-clavulanic acid and metronidazole	22 mg/kg twice daily IV *or* PO and 15 mg/kg twice daily IV *or* PO	22	Not stated	Not stated	Presence of diarrhoea	Yes/No. Time to remission was defined as days to the first normal stool.

PICO, Population, intervention, comparator, outcome; P1, Subgroup of dogs with mild disease and acute non-haemorrhagic diarrhoea; P2, Subgroup of dogs with mild disease and haematochezia; P3, Subgroup of dogs with moderate disease and acute non-haemorrhagic diarrhoea; P4, Subgroup of dogs with moderate disease and acute haemorrhagic diarrhoea.

Effect of probiotic/synbiotic/prebiotic treatment versus no treatment

Duration of diarrhoea was the main outcome for all PICOs, which was assessed by dog owners by predefined unvalidated scoring systems. However, Herstad et al. (2010) did not report a scale, only presence or absence of diarrhoea (Table 7). Gomez-Gallego et al. (2016) described the mean faecal score after 7 and 28 days, but supplementary data were provided by the authors that enabled calculation of mean duration of diarrhoea. The four probiotic trials were included in a pairwise meta-analysis (Fig. 9) that showed a trivial clinical effect in favour of the intervention (MD −0.68 days; 95% CI −1.28, −0.09; $I^2 = 0$). The single synbiotic trial also showed a trivial effect in favour of the intervention (MD −0.62 days; 95% CI −1.07, −0.17). While the prebiotic trial showed a small clinical effect in favour of the intervention (MD −1.2 days; 95% CI −3.77, 1.37). No events of mortality or adverse effects were reported in any of the studies. Progression of disease (additional medical intervention due to non-improvement or worsening) was reported at a higher frequency in the placebo group in comparison to the synbiotic group ($P = 0.04$) with a relative risk of 0.88 (95% CI 0.77, −0.99) by Nixon et al. (2019). Duration of hospitalization was not reported in a population of hospitalised dogs (Ziese et al., 2019), but a null overall effect between the intervention and comparator was found.

Certainty of evidence

Risk of bias was generally low (Supplementary Figs. S3-S4; Supplementary file 4). Allocation concealment was the only domain of unclear or high risk of bias and was not downgraded based on the same rationale as described for PICO 1. Overall certainty of evidence was moderate for PICO 4 and PICO 5, and very low for PICO 6 (Table 4).

Discussion

There is high certainty evidence that antimicrobial treatment does not reduce the duration of diarrhoea in mild and moderate disease irrespective of antimicrobial class or duration of treatment. Antimicrobial treatment also failed to affect other outcomes including mortality, duration of hospitalization, and progression of disease. No evidence was

Study or subgroup	Antimicrobials Mean	SD	Total	Placebo Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
1.3.1 P1-P3								
Langlois et al., 2020	0	0	14	0	0	17	-	Not estimable
Rudinsky et al., 2022	0	0	20	0	0	19	-	Not estimable
Shmalberg et al., 2019	0	0	20	0	0	20	-	Not estimable
Werner et al., 2020	0	0	8	0	0	8	-	Not estimable
Subtotal (95% *CI*)			**62**			**64**		**Not estimable**
Heterogeneity: Not applicable								
Test for overall effect: Not applicable								
1.1.2 P4								
Israiloff 2009	3.59	1.52	24	3.36	1.34	22	15.4%	0.23 (-0.60, 1.06)
Unterer et al., 2011	3.61	0.85	29	3.22	0.42	24	84.6%	0.39 (0.04, 0.74)
Subtotal (95% CI)			**53**			**46**	**100%**	**0.37 (0.04, 0.69)**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.12$, df = 1 ($P = 0.73$); $I^2 = 0\%$								
Test for overall effect: $Z = 2.21$ ($P = 0.03$)								
Total (95% CI)			**115**			**110**	**100%**	**0.37 (0.04, 0.69)**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.12$, df = 1 ($P = 0.73$); $I^2 = 0\%$								
Test for overall effect: $Z = 2.21$ ($P = 0.03$)								
Test for subgroup differences: Not applicable								

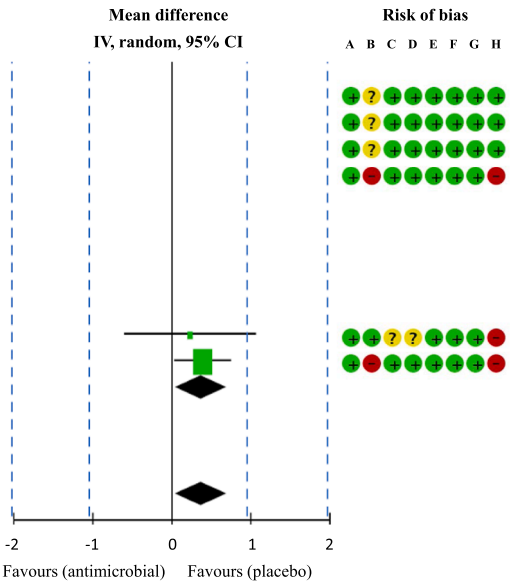

Fig. 3. Forest plot of time of hospitalization (in days) in dogs with acute diarrhoea treated with an antimicrobial or a placebo for individual trials and overall (black diamond). Effects of trials are presented as mean differences (95% confidence interval, CI; represented as whiskers). IV, inverse variance; SD, standard deviation. Dashed vertical lines represent clinical thresholds (<1 day, trivial effect; 1–2 days, small effect; 2–3 days, moderate effect). P1, Subgroup of dogs with mild disease and acute non-haemorrhagic diarrhoea. P2, Subgroup of dogs with mild disease and haematochezia. P3, Subgroup of dogs with moderate disease and acute non-haemorrhagic diarrhoea. P4, Subgroup of dogs with moderate disease and acute haemorrhagic diarrhoea. Risk of bias analysis can be found on the right: A, random sequence generation (selection bias); B, allocation concealment (selection bias); C, masking of participants and personal (performance bias); D, masking of outcome assessment (detection bias); E, incomplete outcome data (attrition bias); F, selective reporting (reporting bias); G, other bias; H, overall. Green dots represent low risk, yellow dots unknown risk, and red dots high risk.

Study or subgroup	Antimicrobials Events	Total	Placebo Events	Total	Weight	Peto odds ratio Peto, fixed, 95% CI
1.2.1 P1-P3						
Langlois et al., 2020	0	14	0	17	-	Not estimable
Rudinsky et al., 2022	0	20	0	19	-	Not estimable
Shmalberg et al., 2019	0	20	0	20	-	Not estimable
Werner et al., 2020	0	8	0	8	-	Not estimable
Subtotal (95% *CI*)		**62**		**64**		**Not estimable**
Total events	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
1.2.2 P4						
Israiloff 2009	2	24	1	22	59.3%	1.84 (0.18, 18.64)
Unterer et al., 2011	1	30	1	30	40.7%	1.00 (0.06, 16.37)
Subtotal (95% *CI*)		**54**		**52**	**100%**	**1.43 (0.24, 8.54)**
Total events	3		2			
Heterogeneity: $Chi^2 = 0.11$, df = 1 ($P = 0.74$); $I^2 = 0\%$						
Test for overall effect: $Z = 0.40$ ($P = 0.69$)						
Total (95% CI)		**116**		**116**	**100%**	**1.43 (0.24, 8.54)**
Total events	3		2			
Heterogeneity: $Chi^2 = 0.11$, df = 1 ($P = 074$); $I^2 = 0\%$						
Test for overall effect: $Z = 0.40$ ($P = 0.69$)						
Test for subgroup differences: Not applicable						

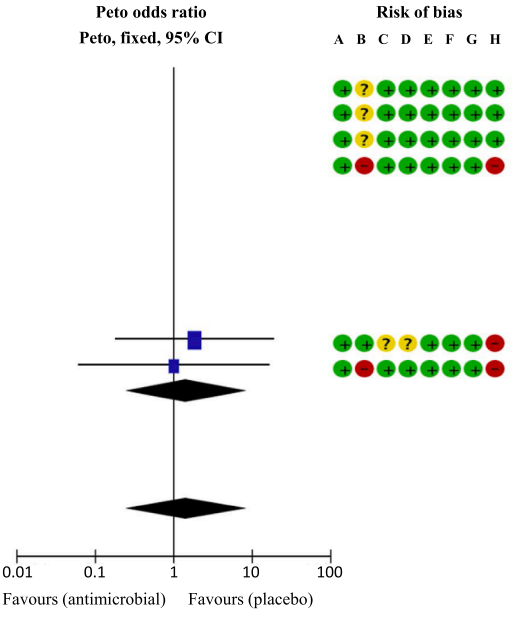

Fig. 4. Forest plot of mortality in dogs with acute diarrhoea treated with an antimicrobial or a placebo in subpopulation 4 (dogs with moderate disease with haemorrhagic diarrhoea), with the overall effect included (black diamond). Effects of trials are presented as Peto odds ratio (95% confidence interval, CI; represented as whiskers). P1, Subgroup of dogs with mild disease and acute non-haemorrhagic diarrhoea. P2, Subgroup of dogs with mild disease and haematochezia. P3, Subgroup of dogs with moderate disease and acute non-haemorrhagic diarrhoea. P4, Subgroup of dogs with moderate disease and acute haemorrhagic diarrhoea. Risk of bias analysis can be found on the right: A, random sequence generation (selection bias); B, allocation concealment (selection bias); C, masking of participants and personal (performance bias); D, masking of outcome assessment (detection bias); E, incomplete outcome data (attrition bias); F, selective reporting (reporting bias); G, other bias; H, overall. Green dots represent low risk, yellow dots unknown risk, and red dots high risk. See Results for absolute effects and Table 1 for clinical thresholds.

found for dogs with severe disease, which reduces the certainty in the interpretation of results for this population. The lack of representation of dogs with severe disease in the included RCTs is not a surprising limitation, since the risk of withholding antimicrobials from animals with potential sepsis could be perceived as clinically and ethically unacceptable. No clinical adverse effects of antimicrobials were found in

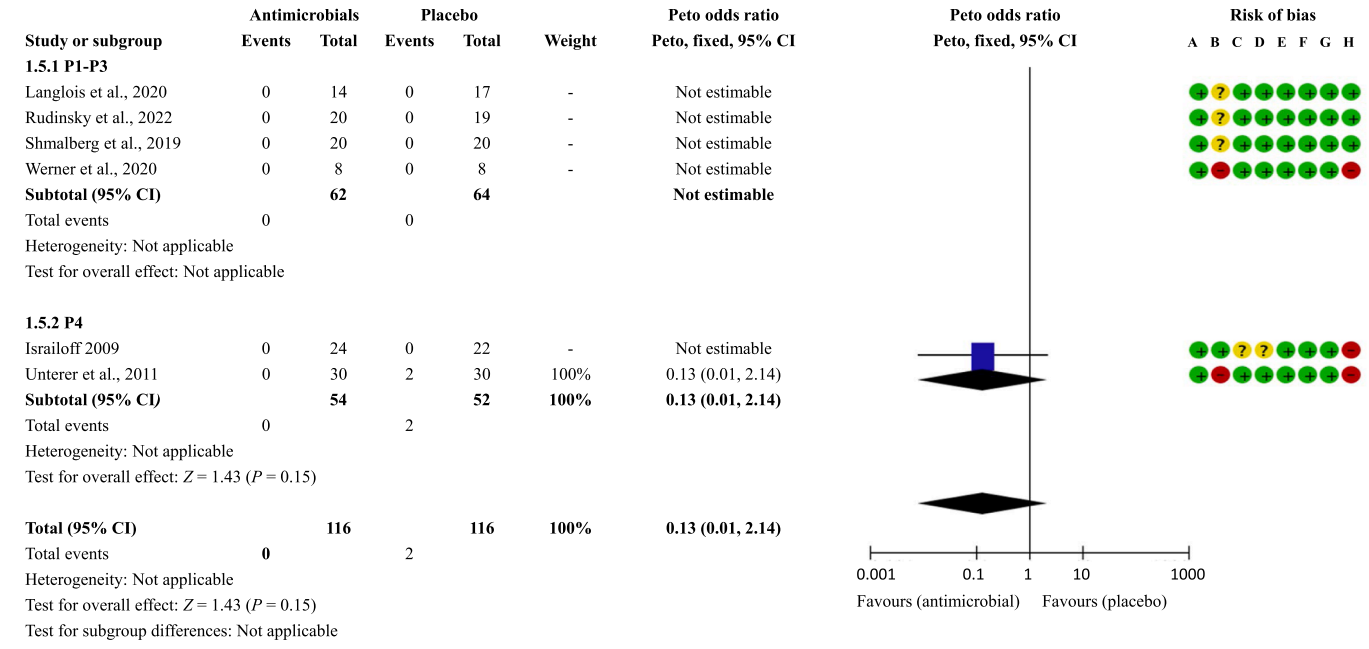

Fig. 5. Forest plot of progression of disease in dogs with acute diarrhoea treated with an antimicrobial or a placebo. Effects of trial is presented as Peto odds ratio (95% confidence interval, CI; represented as whiskers). P1, Subgroup of dogs with mild disease and acute non-haemorrhagic diarrhoea. P2, Subgroup of dogs with mild disease and haematochezia. P3, Subgroup of dogs with moderate disease and acute non-haemorrhagic diarrhoea. P4, Subgroup of dogs with moderate disease and acute haemorrhagic diarrhoea. Risk of bias analysis can be found on the right: A, random sequence generation (selection bias); B, allocation concealment (selection bias); C, masking of participants and personal (performance bias); D, masking of outcome assessment (detection bias); E, incomplete outcome data (attrition bias); F, selective reporting (reporting bias); G, other bias; H, overall. Green dots represent low risk, yellow dots unknown risk, and red dots high risk. See Results section for absolute effects and Table 1 for clinical thresholds.

Table 4
Certainty of evidence for PICOs 1, 2, and 4–6 (PICO 3 was described narratively). Outcomes (duration of diarrhoea, mortality, duration of hospitalization, progression of disease, and adverse effects) are not shown separately here since results were the same for all outcomes but are presented in summary of findings tables in Supplementary file 3. Absolute effects were considered in the mortality (29 fewer to 47 more) and progression of disease (70 fewer to 30 more) outcomes when evaluating imprecision.

PICO	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Overall certainty
PICO 1 (P1-P4)	Not serious	Not serious	Not serious	Not serious	None	High
PICO 1 (P5-P6)	Not serious	Not serious	Very serious[a]	Not serious	None	Low
PICO 2 (P1-P4)	Not serious	Not serious	Not serious	Extremely serious[b]	None	Very low
PICO 2 (P5-P6)	Not serious	Not serious	Very serious[a]	Extremely serious[b]	None	Very low
PICO 4	Not serious	Not serious	Not serious	Serious[c]	None	Moderate
PICO 5	Not serious	Not serious	Not serious	Serious[d]	None	Moderate
PICO 6	Not serious	Not serious	Not serious	Extremely serious[e]	None	Very low

PICO, Population, intervention, comparator, outcome; PICO 1, In dogs with acute diarrhoea, does antimicrobial treatment compared to no antimicrobial treatment have an effect?; PICO 2, In dogs with acute diarrhoea, does metronidazole treatment have a superior effect compared to beta-lactam treatment?; PICOs 4–6: In dogs with acute diarrhoea, does treatment with probiotics (PICO 4), synbiotics (PICO 5), or prebiotics (PICO 6) compared to no treatment shorten the duration of diarrhoea? P1, Subgroup of dogs with mild disease and acute non-haemorrhagic diarrhoea; P2, Subgroup of dogs with mild disease and haematochezia; P3, Subgroup of dogs with moderate disease and acute non-haemorrhagic diarrhoea; P4, Subgroup of dogs with moderate disease and acute haemorrhagic diarrhoea; P5, Subgroup of dogs with severe disease and acute non-haemorrhagic diarrhoea; P6, Subgroup of dogs with severe disease and acute non-haemorrhagic diarrhoea.
[a] As no dogs in subgroups P5 or P6 were represented in any of the included studies, indirectness was downgraded two levels.
[b] As confidence intervals crossed three thresholds (2 days in intervention group and 1 day in comparator), imprecision was downgraded three levels.
[c] As confidence intervals crossed one threshold (1 day), imprecision was downgraded one level.
[d] As confidence intervals crossed one threshold (1 day), imprecision was downgraded one level.
[e] As confidence intervals crossed three thresholds (3 days) in the intervention groups and one threshold (1 day) in the placebo group, imprecision was downgraded three levels.

any of the studies; however, other controlled trials of healthy dogs have reported antimicrobial-associated (metronidazole and enrofloxacin) gastrointestinal signs (Whittemore et al., 2019; Pilla et al., 2020). These results may not be directly generalizable to the target population of the present work. The cited study population presented with diarrhoea prior to antimicrobial administration, which could have masked adverse treatment effects.

No previous systematic review has investigated antimicrobial treatment of CAD; however, authors of several narrative reviews have advised against antimicrobial treatment in dogs with acute diarrhoea for the past decade (Marks et al., 2011, Weese, 2011, Guardabassi et al., 2018; Unterer et al., 2021; Werner et al., 2021; Busch et al., 2022), which is consistent with the results of this study.

With regard to nutraceuticals, no clinically relevant effects on duration of diarrhoea with probiotic (moderate certainty evidence) or synbiotic treatment (moderate certainty), were identified although a small favourable effect was reported in a single study of prebiotics (very low certainty). Use of probiotics in dogs with acute and chronic

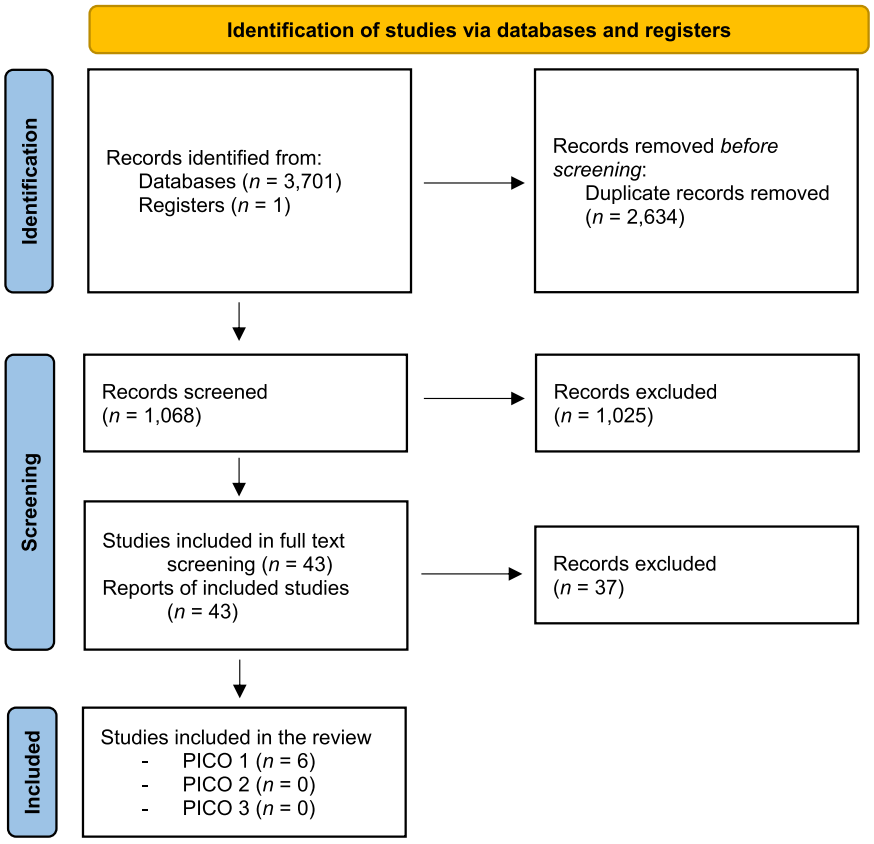

Fig. 6. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. Search PICO 1–3 (using the following databases: Web of Science, CAB, PubMed/Medline): PICO 1: In dogs with acute diarrhoea, does antimicrobial treatment compared to no antimicrobial treatment have an effect? PICO 2: In dogs with acute diarrhoea, does metronidazole treatment have a superior effect compared to beta-lactam treatment? PICO 3: In dogs with acute diarrhoea, does long duration (≥7 days) of antimicrobial treatment have a superior effect compared to short duration (<7 days) of treatment? PICO, Population, intervention, comparator, outcome.

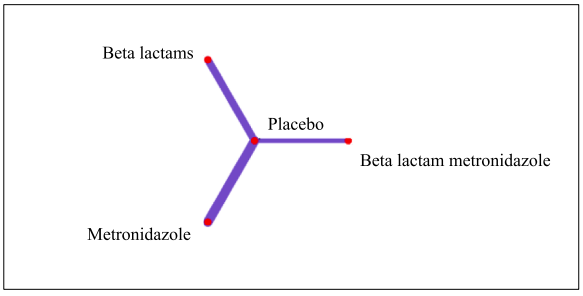

Fig. 7. Network diagram. The nodes in the network represents the different interventions, and the edges connecting the nodes represents direct comparisons between interventions. The size of the nodes was proportional to the number of participants receiving the intervention, and the thickness of the edges was proportional to the number of studies comparing the connected interventions.

Table 5
Results of network meta-analysis. The estimated mean differences in days (95% confidence intervals) from all possible pairwise comparisons in the network meta-analysis of four treatment groups.

Comparison of substances	Mean difference (days)	Confidence interval
Beta-lactam vs. metronidazole	0.29	-2.24; 1.65
Beta-lactam vs. placebo	0.32	-1.76; 1.13
Metronidazole vs. placebo	0.03	-1.33; 1.28
Beta-lactam and metronidazole vs. placebo	-0.29	-2.41; 1.83
Beta-lactam and metronidazole vs. beta-lactam alone	0.03	-2.54; 2.60
Beta-lactam and metronidazole vs. metronidazole alone	-0.26	-2.76; 2.23

diarrhoea has been investigated in a systematic review by Jensen and Bjørnvad (2019), including 12 studies of acute diarrhoea. No meta-analysis was performed due to significant heterogeneity and the authors reported that the narratively synthesised evidence was not sufficiently robust to determine the effectiveness and clinical relevance of probiotics. In this systematic review, the eligibility criteria were more stringent (no preventative studies of healthy dogs or co-interventions with antimicrobials were included) in order to reduce indirectness. This systematic review also includes two of the studies (Herstad et al., 2010; Gomez-Gallego et al., 2016) included in that review and two additional probiotic studies (Ziese et al., 2018; Shmalberg et al., 2019) published since then. In addition, the study described herein evaluated certainty of evidence, performed a pairwise meta-analysis, and established clinical thresholds of importance to end-users, which has enabled an objective assessment of results. The pooling and comparison of probiotic products of different bacterial strains and dosages could be questionable since documented benefits and/or harms might be applicable only to the specific product; however, these were chosen for inclusion in the pairwise meta-analysis since results were similar and pointed in the same direction.

The primary focus of the systematic review was on outcomes that are of immediate clinical relevance to the dogs and end-users (veterinary practitioners and dog-owners), but acknowledge that other outcomes, such as disturbances of the intestinal microbiota and metabolome are

Table 6
Study characteristics for PICO 3 (In dogs with acute diarrhoea, does long duration (≥7 days) of antimicrobial treatment have a superior effect compared to short duration (<7days) of treatment?). Prospective randomised trials that include duration of treatment and resolution of diarrhoea.

Study (author, year)	Sub-group	Treatment duration (days)	Antimicrobial treatment given (number of dogs)	Duration of diarrhoea (days)
Chaitman et al., 2020	P1	7	Metronidazole (7)	7 (median)[a]
Fenimore et al., 2017	P1 (shelter dogs)	7	Metronidazole (16)	3 (median)
Rudinsky et al., 2022	P1 and P2	7–10	Metronidazole (20)	8.5 (median)
Langlois et al., 2020	P1 and P3	7	Metronidazole (14)	2.1 ± 1.6 (mean)
Shmalberg et al., 2019	P1 and P3	10	Metronidazole (20)	4.6 ± 2.4 (mean)
Pignataro et al., 2021	P1	6	Metronidazole and spiramycin (15)	3 (median)
Unterer et al., 2011	P4	7	Amoxicillin-clavulanic acid (30)	3.3 (mean)
Werner et al., 2020	P1	7	Amoxicillin-clavulanic acid (8)	2 (median)

PICO, Population, intervention, comparator, outcome.
P1, Subgroup of dogs with mild disease and acute non-haemorrhagic diarrhoea; P2, Subgroup of dogs with mild disease and haematochezia; P3, Subgroup of dogs with moderate disease and acute non-haemorrhagic diarrhoea; P4, Subgroup of dogs with moderate disease and acute haemorrhagic diarrhoea.
[a] A significantly decreased faecal score (i.e. a more normal stool consistency) was observed on day 7 in comparison to baseline (day 0). There was no reported assessment between baseline (day 0) and day 7.

likely to impact gut health. Moreover, the selective pressure of enteropathogenic resistance is likely to have negative implications on animal and public health. Werner et al. (2020) reported an increase in resistant faecal bacteria in dogs with CAD treated with amoxicillin-clavulanic acid, and Rudinsky et al. (2022) found that metronidazole treatment of CAD negatively affected the faecal dysbiosis index. In contrast, three studies showed a subjectively favourable change in the microbiota in dogs receiving probiotics or prebiotics for CAD in comparison to dogs receiving placebo (Gomez-Gallego et al., 2016; Ziese et al., 2018; Rudinsky et al., 2022). These are important considerations in the balance of benefits and harms, which must be taken into account when making management recommendations in CAD.

A statistical threshold (e.g., $p = 0.05$) cannot measure the clinical importance of a result and should thus not be used by itself to support scientific conclusions and policy decisions as stated by the American Statistical Association (2016). In this systematic review a contextualised approach was used when assessing the evidence as recommended and described by GRADE. The aim of the contextualised approach is to assess the effect of a given intervention in a specific clinical context, using thresholds for clinical relevance. The GRADE methodology engages clinicians and other stakeholders involved in decision- making when generating thresholds, to ensure that the interpretation of results is meaningful to end-users (Hultcrantz et al., 2017). The involvement of general practitioners and dog-owners in the prioritization of outcomes, generation of clinical thresholds, and acceptability of definitions is the primary strength of this study. The systematic approach in finding the best available evidence, and rigorous evaluation of included trials by using the GRADE approach contributes to the robustness of the results.

The low numbers of dogs reported in the available studies could be a limitation in the interpretation of results, but the certainty of evidence and agreement between studies was generally high. There were few events of mortality and progression of disease in the included studies and few events are expected to make statistical results less certain. This could lead to an overinterpretation of results and the authors want to emphasise that results have been interpreted with caution. For example, the authors believe that type 1 statistical error is more likely to account for the higher mortality in the antimicrobial treatment group (PICO 1)

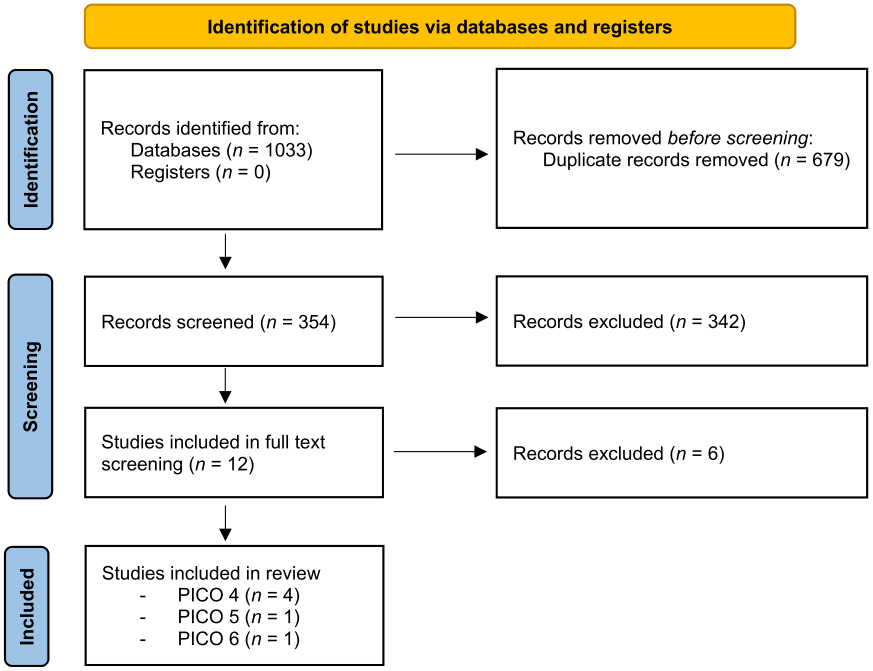

Fig. 8. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram Search PICO 4–6 (using the following databases: Web of Science, CAB, PubMed/Medline). PICO 4–6: In dogs with acute diarrhoea, does treatment with probiotics (PICO 4), synbiotics (PICO 5), or prebiotics (PICO 6) compared to no treatment shorten the duration of diarrhoea? PICO, Population, intervention, comparator, outcome.

Table 7
Study characteristics for PICO 4–6 (In dogs with acute diarrhoea, does treatment with probiotics (PICO 4), synbiotics (PICO 5), or prebiotics (PICO 6) compared to no treatment shorten the duration of diarrhoea?). All were prospective randomised double masked controlled trials. All recruited dogs had acute diarrhoea of unknown aetiology. Faecal flotation and haematology/ serum biochemistry were used to exclude parasitism and other systemic causes of disease in most studies.

Study (author, year)	Subpopulation	Intervention		Comparator		Duration (days)	Clinical outcome assessment (scoring method / assessment criteria)
		n	Type of nutraceutical, bacteria, and dose	*n*	Placebo		
Shmalberg et al., 2019	P1 and P3	20	Probiotic (Vital Vet; *Lactobacillus* spp. and *Bifidobacterium* spp.) Dose of 30 billion CFU (capsule) twice daily.	20	Sucrose	10	Waltham faecal score (scale of 1–5). Time to remission was defined as days to the first normal faecal score (≤3).
Gomez-Gallego et al., 2016	P1	25	Probiotic (sour milk, not commercially available; *Lactobacillus* spp. 2 billion CFU/mL) Dose of 200 mL per dog once (or divided twice) daily.	19	Sterilised water and 10% titanium	7	Waltham faecal score (scale of 1–5). Time to remission was defined as days to the first normal faecal score (≤3; this was calculated by the present authors on data provided by the original authors).
Herstad et al., 2010	P1	15	Probiotic (ZooLac Propaste; *Lactobacillus, Bacillus, Farciminis, Acidilactic, Licheniformis*, and *Pediococcus* spp. 4.2 billion CFU/mL). Dose based on body weight (1–10 kg, 1 mL; 10–25 kg, 2 mL; 25–50 kg, 3 mL) three times daily	21	The same pasta-base with vegetable oil, lecithin, and a stabiliser (E551b) as the probiotic.	Until normalization of stools (all recovered within 8 days)	Date and time for the first and last abnormal stool was recorded but no specific scale was reported.
Ziese et al., 2018	P4	13	Probiotic (Vivomixx; *Lactobacillus, Streptococcus,* and *Bifidobacterium* spp.). Dose based on body weight (1–10 kg, 225 billion CFU; 10–20 kg, 450 billion CFU; 20–40 kg, 900 billion CFU) once daily.	12	Powder containing maltose with trace amounts of silicon dioxide	21	Waltham faecal score (scale of 1–5) and canine haemorrhagic diarrhoea severity index (CHDS)[a]. Time to remission was defined as days to a normal CHDS-Index (≤3).
Nixon et al., 2019	Not stated	51	Synbiotic (Pro-Kolin; probiotic *Enterococcus* spp. 2 $\times 10^8$ CFU/g (CFU/mL not stated); prebiotic psyllium, pectin, and beta glucan; other components kaolin, montmorillonite clay). Dose based on body weight (<5 kg, 2 mL; 5–15 kg, 3 mL; 15–30 kg, 5 mL; 30–45 kg, 7 mL; >45 kg, 10 mL) three times daily	58	The placebo was indistinguishable in packaging, appearance, and sensory properties	Max 10 days	Nestle-Purina faecal score (scale of 1–6). Time to remission was defined as days to three normal sequential faecal scores (≤3).
Rudinsky et al., 2022	P1 and P2	20	Prebiotic (experimental, not commercially available). Easily digestible diet with additional fibre (psyllium husk; total dietary fibre 28.3 g/Mcal). Dogs were fed based on their estimated maintenance energy requirement (1.2–1.4x resting energy requirement; 70x body weight x $kg^{0.75}$) for the entire study period.	19	Same easily digestible diet (total dietary fibre 15.3 g/Mcal).	30	Waltham faecal score (scale of 1–5) and wellness survey. Time to remission was defined as three consecutive days with a normal faecal score (≤3)

PICO, Population, intervention, comparator, outcome; CFU, colony forming units; P1, Subgroup of dogs with mild disease and acute non-haemorrhagic diarrhoea; P2, Subgroup of dogs with mild disease and haematochezia; P3, Subgroup of dogs with moderate disease and acute non-haemorrhagic diarrhoea; P4, Subgroup of dogs with moderate disease and acute haemorrhagic diarrhoea.
[a] Dogs were scored from 0 to 3 on activity, appetite, vomiting (times/day), faecal consistency, defecation (times/day), and admixture of blood in the stool, with the sum of scores yielding a total cumulative score.

than the antimicrobial treatment. We do, however, believe that the few events of mortality reported are representative of real life based on the panel's clinical experience, stakeholder questionnaires (reflected in the relatively high mortality thresholds), and by observational studies (0–4% mortality in treated and untreated dogs with moderate disease) and have therefore not downgraded the certainty of evidence for this outcome (Mortier et al., 2015; Unterer et al., 2015; Dupont et al., 2021). The same rationale was used for the progression of disease outcome, but the authors wish to highlight and acknowledge that end-users prioritised this outcome in dogs from P2-P4 (Supplementary file 1), which could indicate that the outcome is experienced more frequently than was reported in the included studies. It could also be due to the inexperience of treating hospitalised dogs (the panel rarely experience progression of disease in hospitalised dogs, which is also supported by previously referenced observational studies) and the influence of the owner's concern of haemorrhagic diarrhoea. We made the decision not to downgrade since the thresholds chosen by end-users were high. We recommend further studies of progression of disease in primary care settings.

The results of this study will inform ENOVAT's evidence-based treatment guidelines of CAD. The authors also encourage policy-makers to use this study to create national antimicrobial treatment guidelines for this condition.

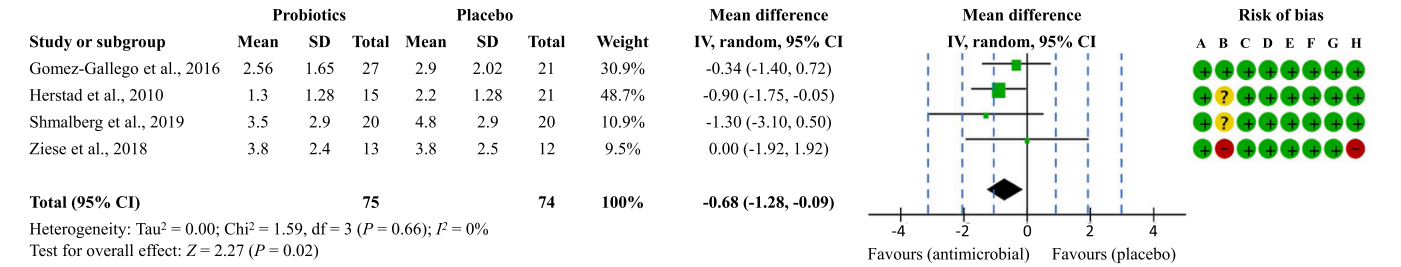

Fig. 9. Forest plot of duration of diarrhoea represented in days in dogs with acute diarrhoea treated with probiotics or a placebo for individual trials and overall (black diamond). Effects of trials are presented as mean differences (95% confidence interval, CI; represented as whiskers). IV, inverse variance; SD, standard deviation. Dashed vertical lines represent clinical thresholds (<1 day, trivial effect; 1–2 days, small effect; 2–3 days, moderate effect; >3 days, large effect). Risk of bias analysis for the included studies on the right: A, random sequence generation (selection bias); B, allocation concealment (selection bias); C, masking of participants and personal (performance bias); D, masking of outcome assessment (detection bias); E, incomplete outcome data (attrition bias); F, selective reporting (reporting bias); G, other bias; H, overall. Green dots represent low risk, yellow dots unknown risk, and red dots high risk.

Conclusion

High certainty evidence showed that antimicrobial treatment did not have a clinically relevant effect on any of the outcomes in dogs with acute diarrhoea with mild and moderate disease. Certainty of evidence was low for dogs with acute diarrhoea with severe disease. Nutraceutical products did not show a clinically relevant effect in shortening the duration of diarrhoea (very low to moderate certainty evidence). No adverse effects were found in any of the studies.

Declaration of Competing Interest

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Author contribution

KS conducted the search, data-extraction, meta-analysis, evidence assessment and drafted the manuscript. LRJ conceived and chaired the study and contributed to the evidence assessment and drafting of the manuscript. MW conducted the search, data-extraction and meta-analysis and contributed to the evidence assessment. CP conducted the search, data-extraction and contributed to the evidence assessment. MB and DS supervised the search and assisted with the evidence assessment. FF created the networking metanalysis and supervised the generation of thresholds. The other ENOVAT panel members contributed to the selection of PICOs and outcomes, generation of thresholds and conducted structured interviews with dog owners and general practitioners. All authors reviewed and approved the final version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2023.106054.

References

Allerton, F., Prior, C., Bagcigil, A.F., Broens, E., Callens, B., Damborg, P., Dewulf, J., Filippitzi, M.-E., Carmo, L.P., Gómez-Raja, J., et al., 2021. Overview and evaluation of existing guidelines for rational antimicrobial use in small-animal veterinary practice in Europe. Antibiotics 10, 1–16.

American Statistical Association, 2016. American Statistical Association releases statement on statistical significance and p-values. ⟨https://www.amstat.org/asa/files/pdfs/p-valuestatement.pdf⟩ (Accessed 26 November 2023).

Busch, K., Unterer, S., 2022. Update on acute hemorrhagic diarrhea syndrome in dogs. Advances in Small Animal Care 3, 133–143.

Chaitman, J., Ziese, A.L., Pilla, R., Minamoto, Y., Blake, A.B., Guard, B.C., Isaiah, A., Lidbury, J.A., Steiner, J.M., Unterer, S., Suchodolski, J.S., 2020. Fecal microbial and metabolic profiles in dogs with acute diarrhea receiving either fecal microbiota transplantation or oral metronidazole. Frontiers in Veterinary Science 7, 192.

De Briyne, N., Atkinson, J., Pokludová, L., Borriello, S.P., Price, S., 2013. Factors influencing antibiotic prescribing habits and use of sensitivity testing amongst veterinarians in Europe. Veterinary Record 173, 475–475.

Dupont, N., Jessen, L.R., Moberg, F., Zyskind, N., Lorentzen, C., Bjørnvad, C.R., 2021. A retrospective study of 237 dogs hospitalized with suspected acute hemorrhagic diarrhea syndrome: Disease severity, treatment, and outcome. Journal Veterinary Internal Medicine 35 (2), 867–877.

Fenimore, A., Martin, L., Lappin, M.R., 2017. Evaluation of metronidazole with and without *Enterococcus faecium* SF68 in shelter dogs with diarrhea. Topics in Companion Animal Medicine 32, 100–103.

Gómez-Gallego, C., Junnila, J., Männikkö, S., Hämeenoja, P., Valtonen, E., Salminen, S., Beasley, S., 2016. A canine-specific probiotic product in treating acute or intermittent diarrhea in dogs: a double-blind placebo-controlled efficacy study. Veterinary Microbiology 197, 122–128.

GRADE, 2023. Grading of Recommendations Assessment, Development and Evaluation GRADEpro. ⟨https://www.gradepro.org/⟩ (Accessed 26 November 2023).

Grindlay, D.J.C., Brennan, M.L., Dean, R.S., 2012. Searching the veterinary literature: a comparison of the coverage of veterinary journals by nine bibliographic databases. The Journal of Veterinary Medical Education 39, 404–412.

Guardabassi, L., Apley, M., Olsen, J.E., Toutain, P.-L., Weese, S., 2018. Optimization of antimicrobial treatment to minimize resistance selection. Microbiology Spectrum 6, 1–36.

Guyatt, G., Zhao, Y., Mayer, M., Briel, M., Mustafa, R., Izcovich, A., Hultcrantz, M., Iorio, A., Alba, A.C., Foroutan, F., et al., 2023. GRADE guidance 36: updates to GRADE's approach to addressing inconsistency. Journal of Clinical Epidemiology 158, 70–83.

Guyatt, G.H., Oxman, A.D., Vist, G.E., Kunz, R., Falck-Ytter, Y., Alonso-Coello, P., Schünemann, H.J., GRADE Working Group, 2008. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. The British Medical Journal 336, 924–926.

Guyatt, G.H., Oxman, A.D., Kunz, R., Woodcock, J., Brozek, J., Helfand, M., Alonso-Coello, P., Glasziou, P., Jaeschke, R., Akl, E.A., et al., 2011. GRADE guidelines: 7. Rating the quality of evidence–inconsistency. Journal of clinical epidemiology 64, 1294–1302.

Herstad, H.K., Nesheim, B.B., L'Abée-Lund, T., Larsen, S., Skancke, E., 2010. Effects of a probiotic intervention in acute canine gastroenteritis-a controlled clinical trial. The Journal of Small Animal Practice 51, 34–38.

Hubbard, K., Skelly, B.J., McKelvie, J., Wood, J.L.N., 2007. Risk of vomiting and diarrhoea in dogs. Veterinary Record 161, 755–757.

Hultcrantz, M., Rind, D., Akl, E.A., Treweek, S., Mustafa, R.A., Iorio, A., Alper, B.S., Meerpohl, J.J., Murad, M.H., Ansari, M.T., et al., 2017. The GRADE working group clarifies the construct of certainty of evidence. Journal of Clinical Epidemiology 87, 4–13.

Israiloff, J.V., 2009. Vergleich von therapieformen der idiopathischen hämorrhagischen gastroenteritis (HGEI) beim Hund. Dissertation. Veterinärmedizinischen Universität. Wien 2009.

Jensen, A.P., Bjørnvad, C.R., 2019. Clinical effect of probiotics in prevention or treatment of gastrointestinal disease in dogs: a systematic review. Journal of Veterinary Internal Medicine 33, 1849–1864.

Langlois, D.K., Koenigshof, A.M., Mani, R., 2020. Metronidazole treatment of acute diarrhea in dogs: a randomized double blinded placebo-controlled clinical trial. Journal of Veterinary Internal Medicine 34, 98–104.
Lehner, C., Hubbuch, A., Schmitt, K., Schuepbach-Regula, G., Willi, B., Mevissen, M., Peter, R., Muentener, C.R., Naegeli, H., Schuller, S., 2020. Effect of antimicrobial stewardship on antimicrobial prescriptions for selected diseases of dogs in Switzerland. Journal of Veterinary Internal Medicine 34, 2418–2431.
Lutz, B., Lehner, C., Schmitt, K., Willi, B., Schüpbach, G., Mevissen, M., Peter, R., Müntener, C., Naegeli, H., Schuller, S., 2020. Antimicrobial prescriptions and adherence to prudent use guidelines for selected canine diseases in Switzerland in 2016. Veterinary Record 7 (1), 15.
Marks, S.L., Rankin, S.C., Byrne, B.A., Weese, J.S., 2011. Enteropathogenic bacteria in dogs and cats: diagnosis, epidemiology, treatment, and control. Journal of Veterinary Internal Medicine 25, 1195–1208.
Mortier, F., Strohmeyer, K., Hartmann, K., Unterer, S., 2015. Acute haemorrhagic diarrhoea syndrome in dogs: 108 cases. Veterinary Record 176, 627.
Nixon, S.L., Rose, L., Muller, A.T., 2019. Efficacy of an orally administered anti-diarrheal probiotic paste (Pro-Kolin Advanced) in dogs with acute diarrhea: a randomized, placebo-controlled, double-blinded clinical study. Journal of Veterinary Internal Medicine 33, 1286–1294.
Ouzzani, M., Hammady, H., Fedorowicz, Z., Elmagarmid, A., 2016. Rayyan-a web and mobile app for systematic reviews. Systematic Reviews 5 (1), 10.
Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., et al., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. British Medical Journal 372, 1–9.
Pignataro, G., Di Prinzio, R., Crisi, P.E., Belà, B., Fusaro, I., Trevisan, C., De Acetis, L., Gramenzi, A., 2021. Comparison of the therapeutic effect of treatment with antibiotics or nutraceuticals on clinical activity and the fecal microbiome of dogs with acute diarrhea. Animals 11, 1484.
Pilla, R., Gaschen, F.P., Barr, J.W., Olson, E., Honneffer, J., Guard, B.C., Blake, A.B., Villanueva, D., Khattab, M.R., AlShawaqfeh, M.K., et al., 2020. Effects of metronidazole on the fecal microbiome and metabolome in healthy dogs. Journal of Veterinary Internal Medicine 34, 1853–1866.
Rayyan, 2023. Faster Systematic Reviews. ⟨https://www.rayyan.ai/⟩. Accessed 26 November 2023.
Robinson, N.J., Dean, R.S., Cobb, M., Brennan, M.L., 2015. Investigating common clinical presentations in first opinion small animal consultations using direct observation. Veterinary Record 176, 463-463.
Rudinsky, A.J., Parker, V.J., Winston, J., Cooper, E., Mathie, T., Howard, J.P., Bremer, C. A., Yaxley, P., Marsh, A., Laxalde, J., et al., 2022. Randomized controlled trial demonstrates nutritional management is superior to metronidazole for treatment of acute colitis in dogs. Journal of the American Veterinary Medical Association 260, S23–S32.
Schünemann, H.J., Neumann, I., Hultcrantz, M., Brignardello-Petersen, R., Zeng, L., Murad, M.H., Izcovich, A., Morgano, G.P., Baldeh, T., Santesso, N., et al., 2022. GRADE guidance 35: update on rating imprecision for assessing contextualized certainty of evidence and making decisions. Journal of Clinical Epidemiology 150, 225–242.
Shmalberg, J., Montalbano, C., Morelli, G., Buckley, G.J., 2019. A randomized double blinded placebo-controlled clinical trial of a probiotic or metronidazole for acute canine diarrhea. Frontiers in Veterinary Science 6, 1–8.
Shojania, K.G., Sampson, M., Ansari, M.T., Ji, J., Garritty, C., Rader, T., Moher, D., 2007. Updating systematic reviews. Agency for Healthcare Research and Quality (US) 16, 1–135.
Singleton, D.A., Noble, P.J.M., Sánchez-Vizcaíno, F., Dawson, S., Pinchbeck, G.L., Williams, N.J., Radford, A.D., Jones, P.H., 2019. Pharmaceutical prescription in canine acute diarrhoea: a longitudinal electronic health record analysis of first opinion veterinary practices. Frontiers in Veterinary Science 6, 1–14.
Stavisky, J., Radford, A.D., Gaskell, R., Dawson, S., German, A., Parsons, B., Clegg, S., Newman, J., Pinchbeck, G., 2011. A case–control study of pathogen and lifestyle risk factors for diarrhoea in dogs. Preventive Veterinary Medicine 99, 185–192.
SYREAF, 2023. Systematic Reviews for Animals and Food, Protocol Registration. ⟨https://syreaf.org/wp-content/uploads/2022/05/GXyTVqtvD7.pdf⟩ (Accessed 26 November 2023).
Unterer, S., Busch, K., 2021. Acute hemorrhagic diarrhea syndrome in dogs. Veterinary Clinics of North America: Small Animal Practice 51, 79–92.
Unterer, S., Strohmeyer, K., Kruse, B.D., Sauter-Louis, C., Hartmann, K., 2011. Treatment of aseptic dogs with hemorrhagic gastroenteritis with amoxicillin/clavulanic acid: a prospective blinded study. Journal of Veterinary Internal Medicine 25, 973–979.
Unterer, S., Lechner, E., Mueller, R.S., Wolf, G., Straubinger, R.K., Schulz, B.S., Hartmann, K., 2015. Prospective study of bacteraemia in acute haemorrhagic diarrhoea syndrome in dogs. Veterinary Record 176, 309.
Weese, J.S., 2011. Bacterial enteritis in dogs and cats: diagnosis, therapy, and zoonotic potential. Veterinary Clinics of North America: Small Animal Practice 41, 287–309.
Werner, M., Suchodolski, J.S., Straubinger, R.K., Wolf, G., Steiner, J.M., Lidbury, J.A., Neuerer, F., Hartmann, K., Unterer, S., 2020. Effect of amoxicillin-clavulanic acid on clinical scores, intestinal microbiome, and amoxicillin-resistant Escherichia coli in dogs with uncomplicated acute diarrhea. Journal of Veterinary Internal Medicine 34, 1166–1176.
Werner, M., Unterer, S., 2021. Use of antimicrobials in acute canine diarrhea - overview of potential risks, indications and alternatives. Tierarztliche Praxis Ausgabe K: Kleintiere -Heimtiere 49, 110–120.
Whittemore, J.C., Moyers, T.D., Price, J.M., 2019. Randomized, controlled, crossover trial of prevention of antibiotic-induced gastrointestinal signs using a synbiotic mixture in healthy research dogs. Journal of Veterinary Internal Medicine 33, 1619–1626.
Ziese, A.L., Suchodolski, J.S., Hartmann, K., Busch, K., Anderson, A., Sarwar, F., Sindern, N., Unterer, S., 2018. Effect of probiotic treatment on the clinical course, intestinal microbiome, and toxigenic *Clostridium perfringens* in dogs with acute hemorrhagic diarrhea. PLoS One 13, 1–16.