**Title**

Antimicrobial and gastrointestinal nutraceutical (probiotic, prebiotic, synbiotic) treatment of canine acute diarrhea: a protocol for a systematic review

**Registration**

Systematic Reviews for Animals and Food (SYREAF) syreaf.org

**Authors**

Cameron Prior[1], Karolina Scahill[2,3*], Melanie Werner[4], David Singleton[5], Marnie Brennan[6], Lisbeth Rem Jessen[7]

[1]Willows Veterinary Centre & Referral Centre, Solihull, West Midlands, United Kingdom
[2]University of Edinburgh, College of Medicine and Veterinary Medicine, Edinburgh, United Kingdom
[3]Anicura Bagarmossen Small Animal Hospital, Bagarmossen, Sweden
[4]Clinic of Small Animal Internal Medicine, Centre for Clinical Veterinary Medicine, LMU, Munich, Germany
[5]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, United Kingdom
[6] Centre for Evidence-based Veterinary Medicine, The University of Nottingham, United Kingdom
[7]Department of Veterinary Clinical Science, University of Copenhagen, Denmark

*Corresponding author: Karolina Scahill karolina.scahill@anicura.se

**Contributions**

All authors will contribute to protocol and search strategy development, review of manuscripts and data extraction.

**Support**

This systematic review is based upon work from the COST Action European Network for Optimization of Veterinary Antimicrobial Treatment (CA18217), supported by European Cooperation in Science and Technology (COST); see: www.enovat.eu and www.cost.eu,

**Rationale**

Optimizing antimicrobial use in human and animal health is one of the main objectives in the World Health Assembly's global action plan to fight antimicrobial resistance (AMR) (World Health Organization, 2015). Antimicrobial usage (AMU) and AMR have received considerable attention in food-producing animals whereas the role of companion animals is not fully known; cats and dogs are, however, known reservoirs of resistant bacteria (Joosten et el, 2020; Li et al, 2021). The close contact between companion animals and humans is thought to facilitate zoonotic transmission of resistant bacteria (Miranda et al., 2021; Zhang et al, 2016), which highlights the importance of limiting AMU in companion animals (European Medicines Agency, 2015).

Gastrointestinal disease and diarrhea is common in the general dog population, and in clinical practice diarrhea is one of the most frequent clinical signs that is presented in dogs (Hubbard *et al,* 2007; Robinson *et al*., 2015). Canine acute diarrhea (CAD) is generally mild and self-limiting and in most cases, dogs are not presented for veterinary examination (Pugh *et al.*, 2017). The aetiology of CAD is poorly understood and often considered to be multi-factorial, but dietary indiscretion, pathogens and toxins have been associated with signs of acute diarrhea (Armstrong, PJ., 2013). It has been suggested that lifestyle factors such as scavenging, kennel stays, diet change and home-cooked diets could be a greater risk factor than specific pathogens in the development of canine diarrhea (Stavisky *et al*, 2011).

Although a bacterial cause has not been established, systemic antimicrobials are frequently prescribed for dogs with diarrhea, especially in dogs presenting with haemorrhagic diarrhea (Singleton *et al*, 2019; Lehner *et a*l, 2020). The acute haemorrhagic diarrhea syndrome (AHDS) is characterised by acute onset of haemorrhagic diarrhea that can result in significant fluid losses and hypovolemia (Mortier *et al*., 2015). The aetiology of AHDS is not fully established and diagnosis is generally made by excluding other causes, but *Clostridium perfringens* and their toxins has been implicated in the pathogenesis (Sindern *et al*., 2019). Antimicrobial treatment is, however, not recommended in dogs with AHDS or non-haemorrhagic diarrhea without accompanying signs of sepsis (Allerton *et al,* 2021, Marks *et a*l, 2011; Unterer *et a*l., 2021). Gastrointestinal nutraceuticals, such as probiotics, prebiotics and synbiotics, are frequently provided by veterinarians for dogs with acute diarrhea and do not require a

prescription (Singleton *et al*, 2019). A systematic review on probiotics in dogs with diarrhea concluded that the clinical importance in acute gastrointestinal disease was limited, and the studies that are currently available are often underpowered (Jensen and Bjornvad, 2019).

CAD is a common occurrence in clinical practice and antimicrobials are frequently prescribed to this patient group, despite a lack of consensus about the necessity of these treatments and the potential contribution to AMR and zoonotic transmission of resistant bacteria, which emphasizes the importance of this systematic review and subsequent treatment guidelines. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P) methodology will be followed in this systematic review of current literature (Shamsheer et al., 2015) and the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) guidelines will be used to critically evaluate and interpret current evidence (Guyatt *et al*., 2008). To the best of our knowledge, no systematic review has investigated the usage of antimicrobial treatment and gastrointestinal nutraceuticals (probiotics, prebiotics and synbiotics) compared to no treatment in CAD.

**Objectives**

This protocol describes the methodology for a systematic review that will synthesize and critically evaluate the quality of evidence related to the question: Are antimicrobial or gastrointestinal nutraceutical (probiotics, prebiotics, synbiotics) preparations necessary for the successful management of CAD? The systematic reviews described in this protocol are conducted as part of the ENOVAT Antimicrobial Guidelines in Canine Acute Diarrhea (CAD) project (https://enovat.eu/link-1-wg4/). The included PICOs are based on a selection and prioritization process among all CAD drafting group members (Delphi method) and end-user interviews finalized in June 2021.

**METHODS**

The review team will include three members (CP, KS, MW). In case of doubt, the Review/Methodology team will consult the chairs of the drafting group (LRJ and DS) and the methodological task force representative (MB).

**5 Eligibility criteria**

The eligibility criteria will be based on the PICO format framework. Dogs in all settings, ranging from individually owned to homeless animals, can be included. The included studies consist of randomized controlled trials, clinical trials, cohort and case-control studies and cross-sectional studies. Existing systematic reviews can help provide information about previously published studies. Grey literature will be searched. Observational studies and case series will be considered if no other evidence is available. The studies must be published in the English, Swedish, French, Spanish or German language.

The detailed definition of the subpopulation is stated as follows:

- **P1 + P2:** mild disease; may be treated as outpatient; Mental status: bright, alert and responsive; systemic response to disease: circulatory stable, no clinical signs of dehydration or hypovolemia, absence of fever
- **P3 + P4:** moderate disease; Hospitalization/fluid demanding; Mental status: mildly to moderately depressed; systemic response to disease: clinically detectable dehydration or hypovolemia; but rapidly improve in response to appropriate fluid therapy *; absence of fever
- **P5 + P6:** severe disease; Hospitalization/fluid demanding; Mental status: moderately to severely depressed; systemic response to disease: clinically detectable dehydration or hypovolemia, but no rapid response to appropriate fluid therapy *, severe circulatory compromise (hypovolemic/hypotensive or septic shock), fever (T > 39.5°C)

*adequate rapid response to fluid therapy = alert and responsive, circulatory stable (absence of tachycardia, tachypnoea, prolonged CRT) and afebrile (≤39.5°C)

PICO-questions have been designed for which a systematic review will be conducted:

**PICO 1**

**In dogs with acute diarrhea, does antimicrobial treatment compared to no antimicrobial treatment have an effect?**

| | |
|---|---|
| Population | Dogs with acute diarrhea<br>Subpopulation:<br>P1 – mild non-haemorrhagic diarrhea<br>P2 – mild haemorrhagic diarrhea<br>P3 - moderate non-haemorrhagic diarrhea<br>P4- moderate haemorrhagic diarrhea<br>P5 - severe non-haemorrhagic diarrhea<br>P6 - severe haemorrhagic diarrhea |
| Intervention | Antimicrobial treatment (beta-lactams, metronidazole, trimethroprim sulphonamides and fluoroquinolones) |
| Comparator | No antimicrobial treatment or placebo* |
| Outcome | <u>Main</u><br>P1: Shorten the duration of diarrhea (by 24-48h)<br>P2-P4: Reduce disease progression<br>P5-P6: Prevent mortality<br><u>Secondary (P1-P6)</u><br>P2-P6: Shorten the duration of diarrhea (by 24-48h)<br>P1, P5, P6: Reduce disease progression<br>P1-P4: Prevent mortality<br>Shorten the duration of hospitalization (by 24-48h)<br>Adverse effects** |

**PICO 2**

**In dogs with acute diarrhea does metronidazole treatment have a superior effect compared to beta-lactam treatment?**

| | |
|---|---|
| Population | Dogs with acute diarrhea (P1-P6) |
| Intervention | Metronidazole treatment |

| | |
|---|---|
| Comparator | Beta-lactam treatment |
| Outcome | Main<br>P1: Shorten the duration of diarrhea (by 24-48h)<br>P2-P4: Reduce disease progression<br>P5-P6: Prevent mortality<br>Secondary (P1-P6)<br>P2-P6: Shorten the duration of diarrhea (by 24-48h)<br>P1, P5, P6: Reduce disease progression<br>P1-P4: Prevent mortality<br>Shorten the duration of hospitalization (by 24-48h)<br>Adverse effects** |

**PICO 3**

**In dogs with acute diarrhea, does long duration of antimicrobial treatment have a superior effect compared to short duration of treatment?**

| | |
|---|---|
| Population | Dogs with acute diarrhea (P1-P6) |
| Intervention | Antimicrobial treatment > 7 days |
| Comparator | Antimicrobial treatment < 7 days |
| Outcome | Main<br>P1: Shorten the duration of diarrhea (24-48h)<br>P2-P4: Reduce disease progression<br>P5-P6: Prevent mortality<br>Secondary (P1-P6)<br>P2-P6: Shorten the duration of diarrhea (24-48h)<br>P1, P5, P6: Reduce disease progression<br>P1-P4: Prevent mortality<br>Shorten the duration of hospitalization (24-48h)<br>Adverse effects** |

**PICO 4**

**In dogs with acute diarrhea, does treatment with probiotics compared to no treatment with probiotics have an effect?**

| | |
|---|---|
| Population | Dogs with acute diarrhea (P1-P6) |
| Intervention | Probiotic treatment |
| Comparator | No probiotic treatment or placebo* |
| Outcome | <u>Main</u><br>P1-P6: Shorten the duration of diarrhea (24-48h)<br><u>Secondary (P1-P6)</u><br>Reduce disease progression<br>Prevent mortality<br>Shorten the duration of hospitalization (24-48h?)<br>Adverse effects** |

**PICO 5**

**In dogs with acute diarrhea, does treatment with prebiotics compared to no treatment with prebiotics have an effect?**

| | |
|---|---|
| Population | Dogs with acute diarrhea (P1-P6) |
| Intervention | Prebiotic treatment |
| Comparator | No prebiotic treatment or placebo* |
| Outcome | <u>Main</u><br>P1-P6: Shorten the duration of diarrhea (24-48h)<br><u>Secondary (P1-P6)</u><br>Reduce disease progression<br>Prevent mortality<br>Shorten the duration of hospitalization (24-48h?)<br>Adverse effects** |

**PICO 6**

**In dogs with acute diarrhea, does treatment with synbiotics compared to no treatment with synbiotics have an effect?**

| | |
|---|---|
| Population | Dogs with acute diarrhea (P1-P6) |
| Intervention | Synbiotic treatment |
| Comparator | No synbiotic treatment or placebo* |
| Outcome | <u>Main</u><br>P1-P6: Shorten the duration of diarrhea (24-48h)<br><u>Secondary (P1-P6)</u><br>Reduce disease progression<br>Prevent mortality<br>Shorten the duration of hospitalization (24-48h?)<br>Adverse effects** |

* If both comparators are included in the retrieved studies a sub-analysis will be undertaken

**Adverse effects include clinical side effects, alteration of microbiome, overgrowth of enteropathogens and selection of resistant bacteria.

**Information sources**

The date of the search process will be the day before the start of the systematic review. There will be no geographical restrictions.

**Databases:**

- CAB Abstracts
- Web of Science
- MEDLINE

On the Web of Science platform three databases will be searched: WoS Core collection, Medline & CAB Abstracts. The data below was taken from the database to show the periods covered.

**Web of Science Core Collection** (1956-present)
Search the world's leading scholarly journals, books, and proceedings in the sciences, social sciences, and arts and humanities and navigate the full citation network.
All cited references for all publications are fully indexed and searchable.

Search across all authors and all author affiliations.
Track citation activity with Citation Alerts.
See citation activity and trends graphically with Citation Report.
Use Analyze Results to identify trends and publication patterns.
Data updated 2021-07-14

**CABI: CAB Abstracts® and Global Health®** (1910-present)
Provides authoritative research information on agriculture, environment, and related applied life sciences.
Search using unique CABI indexes including CAB Thesaurus, CABICODES, and subject descriptors.
Includes data from journals, books, proceedings, monographs, technical reports, and more.

**MEDLINE®** (1950-present)
The U.S. National Library of Medicine® (NLM®) premier life sciences database.
Explore biomedicine and life sciences, bioengineering, public health, clinical care, and plant and animal science.
Search precisely with MeSH terms and CAS registry numbers.
Link to NCBI databases and PubMed Related Articles

**Search strategy**
Relevant index terms from medical subject headings (MeSH) will be searched in the databases described above.

**PICO 1, 2 and 3**

| **Search strategy number** | **Key terms with synonyms:** |
|---|---|
| 1. | Dog* OR Canine* |
| 2. | Acute diarrh* OR diarrh* OR enteritis OR gastroenteritis |
| 3 | Antimicrob*OR antibiotics* OR anti-microb* OR antibacterial OR beta-lactam* OR Amoxicillin* OR metronidazole OR trimethoprim sulphonamide* OR enrofloxacin OR fluoroquinolone* |

The combination will be 1 AND 2 AND 3

**PICO 4, 5, AND 6**

| Search strategy number | Key terms with synonyms: |
|---|---|
| 1. | Dog*OR Canine* |
| 2. | Acute diarrh* OR diarrh*OR enteritis OR gastroenteritis |
| 3. | Probiotic*OR prebiotic* OR symbiotic*OR microbio* OR synbiotic* OR lactobacill* OR bifidobacter* OR VSL* OR enterococc* |

The combination will be 1 AND 2 AND 3

**Selection process**

Literature references will be downloaded to EndNote (EndNote X9, Clarivate Analytics, Philadelphia) from the literature databases and duplicates will be removed. Then the title, abstract and full text screening will be carried out using Rayyan (https://www.rayyan.ai/). Risk of bias will be documented in Microsoft Excel and the GRADE-Software. If statistical analysis/metaanalysis will be performed, GraphPadPrism8 and RevMan 5.3 will be used.

The citations will be screened in two independent stages. The first stage of the selection process will consist of titles and abstract screening. Three independent reviewers (KS, CP, MW) will screen the publications independently using Rayyan (e.g. at least two reviewers will assess each citation). The studies that meet inclusion criteria will pass to the next phase.

First stage

The title and the abstract will be assessed for relevance for the review question and language and will be assigned ‘yes’, ‘no’ or ‘unclear´,the last meaning that the relevance cannot be answered by only the information given in the abstract. Articles classified as ‘no’ by at least two reviewers will be excluded. Articles with ‘unclear’ or ‘yes’ answers by at least two reviewers will go the next phase.

Second stage

In the second stage the full texts will be evaluated against the eligibility criteria and for relevance regarding the PICO questions. Three independent reviewers (KS, CP, MW) will carry out this task using Rayyan. Studies will only be included if they receive 'yes' or 'unclear' for the inclusion criteria. All of those which receive at least one 'no' will be excluded.

We will report on reasons for study exclusion using a flow diagram as outlined by PRISMA.

**Data collection process**

Two members will collect and extract the relevant data from the included studies independently using Microsoft Excel. A third author will check/supervise the summaries of the first two members.

**Data items**

Data to be extracted include:

- General information: bibliographic information (journal name, language, country, year, authors, funding information)
- Study design: type of study, sample size
- Population characteristics: breed, sex, age, which subgroup of CAD is addressed, setting
- Intervention assessed and comparator: characteristics of the intervention
- Outcomes: outcome definition estimate (adjusted and unadjusted) confidence intervals, p-values, odds ratios, risk ratios/relative risk.

**Outcome Prioritization**

**PICO 1:-3**

- P1: Shorten the duration of diarrhea (24-48h)

  P2-P4: Reduce disease progression

  P5-P6: Prevent mortality

**PICO 4-6:** P1-P6: Shorten the duration of diarrhea (24-48h)

**PICO 7:** Clinical side effects

**Study risk of bias assessment**

This process will be carried out by three independent reviewers (CP,KS, MW)

The risk of bias will be evaluated using the Revised Cochrane risk-of-bias tool for randomized trials and for observational studies using Microsoft Excel. Studies will be evaluated individually in terms of internal validity by three reviewers qualitatively (judged as low, high, and some concerns).

Responses to the questions will be registered to justify the responses given. After answering the questions, a risk-of-bias judgement will be made for each domain.

Finally, a global risk of bias will be determined by allocating the lowest risk of bias in any of the evaluated domains.

**Effect measures**

The effect measures to be extracted (where available) or calculated risk difference, risk ratio and odds ratio.

**Synthesis methods**

The evidence will be synthesized into a summary of findings table using the GRADE-Software. We will investigate the studies for possible methodological heterogeneity and statistical heterogeneity using the I-squared and Q-statistics. If there is sufficient data from homogeneous studies that report quantitative outcomes, a meta-analysis will be carried out. The strength of the overall body of evidence will be assessed using Grading of Recommendations, Assessment, Development and Evaluation (GRADE).

**Reporting bias assessment**

In order to assess small-study effects, it is planned to generate funnel plots for reports that are eligible for performing a meta-analysis. If asymmetry in the funnel plot was detected, the characteristics of the trials will be evaluated to assess whether the asymmetry was likely due to publication bias or other factors such as methodological or clinical heterogeneity of the trials.

**Certainty assessment**

All members of the CAD-drafting group will independently assess the certainty in the body of evidence by using the GRADE-approach. The confidence of evidence will be defined as high, moderate, low, or very low

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