A protocol for a systematic review and meta-analysis

Title:

Is biosecurity efficacious to prevent or control colibacillosis in broiler production? 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 and Ronald Vougat Ngom drafted the protocol and all authors provided their input.

Registration

This protocol is archived at Padua Research Archive (handle code: 11577/3439978) 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). Biosecurity measures play a crucial role in protecting the flock against colibacillosis since it has been widely demonstrated that high biosecurity measures and correct management of flocks reduce diseases and consequently reduce condemnation rates at the slaughterhouse (Schulze Bernd et al., 2020). Correct implementation of biosecurity such as limited number of people entering the house, rigorous cleaning and disinfection procedures between production cycles (Mo et al., 2016), all in-all out system (Christensen et al., 2021), use of transition zone, pest control (Lutful Kabir, 2010), systematic culling of weak chicks at first week (Christensen et al., 2020), and

disinfestation protocols (Schiavone et al., 2020) may lead to a reduction of contamination within the farm. Cleaning and disinfection procedures, if well executed, represent an important tool for *E. coli* reduction (Hao et al., 2013; Bragg et al., 2014; Luyckx et al., 2014; 2015), especially if wet rinsing is used (steam treatment of ≥60°C and 100% RH during a 24h period (Gradel et al., 2003; 2004).
When implementing biosecurity measures, other potential transmission routes like insects (Souillard et al., 2014; Sandberg et al., 2017), personnel (Chowdhury et al., 2012), rodents (Backhans & Fellström, 2012; Velkers et al., 2017) or other species (Fujimoto et al., 2015) should be taken into account as well as egg management. Correct egg management represents an important means to prevent and control colibacillosis and should be ensured from the breeders, in order to avoid vertical transmission (Giovanardi et al., 2005). In this context, nest eggs are preferable to floor eggs since the latter may cause a higher mortality and higher contamination compared to nest eggs (Adikari, 2020). In addition, the use of floor eggs (that are usually dirtier than nest eggs) poses a higher risk of *E. coli* contamination inside the hatchery (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).
Finally, in biosecurity implementation also hatcheries should be taken into account since, as Zhao et al. (2019) reported, inadequate sanitation of the hatcheries leads to a higher incidence of poultry colibacillosis.

The protocol is established as an essential and basic tool to perform a systematic review on the efficacy of biosecurity measures to prevent or reduce 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 review question: "*In broilers at risk of colibacillosis, does biosecurity versus no biosecurity 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: Biosecurity measures to prevent colibacillosis in broilers.
3. **C**omparator: Low biosecurity.
4. **O**utcomes: Production losses (FCR), mortality, condemnation 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 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, KCI-Korean Journal Database, Medline, Russian Science Citation Index and SciELO Citation Index). However, we will exclude the following editions: Arts & Humanities Citation Index (A&HCI), Conference Proceedings Citation Index-Science (CPCI-S), Conference Proceedings Citation Index-Social Science & Humanities (CPCI-SSH) and Social Sciences Citation Index (SSCI).

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 backward 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 [**Biosecurity**] 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 (Biosecurity OR Clean* OR Disinfect* OR Disinfest* OR Pest OR Insect* OR Vermin* OR Rodent* OR Fomites OR Sanit* OR Hygien* OR "All in-all out" OR Downtime OR Turnaround OR "Biological break" OR "Filter zone" OR "Danish entry system" OR "Footdips" OR Visitor* OR Thinning OR Depopulation)

#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 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
The citations will be screened in two independent stages.
The first stage of the selection process will consist of titles and abstract screening. Two independent reviewers (Giuditta Tilli and Ronald Vougat Ngom) will carry out this task using Rayyan. Conflict will be resolved with a third reviewer (Alessandra Piccirillo) if

consensus between the two reviewers cannot be reached. Eligibility of studies will be assessed with the following questions, as suggested by Sargeant et al. (2019):

1. Is the study an original research assessing the use of biosecurity measure(s) to prevent or control colibacillosis in broilers? YES [PASS], NO [EXCLUDE], UNCLEAR [PASS]
2. Does the study include an eligible comparator via a controlled trial, disease challenge study or observational study? YES [PASS], NO [EXCLUDE], UNCLEAR [PASS]

The studies that meet inclusion criteria will pass to the next phase. The concordance among the reviewers will be evaluated by randomly selecting 25 of the citations entering each stage of the process prior to screening all papers. This calibration study will enable discussion and solve disagreement before carrying out the full selection process by the two reviewers (Sanguinetti et al., 2021).

The second stage will involve the full-text screening. Two independent reviewers (Giuditta Tilli and Ronald Vougat Ngom) will carry out this task using Rayyan. Conflict will be resolved with a third reviewer (Alessandra Piccirillo) if consensus between the two reviewers cannot be reached. 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 and/or Spanish? YES [PASS], NO [EXCLUDE]
3. Is the **Population** of the study broilers? YES [PASS], NO [EXCLUDE], UNCLEAR [NO]
4. Is the **Intervention** of the study the use of biosecurity measure(s) to prevent or control colibacillosis in broilers? YES [PASS], NO [EXCLUDE], UNCLEAR [NO]
5. Is at least one of mortality, FCR, or condemnations due to colibacillosis the **Outcome(s)** described? YES [PASS], NO [EXCLUDE]
6. Is the study design a controlled trial with natural disease exposure? YES [PASS to data extraction process], NO [this is a disease challenge study, indicate the biosecurity measure(s) assessed and extract data]
7. Is the study design a controlled trial with natural disease exposure? YES [PASS to data extraction process], NO [this is an observational study, indicate the biosecurity measure(s) assessed and extract data]

Data extraction

Two independent reviewers (Giuditta Tilli and Ronald Vougat Ngom) will carry out this task using Revman. Conflict will be resolved with a third reviewer (Alessandra Piccirillo) if consensus between the 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:
· Characteristics such as biosecurity practices 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?

2.7. 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; Marvridis *et al*., 2014).

Conclusions

The overall objective of this systematic review is to examine the efficacy of biosecurity 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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