An amendment protocol for a systematic review and meta-analysis

Title

Is antibiotic treatment efficacious to treat or prevent/control colibacillosis in broiler production? An amendment protocol for a systematic review and meta-analysis.

Authors and their affiliations

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Author contributions

The review (PICO) question and protocol described in this document were adapted from Sargeant et al. (2018) and developed with the contribution and final approval of all co-authors. Ronald Vougat Ngom and Alessandra Piccirillo drafted the protocol and all authors provided their input.

Registration

This amendment protocol is archived at Padua Research Archive (handle code: ...) and published online with Systematic Reviews for Animals and Food (SYREAF) available at: http://www.syreaf.org/. This protocol is reported using the items (headings) recommended in the PRISMA-P guidelines (Moher *et al.,* 2015).

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Amendments

This review is an amendment of a previously completed published protocol. The original protocol can be found here http://hdl.handle.net/10214/14349. In case any

amendments are made to this protocol after its registration, they will be adequately documented in the systematic review as Protocol Deviations.

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1. Introduction

1.1. Rationale

Avian pathogenic *Escherichia coli* (APEC) is the causative agent of colibacillosis, a disease with significant economic losses for the broiler industry, and can act as a primary or secondary pathogen when the host immune system is compromised (Nolan *et al*., 2020). Colibacillosis is manifested as a localised or systemic infection resulting in various disease syndromes that affect all stages of the broiler production. In broiler breeders, increased mortality and decreased egg production due to the salpingitis-peritonitis syndrome can reach the cost of 1.87 euros per housed hen (Landman *et al*., 2015). At the slaughterhouse, condemnations as a result of cellulitis lead to losses of 0.14%-1.4% of poultry meat and increased labour costs for the process of affected carcasses (Barbieri *et al*., 2013; Nolan *et al*., 2020).

E. coli infection of broilers is a global challenge with potential threats to human health, which is probably one of the major reasons for the use of antibiotics in the poultry industry. Importantly, it has been identified as one of the most relevant antimicrobial resistant bacterial pathogens from poultry in a recent report by the European Union (EFSA, 2021).

Antibiotics are used to prevent illness (prophylaxis) or in flocks where some birds are already ill with the intention to prevent further illness or mortality (metaphylaxis) (Chauvin *et al*., 2005; Singer and Hofacre, 2006; Dziva and Stevens, 2008). Antibiotics are typically used to reduce early mortality. Those with severe infection are unlikely to survive, however appropriate treatment reduces transmission between birds and improves the suitability of those with a mild infection. In addition to the limited availability of drugs, not every labelled drug for *E. coli* is efficacious, resistance is common (Johnson *et al.,* 2005, 2006; Kabir, 2010) and effectiveness can vary from flock to flock, even within a flock, with more than one strain and more than one treatment. In this regard, to address this specific question, a review on the efficacy of antibiotics to prevent colibacillosis in broilers have been conducted by Sargeant *et al.* (2019).

This protocol is an amendment of the previous study conducted by Sargeant *et al.* (2019) on the same topic. The description below will mainly focus on modifications provided.

1.2. Objectives

This protocol defines the methodology of the systematic review and meta-analysis to address the following PICO question: "*In broilers at risk of colibacillosis, does antibiotic treatment versus no antibiotic treatment result in higher FCR/fewer condemnations/lower mortality/total antibiotic use*?". The specific PICO elements are:

1. **P**opulation: Broilers (including the whole production chain).
2. **I**ntervention: Any antibiotic licensed for use in chickens *in ovo*, by injection, in feed, or in water at doses consistent with therapeutic or prophylactic use. Eligible antibiotic include any antibiotic for use in treating or preventing colibacillosis in poultry included in Sargeant et al. (2019) and the OIE list of antibiotic agents of veterinary importance.
3. **C**omparator: Placebo or untreated control group or an alternative antibiotic treatment.
4. **O**utcomes: Mortality, Feed Conversion Ratio (FCR), condemnations due to colibacillosis at the slaughterhouse, and total antibiotic use.

2. Methods

2.1 Eligibility criteria

1. Criteria related with the elements of the PICO question (Population, Intervention, Comparator and Outcomes).
2. Language: Publications in English, French and/or Spanish.
3. Publication types: Journal articles and any other form of research publication that provides results of original research, fulfills the study design eligibility criteria and has a full text of more than 500 words.
4. Publication date: No limits.
5. Geographical location of studies: No limits.
6. Studies reporting controlled trials with natural disease exposure will be the primary type of study for inclusion. Disease challenge studies and observational studies will be documented as well and assessed during full-text screening for the reported intervention and measured outcomes of interest.

2.2. Information sources

Bibliographic databases that provide a high level of article recall across biomedical articles (Bramer *et al*., 2017) will be used. Table 1 lists the databases to be searched. CAB abstract and Agricola will be searched via the University of Bern (Switzerland) and Pubmed and Web of Sciences (WOS) will be conducted via the University of Padova

(Italy). All the databases of WOS will be used (Web of science core collection, BIOSIS Citation Index, Current Contents Connect, Data Citation Index, Derwent Innovations Index, KCI-Korean Journal Database, Medline, SciELO Citation Index, Zoological Record). However, we will exclude the following editions: Social Sciences Citation Index (SSCI), Arts & Humanities Citation Index (AHCI), Conference Proceedings Citation Index-Social Science & Humanities (CPCI-SSH), Book Citation Index Science (BKCI-S) and Book Citation Index Social Sciences & Humanities (BKCI-SSH).

The method described by Sargeant *et al*. (2018) with slight revisions will be used.

Table 1: List of databases to be searched.

Database	**Interface**	**URL**
MEDLINE	PubMed	https://pubmed.ncbi.nlm.nih.gov/
CAB abstracts	Ovid	https://www.wolterskluwer.com/en/solutions/ovid/cab-abstracts-31
Web of science	Web of Science	http://webofknowledge.com/
AGRICOLA	Proquest	https://www.proquest.com/

2.3. Search strategy

The search strategy will involve a multi-stranded approach that uses a series of searches, with different combinations of concepts to gather all possibly related research and thus achieve high sensitivity (Higgins *et al*., 2021). If only few papers are found to be relevant to the review, in addition to the database, citations will be extracted from a selection of important papers and reviews. In the event of using search reviews, Scopus or Google scholar databases will be used for backward searching.

The concept of the search strategy will be the following:

[**Broilers**] AND [**Antimicrobials**] AND [**Colibacillosis**].

Search terms will be amended appropriately to reflect the functionality differences in each database. The general search strategy to identify studies relevant to the PICO of this review will be the following:

#1 (chicken* OR poultry* OR flock* OR gallus OR broiler*)

#2 (antimicrobial* OR anti-microbial* OR antibiotic* OR antibacterial* OR anti-bacterial* OR apramycin OR amoxicillin OR avilamycin OR enrofloxacin OR neomycin OR neomicin

OR salinomicyn OR salinomicin OR spectinomycin OR sulfaquinoxaline OR ceftiofur OR gentamycin OR gentamicin OR lincomycin OR oxytetracycline OR bacitracin OR sulfadimethoxine OR virginiamycin OR chlortetracycline OR tylosin OR tetracycline OR trimethoprim OR sulfamethoxazole OR penicillin OR flumequine OR ampicillin OR colistin OR ciprofloxacin)

#3 (colibacillosis OR colisepticaemia OR peritonitis OR coli OR Escherichia OR coliform OR colisepticemia OR coligranuloma OR "Hjarre's" OR "air sac disease" OR cellulitis OR osteomyelitis OR "brittle bone disease" OR salpingitis OR synovitis OR omphalitis OR enteritis OR "hemorrhagic septicemia" OR "chronic respiratory disease" OR "swollen head syndrome" OR "venereal colibacillosis" OR "coliform cellulitis" OR "yolk sac infection" OR APEC OR "pathogenic E. coli" OR "primary infection" OR "secondary infection" OR multifactorial OR multicausal)

#1 AND #2 AND #3

2.4. Study Records

Data management
Database records of the articles recovered will be imported into Zotero and duplicates will be deleted. Abstract and full screening will be recorded in Rayyan. Data extraction and risk of bias assessment will be performed in Revman. Summary of findings table will be done in GradePro.

Selection process
The citations will be screened in two independent stages. Four independent reviewers (Ronald Vougat Ngom, VN; Alessandra Piccirillo, AP; Gaspard J. Ayissi, GA; and Akenghe Tanyienow; AT) will carry out the title and abstract screening using Rayyan. Half of the citations will be assigned to two authors (VN and AT) and the other half to other two authors (AP and GA). This will guarantee that each reference is screened by two independent reviewers. Conflicts will be resolved with a third reviewer (Helena C. de Carvalho Ferreira) if consensus between two reviewers of the pair cannot be reached. The concordance among the reviewers will be evaluated by randomly selecting 100 citations entering in the first stage of the process prior to screening all papers. For the second phase, 10% of the total number of papers will be used for the calibration exercice. This calibration study will enable discussion and solve disagreement before carrying out the full selection process by reviewers (Sanguinetti *et al*., 2021).
Eligibility of studies will be assessed with the following questions, as (partly) suggested by Sargeant *et al*. (2019):

1. Is the abstract of the study available? YES [INCLUDE], NO [EXCLUDE]

2. Is the study an original research assessing the use of one or more antibiotic(s) to treat or control/prevent colibacillosis in broilers? YES [PASS], NO [EXCLUDE], UNCLEAR [PASS]
3. Is there a concurrent comparison group? (i.e., controlled with natural or deliberate disease exposure or analytical observational study?) YES [PASS], NO [EXCLUDE], UNCLEAR [PASS].

The studies that meet inclusion criteria will pass to the next phase.

Like the previous step of the screening, the same four independent reviewers (VN, AT, AP and GA) will carry out the full-text screening using Rayyan according to the method used during the previous phase. Conflicts will be resolved with a third reviewer (Helena C. de Carvalho Ferreira) if consensus between two reviewers cannot be reached. Eligibility of studies will be assessed with the following questions:

1. Is a full text available in English, French and/or Spanish? YES [PASS], NO [EXCLUDE]
2. Is the **Population** of the study broilers? YES [PASS], NO [EXCLUDE], UNCLEAR [EXCLUDE]
3. Is the **Intervention** of the study the use of antibiotic(s) to treat or prevent or control colibacillosis in broilers? YES [PASS], NO [EXCLUDE], UNCLEAR [EXCLUDE]
4. Is at least one of mortality, FCR, condemnations due to colibacillosis at the slaughterhouse or indicator of total antibiotic use due to colibacillosis the **Outcome(s)** described? YES [PASS], NO [EXCLUDE]
5. Is the study design a controlled trial with natural or experimental disease exposure? YES [PASS to data extraction process], NO [this is a disease challenge study, indicate the antibiotic(s) assessed and extract data]

Data extraction
Four independent reviewers (VN, AT, AP and GA) will carry out this task using Revman. Conflicts will be resolved with a third reviewer (Helena C. de Carvalho Ferreira) if consensus between the each pair of reviewers cannot be reached. Data to be extracted from eligible studies will include the following items as (partly) suggested by Sargeant *et al.* (2019):

General information:
1. Country (where the trial study was conducted). If not stated, use country affiliation of corresponding author
2. Number and type of flocks (commercial broilers or experimental flocks)
3. Breed
4. Sex
5. Production type (conventional, organic, antibiotic-free)
6. Duration and year(s) of study
7. Production stage/age of birds when treatment was applied

8. Production stage/age of birds when outcome(s) were measured

Intervention data:

1. Commercial name and type of antibiotic
2. Route and dose of administration
3. Unit of population participants (e.g., flock, house/barn/pen)
4. Description of the comparator group (non-treated or placebo treated)
5. Number of birds enrolled in the participating unit
6. Number of flocks/house/barns/pens enrolled
7. Number of flocks/house/barns/pens enrolled lost until the end of trial study
8. Number of flocks/house/barns/pens enrolled analyzed
9. Method to account for non-independent observations

Outcome data:

1. Mortality
    a. Level at which mortality was measured (e.g., flock, house/barn/pen)
    b. Time period of measured outcome
2. Feed conversion ratio (FCR)
    a. Feed conversion ratio
    b. Age and/or weight of slaughtered participant birds
3. Condemnations due to colibacillosis
    a. Age and/or weight of slaughtered participant birds
4. Quantity of antibiotic used (dependent on the indicator of the included study)

For all relevant outcomes, measures of association (e.g., risk ratio, odds ratio, mean differences for continuous outcomes) will be extracted or calculated only if variance measures are available or if they can be calculated from the study outcome data.

2.5. Risk of Bias Assessment and Data synthesis

Risk of bias will be assessed only for controlled trials for each of the measured outcomes and according to the Cochrane risk of bias instrument (Higgins *et al.,* 2021). Details on the risk of bias assessment follow below:

Selection bias is caused by factors affecting the selection of study subjects (Dohoo *et al.,* 2009). The selection bias associated with external validity will not be taken into account.

Information bias is caused by factors relating to attaining precise information on the exposure, outcome, and covariates (Dohoo *et al*., 2009). This domain will be approached using the following questions:

· Have the definitions of cases of colibacillosis been clearly defined?

· Have the methods used to determine colibacillosis been carried out in such a way that assure truthfulness in the diagnosis?

Low risk of information bias example:
· The diagnosis has been carried out by the combination of clinical disease and laboratory methods.
Examples of low risk of confounding:
· Treatment was randomly assigned to broilers;
Characteristics such as antibiotic were matched between control and treatment groups;
· The statistical approaches used adjusted for potential confounding.

Confounding bias is caused by the effects of factors other than the exposure of interest on the observed association (Dohoo *et al*., 2009). The question that will address this type of bias is the following: Were measures taken into account to reduce potential confounding?

2.6. Data synthesis

The intention of this review is to conduct a quantitative synthesis of results via a (network) meta-analysis if an adequate number of eligible studies are captured with the literature search. If quantitative analysis is not possible, qualitative summary will be made. Furthermore, publication bias will be evaluated using previous approaches (Mavridis *et al*., 2013; Marvridis *et al*., 2014).

Conclusions

The overall objective of this systematic review is to examine the efficacy of antimicrobials in the treatment or prevention/control of colibacillosis in broilers. This will help the decision-making process when applying interventions in broilers by producers and field veterinarians and the suggestions made by policymakers. Moreover, the systematic review will suggest gaps in knowledge that require more research in the future.

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