A protocol for a systematic review and meta-analysis

Title

Are management measures efficacious to prevent or control colibacillosis in broilers production chain? A protocol for a systematic review and meta-analysis.

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Author contributions

The review (PICO) question and protocol described in this document were developed with the contribution and final approval of all co-authors. Giuditta Tilli, Ronald Vougat Ngom and Alessandra Piccirillo drafted the protocol and all authors provided their input.

Registration

This 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 not an amendment of a previously completed or published protocol. 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 manifests 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).
To date, several strategies have been adopted to prevent or control colibacillosis (i.e., antibiotics, vaccination, management and biosecurity, nutritional modulations and nutraceuticals) and it has been widely demonstrated that high biosecurity measures and correct management of the flocks can reduce diseases and consequently reduce condemnation rates at the slaughterhouse (Schulze Bernd *et al*., 2020). In this context, management plays a crucial role to keep the flock in good housing conditions and to protect it against colibacillosis and other possible coinfections (Luftul Kabir, 2010; Nolan *et al*., 2020), such as other respiratory viral infections or Mycoplasma (Alber *et al*., 2021) that can predispose to colibacillosis.
Among the different management measures, microclimatic housing conditions are likely to be the most relevant factors. Birds' density, humidity rate, ventilation, dust and ammonia should be monitored (Luftul Kabir, 2010), in particular high ammonia

concentrations can cause damages of the respiratory mucosa and, as a consequence, can increase bird's susceptibility to bacterial respiratory infections (Soliman *et al*., 2017). Additionally, a good quality of the air and the bedding material should be taken into account since *E. coli* can persist in ventilation and proliferate in wet litter (Li *et al*., 2019). Drinking water control can also reduce the dissemination of *E. coli* through water, in particular by using a nipple watering system and chlorination of water (Kunert Filho *et al*., 2015; Di Martino *et al*., 2018; Nolan *et al*., 2020).
Attention should be paid also to eggs, since a correct management and also disinfection of the eggs (Motola *et al*., 2020) represent an important tool to prevent and control colibacillosis and should be followed from the breeders, in order to avoid vertical transmission (Giovanardi *et al*., 2005). Among the different types of eggs, nest eggs are preferable to floor eggs since floor eggs have a higher mortality and higher contamination compared to nest eggs (Ahamed *et al*., 2019). Additionally, the use of floor eggs (that are usually dirtier than nest eggs) poses a high risk of *E. coli* contamination inside the hatcher (Christensen *et al.,* 2021) and hatchability and fertility of floor eggs is lower if compared to clean nest eggs (Van Den Brand *et al*., 2016). Therefore, management measures should be controlled also in the hatcheries, since they represent an important source of APEC (Ozaki *et al*., 2013).
This protocol is established as an essential and basic tool to perform a systematic review on the efficacy of management to prevent or control colibacillosis in broilers.

1.2. Objectives

The objective of this systematic review and meta-analysis, if supported by the data, is to address the following PICO question: *"In broilers at risk of colibacillosis, does management versus no management result in higher FCR/fewer condemnations/lower mortality?"*. The specific PICO elements are:

1. **P**opulation: Broilers (including the whole production chain).
2. **I**ntervention: Management practice(s) to prevent or control colibacillosis in broilers.
3. **C**omparator: No management practice(s).
4. **O**utcomes: Mortality, Feed Conversion Ratio (FCR) and condemnations due to colibacillosis at the slaughterhouse.

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.

2.2. Eligibility of study designs

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.

2.3. 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 (A&HCI), Conference Proceedings Citation Index-Social Science & Humanities (CPCI-SSH), Book Citation Index Science (BKCI-S) and Book Citation Index Social Sciences & Humanities (BKCI-SSH).

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/

2.4. 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 database will be used for this backwards searching. Alerts (also known as literature surveillance services) will be set up in the databases (when available) to monitor published studies relevant to the review question after the original search has been conducted.

The concept of the search strategy will be the following:

[**Broilers**] AND [**Management**] 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 (management OR stockmanship OR macroclimatic OR microclimatic OR climat* OR humidity OR wet OR drainage OR ammonia OR "carbone dioxide" OR light OR ventilation OR fumigat* OR temperature OR environment OR "stocking density" OR litter OR dust OR stress OR genetic* OR feed OR water OR "free range" OR organic OR conventional OR hatch* OR damage OR "skin scratching" OR injury OR "fast growing" OR "slow growing")

#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

2.5. Study Records

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

Selection process
Eleven independent reviewers (Gunther Antonissen, GA; Yewande Fasina, YF; Alessandra Piccirillo, AP; Karolina Scahill, KS; Giuditta Tilli, GT; Ronald Vougat Ngom, RN; Jeanine

Wiegel, JW; Gaspard Junior Ayissi, GJ; Niamh Cleiren, NC; Akenghe Tanyienow , AT and Jane Njaramba, JN) will carry out the screening using Rayyan. One-sixth of the citations will be assigned to each pair of reviewers (GA and AP, RN and GT, YF and KS, AT and NC, GJ and JN, GT and JW). This will guarantee that each reference is screened by two independent reviewers. During the screening phase, conflict will be resolved with a third reviewer (Helena C. de Carvalho Ferreira) if consensus between two reviewers of the pair cannot be reached.

The citations will be screened in two independent stages.

The concordance among all the reviewers will be evaluated by randomly selecting 100 of the citations entering title and abstract screening phase prior to screening all papers. For the full text screening, 10% of the papers will be used for the calibration exercise. These calibrations will enable discussion and solve disagreement before carrying out the full selection process (Sanguinetti *et al*., 2021).

For the title and abstract screening, eligibility of studies will be assessed with the following questions:

1. Is the study an original research assessing the use of management measure(s) to prevent or control colibacillosis in broilers? YES [PASS], NO [EXCLUDE], UNCLEAR [PASS]
2. 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.

For the full text screening, the eligibility of studies will be assessed with the following questions:

1. Is a full text of more than [500] words available? YES [PASS], NO [EXCLUDE]
2. Is a full text available in English, French and/or Spanish? YES [PASS], NO [EXCLUDE]
3. Is the **Population** of the study broilers? YES [PASS], NO [EXCLUDE]
4. Is the **Intervention** of the study the use of management measure(s) to prevent or control colibacillosis in broilers? YES [PASS], NO [EXCLUDE]
5. Is at least one of mortality, FCR, or condemnations due to colibacillosis at the slaughterhouse the **Outcome(s)** described? YES [PASS], NO [EXCLUDE]
6. 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 management measure(s) assessed and extract data] [EXCLUDE]

Data extraction

Four independent reviewers (GA, AP, GT and RN) will carry out this task using Excel or Revman. Conflict will be resolved with a third reviewer (Helena C. de Carvalho Ferreira) if consensus between two 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 intervention was applied
8. Production stage/age of birds when outcome(s) were measured

Intervention data:

1. Unit of population participants (e.g., flock, house/barn/pen)
2. Description of the comparator group
3. Number of birds enrolled in the participating unit
4. Number of flocks/house/barns/pens enrolled
5. Number of flocks/house/barns/pens enrolled lost until the end of trial study
6. Number of flocks/house/barns/pens enrolled analyzed
7. 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

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

2.6 Risk of Bias Assessment

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:
· 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?

2.7 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, 2014).

Conclusions

The overall objective of this systematic review is to examine the efficacy of management measures in the 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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