THE APPLICATION OF RESEARCH SYNTHESIS METHODS FOR EVALUATING PRIMARY RESEARCH ON *SALMONELLA* IN BROILER CHICKENS

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ABSTRACT

THE APPLICATION OF RESEARCH SYNTHESIS METHODS FOR EVALUATING PRIMARY RESEARCH ON *SALMONELLA* IN BROILER CHICKENS

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Research synthesis methods were applied to identify, appraise and summarize the primary research on *Salmonella* in broiler chickens from farm-to-secondary processing pertaining to three sub-topics: interventions, risk factors, and prevalence. A scoping review was utilized to develop evidence maps for these sub-topics and prioritize *a priori* determined questions for rigorous systematic reviews. Of 12, 982 potentially relevant citations, 748 studies addressed interventions, risk factors (n=30) or prevalence (n=200). Evidence maps of sub-topics indicated substantial heterogeneity in study methods. Among studies evaluating an on-farm intervention, competitive exclusion (CE, n=192) was the most frequently studied. The results of a systematic review-meta-analysis (SR-MA) indicated that various CE products reduced *Salmonella* colonization in broilers, for up to13 weeks post-treatment. The methodological soundness of these studies was limited. The scoping review-SR-MA approach is useful for characterizing broad topics and prioritizing questions for SR, and should be considered for routine use in microbial food safety.

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CHAPTER ONE

INTRODUCTION, STUDY RATIONALE AND OBJECTIVES

Foodborne illness

It is estimated that more than 200 diseases can be transmitted to humans through food (Mead et al., 1999). In 2005, approximately 1.8 million people worldwide died from diarrheal diseases, largely attributed to the consumption of contaminated food and drinking water (Anonymous, 2007a). Although the majority of these deaths occurred in developing countries, up to 30% of people living in industrialized countries are estimated to be affected by foodborne illnesses each year (Anonymous, 2007a).

In the United States, it is estimated that foodborne diseases cause between 6 million and 81 million illnesses, resulting in 325,000 hospitalizations, and up to 9,000 deaths each year (Mead et al., 1999; Anonymous, 2007a). In Canada, this number ranges between 11 and 13 million estimated cases of foodborne illness per year (Anonymous, 2006a). While the majority of foodborne disease cases are sporadic and often underreported, large outbreaks are frequently observed and documented. For example, an outbreak of salmonellosis in 1994 due to contaminated ice cream affected 224,000 individuals in the United States (Anonymous, 2007a).

Foodborne salmonellosis

In 2004, a total of 2,501 unique *Salmonella* serovars were identified, but only around 100 were routinely isolated from food, animals, and humans (Anonymous, 2005). Some

serovars have a limited host-spectrum, affecting only one or a few animal species; for example, *Salmonella* Gallinarum and *S.* Pullorum in poultry (Anonymous, 2005; Cox et al., 2005). The majority of serovars have a broad host-spectrum and *Salmonella* Enteritidis and *S.* Typhimurium are the most important serovars transmitted to humans from animals, mostly through contaminated foods of animal origin (Anonymous, 2005).

Worldwide, *Salmonella* is recognized as one of the most common causes of foodborne illness in humans (Mead et al., 1999; Wegener et al., 2003; Anonymous, 2007a). In recent years, the incidence and severity of human salmonellosis cases has increased significantly, in part due to the emergence of antimicrobial resistance in certain *Salmonella* serovars and improved reporting systems (Anonymous, 2005). Approximately 6,000 to 12,000 cases of human salmonellosis are reported annually in Canada (Anonymous, 2006b); however, these data have to be interpreted with caution because of high levels of underreporting (Todd, 1992; Todd, 1997; Mead et al., 1999; Campbell et al., 2003). In the United States, the total cost associated with *Salmonella* is estimated to be US $3 billion annually, where foodborne salmonellosis is thought to account for $2.9 billion (Anonymous, 2005; Miller et al., 2005; Frenzen, 2009).

Salmonella can cause illness in people of all ages, but the incidence is highest in infants (White et al., 1997). In affected individuals, gastrointestinal symptoms develop approximately 12 to 72 hours after ingestion of the bacterium, and generally last 4 to 7 days (Anonymous, 2006b). Common symptoms include fever, chills, abdominal cramps, nausea, and diarrhea (White et al., 1997; Anonymous, 2006b). Most individuals infected

by a broad host-spectrum serovar recover without treatment, but infection can be severe in the young, elderly or patients with weakened immune systems (Anonymous, 2005).

Salmonella: A global challenge in broiler chicken production

Salmonella can colonize the intestinal tracts of mammalian, avian and amphibian hosts (Anonymous, 2006b). As a result, salmonellosis in humans is often associated with foods of animal origin, such as eggs and meat (White et al., 1997; Anonymous, 2006b).

Broiler chickens can be colonized with *Salmonella* by one of two types of transmission, horizontal or vertical (Mead et al., 1999; Poppe, 2000). Horizontal transmission involves fecal-oral transmission, directly among broilers, or more frequently, indirectly through a contaminated environment, such as feed, water, dust, rodent droppings, or footwear or clothing of farm personnel contaminated with *Salmonella* (White et al., 1997; Poppe, 2000; Cox et al., 2005). Vertical transmission occurs directly to the broiler via the egg, when broiler breeders pass the infection on to their developing ova, usually due to an infection in the ovary or oviduct (Poppe, 2000). Once colonized, broilers are most likely to be asymptomatic carriers, although, in some cases, clinical illness can occur (Poppe, 2000). Asymptomatic carriers can continue to shed *Salmonella* in their feces, contaminating their environment and other broilers (Poppe, 2000).

Broilers can become infected with *Salmonella* at any point in the production chain, although the majority become infected as young chicks (White et al., 1997; Mead et al., 1999; Cox et al., 2005; Van Immerseel et al., 2005). Young chicks are particularly

vulnerable to infection because at hatching they lack protective microflora in the gut and their immune system is immature (White et al., 1997; Mead et al., 1999; Cox et al., 2005; Van Immerseel et al., 2005). This susceptibility can lead to colonization if chicks are exposed to a sufficient dose of bacteria because of persistent hatchery or farm contamination (Poppe, 2000; Van Immerseel et al., 2005).

The highest level of intestinal colonization of *Salmonella* in broilers is typically during the second or third week of grow-out (White et al., 1997; Cox et al., 2005). After the third week there is a gradual decline in prevalence, which continues until processing, where carcasses can be accidentally exposed to external contamination from skin, feathers or contaminated intestinal or crop contents during slaughter (Hargis et al., 1995; Cox et al., 2005). It is estimated that *Salmonella* prevalence near the end of the grow-out phase is between 5 and 10%, but 30 to 50% during processing (Jones et al., 1991; Hargis et al., 1995; White et al., 1997). This increase in prevalence is a result of the multiple points of cross-contamination that can occur during broiler chicken processing (Figure 1.1.) (White et al., 1997).

Carcasses that become contaminated during processing can be distributed for retail, posing a public health risk. In 2002, approximately 10 to 15% of poultry meat at the retail level was positive for several serovars of *Salmonella* in all European countries except Scandinavia (Van Immerseel et al., 2005). A recent study conducted in Alberta, Canada reported that 30% of raw chicken legs from the retail marketplace were contaminated with *Salmonella* (Bohaychuk et al., 2006). This coincides with the

prevalence estimate of 20% in federal broiler processing facilities, provided by the Canadian Food Inspection Agency (Canadian Food Inspection Agency, 2002; Rajić et al., 2007).

Salmonella control in broiler chickens: What works?

The approaches used to control *Salmonella* in broiler chickens vary among regions and countries. In North America, *Salmonella* control primarily occurs at the processing level (Cox et al., 2005). In the US, a Hazard Analysis and Critical Control Point (HACCP)/pathogen reduction approach requires that all poultry processing plants to adopt a system to prevent food safety hazards (Anonymous, 2009b). The program has set *Salmonella* performance standards, based on nationwide averages determined in pre-HACCP studies, to verify whether it reduces *Salmonella* contamination (Roos, 2009). Post-chill broiler carcasses are tested in sets of 51 samples, and 12 samples are permitted to test positive for *Salmonella* (Food safety and inspection service, 1996; Anonymous, 2009b). If an establishment does not meet these standards, corrective action includes coordination among consumer safety officers, district managers, circuit supervisors, compliance officers and inspection personnel (Anonymous, 2009b). To date, this approach has been successful at reducing *S.* Pullorum and *S.* Gallinarum in broiler carcasses, but not serovars of main public health importance (Sternberg Lewerin et al., 2005).

In contrast, a farm-to-fork approach is officially legislated in the European Union (EU) (Anonymous, 2007b) and resembles the main features of the well-known Scandinavian

approach (summarized below). Results of the latter suggest that the elimination or significant reduction of *Salmonella* serovars of public health importance at processing requires the delivery of *Salmonella*-free chicks from the hatchery and grow-out farms (Cox et al., 2005).

Since 1970, Sweden has had a national control program aimed at the eradication of all *Salmonella* serovars in poultry, including broiler chickens (Sternberg Lewerin et al., 2005). The program starts from the top of the broiler breeding pyramid, and includes extensive regulations on poultry feed, breeding flocks, hatcheries, rearing, egg production, slaughter and processing (Sternberg Lewerin et al., 2005). This mandatory program includes the monitoring of all poultry flocks using a *Salmonella* culture method approved by the EU, reporting to the veterinary authorities if *Salmonella* positive samples are detected, and submission of all isolates to the National Veterinary Institute for typing and antimicrobial resistance testing (Sternberg Lewerin et al., 2005). All *Salmonella*-contaminated flocks are killed by euthanasia, regardless of serovar, and considered unfit for human consumption (Sternberg Lewerin et al., 2005). A voluntary portion of the program consists of a high level of biosecurity and hygiene practices (Sternberg Lewerin et al., 2005). Although this approach has achieved virtually *Salmonella*-free broiler populations in Sweden (Sternberg Lewerin et al., 2005), the feasibility of such a program elsewhere is questionable. Sweden has a relatively small broiler chicken production industry, compared to major chicken producing countries, such as the US, China and Brazil (Evans, 2008).

Although some EU member-states questioned the feasibility of the Scandinavian approach, *Salmonella* in food-animal primary production was identified as the main food safety priority in the EU (Lars Plym Forshell, National Food Administration, Sweden, personal communication). As a result, the EU Zoonoses legislation was developed, initially targeting pigs, turkeys and broiler chickens. For each of these populations, each EU member-state developed a *Salmonella* reduction target, based on EU-wide national baseline prevalence surveys that were conducted between 2005 and 2006. Broiler chicken surveys were conducted in a representative sample of commercial broiler flocks with at least 5,000 birds (Anonymous, 2007b). The *Salmonella* flock prevalence varied among the member-states from 0 to 68.2%, with Sweden as the only country with no *Salmonella* positive flocks (Anonymous, 2007b). The survey determined that 11% (range of 0% to 39%) of broiler flocks were positive for *S.* Enteritidis or *S.* Typhimurium, the two most common serovars found in humans (Anonymous, 2007b). As a result, the EU set a reduction target in broilers only for these two serovars (Anonymous, 2007b), and it was left up to member-states to consider addressing other serovars of public health importance within the context of their country (Anonymous, 2007b).

In Canada, the Chicken Farmers of Canada introduced an on-farm food safety (OFFS) program called Safe, Safer, Safest in 1998 (Rajić et al., 2007; Anonymous, 2009a). The program is voluntary, industry-driven and overseen by government. It incorporates mandatory and recommended good production practices (GPP), partially based on HACCP principles, to ensure the safety of broilers leaving farms (Rajić et al., 2007; Anonymous, 2009a). Specific biological, chemical, and physical hazards are mentioned, but not pathogen-specific monitoring or control practices as a means to control these

hazards (Rajić et al., 2007). To date, producers are not penalized for violation of the practices prescribed by the program, which might affect the compliance of producers, as well as the success of the program (Rajić et al., 2007).

The need for scoping reviews and global evidence mapping

Various stakeholders frequently conduct or commission out comprehensive reviews of primary research addressing broad microbial food safety topics, such as *Salmonella* in broiler chickens, in order to generate position statements or issue-specific guidelines or identify research gaps and support future research. These substantial and costly efforts rarely employ systematic and transparent methods for identifying, critically evaluating and summarizing existing evidence. Haphazard reviews are often not repeatable, and a lack of transparency in review methodology can compromise agreement among various stakeholders concerning the body of evidence on a given subject, as well as their support for recommended actions, particularly if the evidence behind the recommendations is ambiguous or contradictory.

Systematic review (SR) (described below) is a transparent and well-established approach used to review the primary research on a focused question (Borenstein et al., 2009). A relatively new type of literature review applied primarily in nursing and health care sectors is called a scoping review and is also referred to as evidence mapping. This approach is reported to be more useful for addressing broader topics or fields of interest than a SR (Katz et al., 2003; Arksey and O'Malley, 2005; Anderson et al., 2008; Davis et al., 2009). The main purposes of scoping reviews are to: examine the quantity, scope and

characteristics of available primary research (evidence mapping); determine if a full SR is feasible on specific, focused questions, relevant and/or cost-effective; summarize and distribute research findings; and identify knowledge gaps (Arksey and O'Malley, 2005; Anderson et al., 2008; Davis et al., 2009).

The methodological framework for this approach is still evolving because the methods, interpretation and expectations applied in various scoping reviews are highly variable, even within a sector (Davis et al., 2009). Scoping reviews share certain characteristics with SRs, such as a replicable and structured literature search and explicit study selection criteria (Arksey and O'Malley, 2005). The main difference is that scoping reviews are primarily used to address broader topics with a wider range of study designs and diverse evidence (Katz et al., 2003; Arksey and O'Malley, 2005; Davis et al., 2009). For these reasons, scoping reviews are often used as a preliminary stage to a full SR (Katz et al., 2003; Anderson et al., 2008). Another useful outcome of this approach is identification of knowledge gaps, which is useful for guiding future research and resource allocations and informing decision-makers (Katz et al., 2003; Anderson et al., 2008). An example of the scoping review approach, as used by the Global Mapping Initiative Network, is outlined in Figure 1.2. (Clavisi et al., 2008). This review retrieved, evaluated and summarized research evidence addressing a range of clinical questions in the pre-hospital, hospital and rehabilitation phases of care for individuals with traumatic brain and spinal cord injuries, using evidence maps (Clavisi et al., 2008).

Scoping reviews are intuitively appealing for broad microbial food safety topics that comprise a wide range of evidence and study designs. An enormous quantity of primary research exists even for a single microbial food safety topic, such as *Salmonella* in broiler chickens. For example, some studies report the prevalence or accuracy of diagnostic tests, others risk factors or interventions for reducing *Salmonella* in various broiler sub-populations and still others investigate the public health aspect of the issue. The complexity of the food chain increases the variation within each topic area because studies are implemented at different levels of the chain from farm-to-processing. Concurrently, food safety experts tend to be highly specialized in one or more topic areas and less often across all areas. For these reasons, the scoping review approach might assist various stakeholders and experts in understanding the characteristics of the available evidence on a selected topic, and through increased transparency and replicability of the review process, might contribute to the overall trust and communication among various stakeholders.

The benefits of using systematic reviews in microbial food safety

Traditional narrative reviews addressing broad or narrow topics in clinical care, microbial food safety or zoonotic public health are often of limited usefulness. They rarely employ a systematic and transparent approach to identify relevant literature, distinguish between valid and unacceptable study conduct and reporting, or support quantitative syntheses (Mulrow, 1987; Oxman and Guyatt, 1988; Egger et al., 2001; Waddell et al., 2009). For this reason, it is believed that narrative reviews are open to expert bias and not

informative for policy decision-makers (Egger et al., 2001; Sargeant et al., 2006; Borenstein et al., 2009; Davies and Crombie, 2009; Waddell et al., 2009).

Systematic review is a transparent and replicable method used to identify, appraise and synthesize the existing research on a focused topic or question (Borenstein et al., 2009). This method is most frequently used to evaluate the effectiveness of interventions, and to a lesser extent, the accuracy of diagnostic tests and to better understand the consistency of reported risk factors and/or prevalence estimates (Sargeant et al., 2006; Sanchez et al., 2007; Centre for Reviews and Dissemination, 2009). Systematic reviews might also be used to generate evidence-based inputs for risk assessments and to identify research gaps and needs (Sargeant et al., 2006; Centre for Reviews and Dissemination, 2009). The main principles of the SR method are shown in Figure 1.3. Appropriately executed and reported SRs provide more transparent, accurate and complete information to decision-makers for various contextual uses than traditional narrative reviews (Mulrow, 1994; Sargeant et al., 2006). The main differences between traditional narrative and SRs are listed in Table 1.1. (Cook et al., 1997).

Although SRs are most common in the human health care literature, several have recently been published in the food safety and veterinary public health fields, addressing a single or a few very focused questions (Sanchez et al., 2007; Sargeant et al., 2007; O'Connor et al., 2008; Waddell et al., 2008; Wilhelm et al., 2009; Young et al., 2009). Their more frequent use may assist in more transparent and accountable, evidence-based policy and decision-making in those fields.

The use of meta-analysis for better understanding global evidence

Meta-analysis is a statistical technique that combines the results from multiple independent studies in order to generate a more precise overall effect estimate (Borenstein et al., 2009; Crombie and Davies, 2009). The weights that are assigned to each included study are based on pre-determined mathematical criteria that account for varying sample sizes (Deeks et al., 2001; Borenstein et al., 2009). The effect estimates reported in studies vary because of chance and individual study characteristics, such as differences among studied populations, study settings and methods of delivery of the intervention (Deeks et al., 2001; Crombie and Davies, 2009). This heterogeneity is measured, and if large (p-value for Q statistic <0.1), the pooled estimate should not be reported, or the heterogeneity should be explained through methods such as a stratified meta-analysis or meta-regression (Sutton et al., 2000). In the latter, potential associations between the study design, methodological soundness characteristics and the reported effect estimate are evaluated (Deeks et al., 2001; Crombie and Davies, 2009).

A high quality meta-analysis requires a high quality SR, through which the studies are comprehensively and critically appraised, generating valid data (Borenstein et al., 2009; Crombie and Davies, 2009). To date, mostly qualitative SRs have been published in microbial food safety; the use of meta-analysis was often precluded because of a low number of studies suitable for inclusion in the review and/or large heterogeneity among the included studies (Sargeant et al., 2007; Wilhelm et al., 2009).

Study rationale

Despite the long history and overall importance of *Salmonella* in the broiler chicken industry and the relatively extensive global research efforts in this field, the extent of the problem within the Canadian context is currently unknown, particularly concerning the most relevant risk factors and interventions for reducing *Salmonella* in broiler chickens, specifically at the farm level. Currently, there is no control program targeting *Salmonella* in broiler chickens in Ontario or the rest of Canada. Before various control options are considered, it is important that the existing research evidence is comprehensively and critically summarized, evaluated and well-understood. Concurrently, various expert groups, appointed by the World Health Organization, Food and Agriculture Organization and the Codex Committee for Food Hygiene, are in the process of developing international guidelines for controlling *Salmonella* in broiler chickens, at the farm and processing levels (Anonymous, 2002; Lammerding and Fazil, Public Health Agency of Canada, personal communication).

For the above reasons, it is important and timely to map out the existing primary research on *Salmonella* in broiler chickens using a scoping review. To the best of our knowledge, this method has not been attempted in the food safety arena. In this thesis, the breadth of primary research underpinning selected sub-topics of *Salmonella* in broilers was evaluated and summarized using this approach, including relevance of the evidence within the Ontario/Canadian context and major research gaps.

Potential questions for rigorous and focused SRs were identified from an *a priori* determined list of potential questions, refined and prioritized based on the results of the scoping review and inputs from the review team, taking into consideration the available manpower and time. Where sufficient data was available, a meta-analysis-meta-regression approach was used to quantitatively present the data. Thus, the two methodologies, scoping review and SR-MA, compliment each other within the context of this thesis.

The results of the scoping review will also be utilized in a companion research project to identify questions for rigorous SRs, and generate inputs and determine data gaps for a quantitative risk assessment. Through the latter, various options will be evaluated for controlling *Salmonella* in broiler chickens within the Ontario context (Bucher et al., University of Guelph, in progress). The complementary aspects of this and another project (Bucher et al., University of Guelph, in progress) are indicated in Figure 1.4.

Thesis objectives and format

The main objectives were:

1. To identify, evaluate and summarize the available primary research on *Salmonella* in broiler chickens for three sub-topics: interventions, risk factors, and prevalence from farm-to-secondary processing, using a scoping review. The opportunities and challenges for using this approach on broader topics in microbial food safety were also evaluated.

2. Based on the results of the scoping review, to select the most promising on-farm intervention, and using a SR-MA approach, to evaluate if the intervention reduces *Salmonella* colonization in broiler chickens.

This thesis is written using a paper style format. Chapters 2 and 3 are formatted according to the style of the journal to which the manuscripts will be submitted. As such, there is unavoidably some repetition of information between chapters. Since research synthesis methods, namely a scoping review and SR-MA, and their application on *Salmonella* topics in broiler chickens are the main focus of this thesis (Chapters 2 and 3), a traditional literature review is not part of the thesis.

Table 1.1. Differences between narrative and systematic reviews

Feature	**Narrative review**	**Systematic review**
Question	Broad in scope	Focused
Search strategy	Not usually specified	Comprehensive, explicit
Selection	Not usually specified	Criterion-based
Appraisal	Variable	Rigorous, critical
Synthesis	Often a qualitative summary	Often a quantitative summary
Inferences	Sometimes evidence-based	Usually evidence-based

Adapted from (Cook et al., 1997)

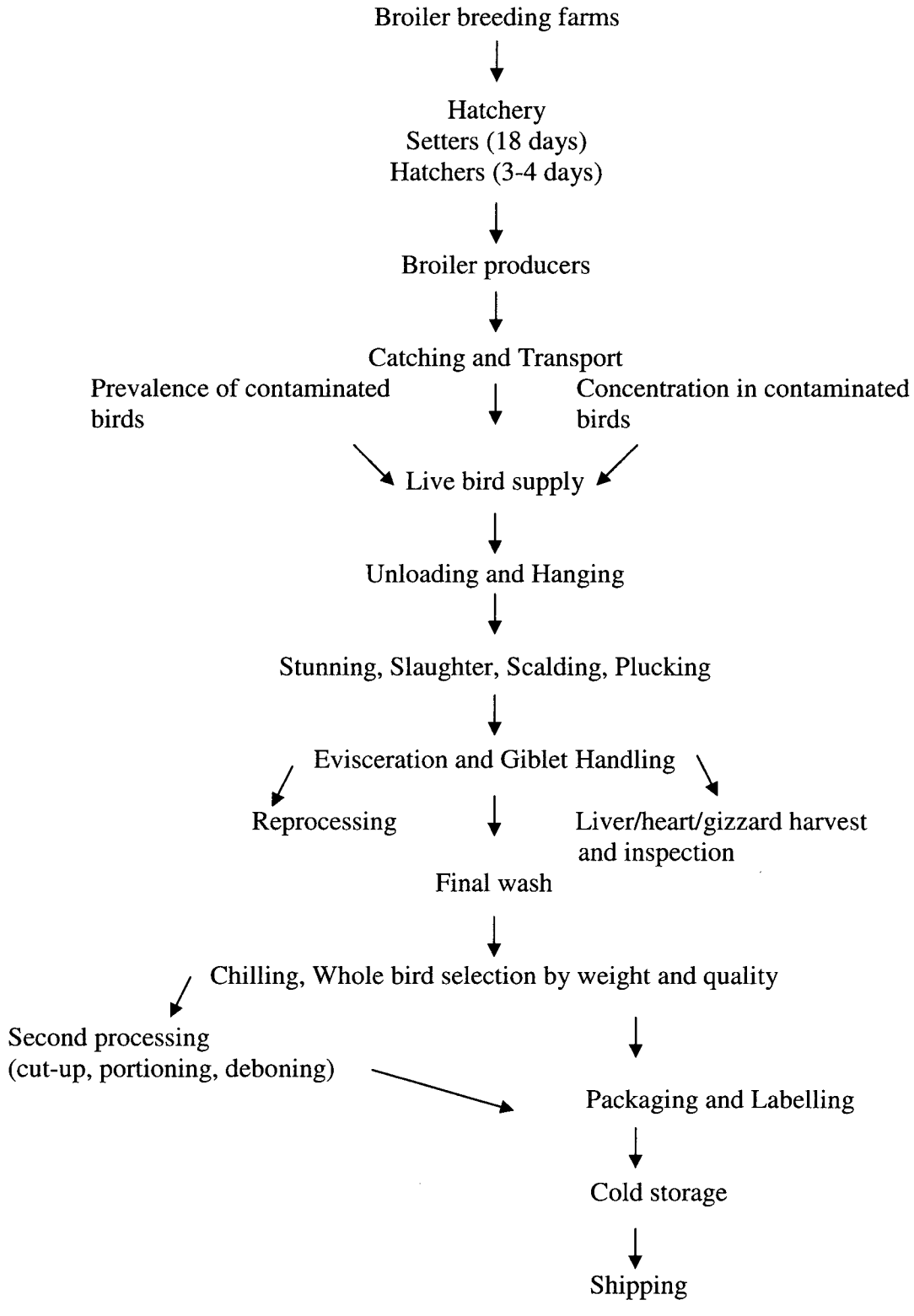

Figure 1.1.

Process flow diagram of broiler chicken production from farm-to-fork

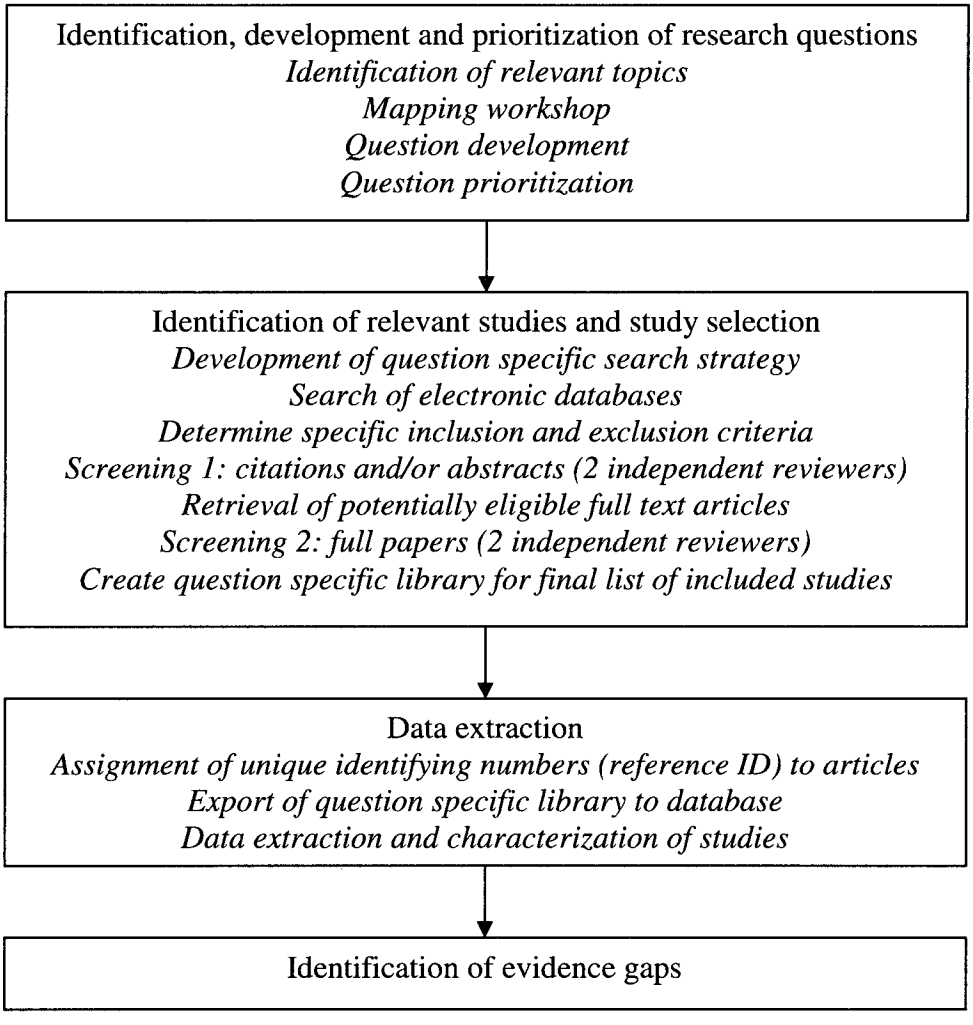

Figure 1.2.

Scoping review method used to evaluate the rehabilitation of patients with traumatic brain or spinal cord injury by the Global Evidence Mapping Initiative Network

Adapted from (Clavisi et al., 2008)

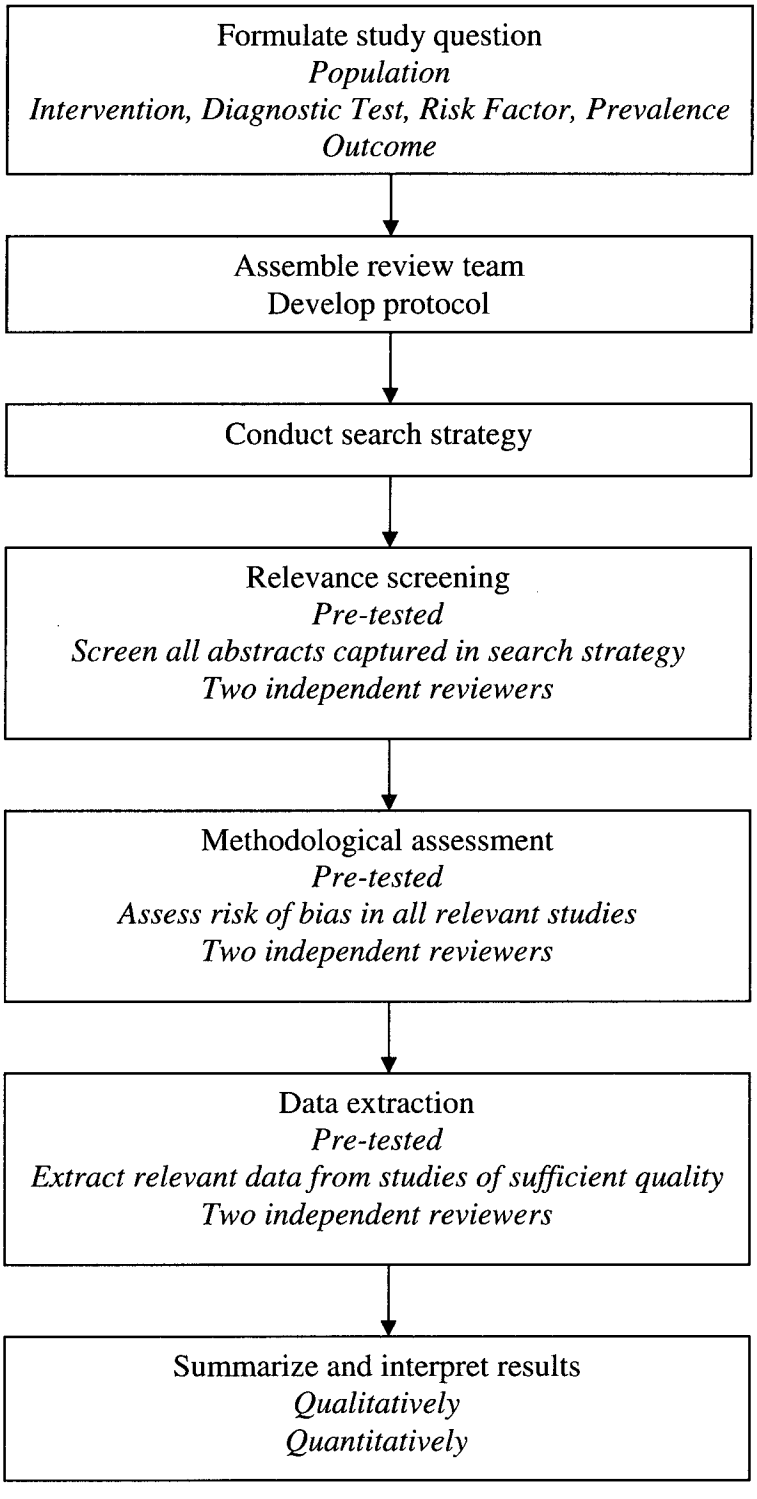

Figure 1.3.

Steps to conducting a systematic review

Adapted from (Sargeant et al., 2006)

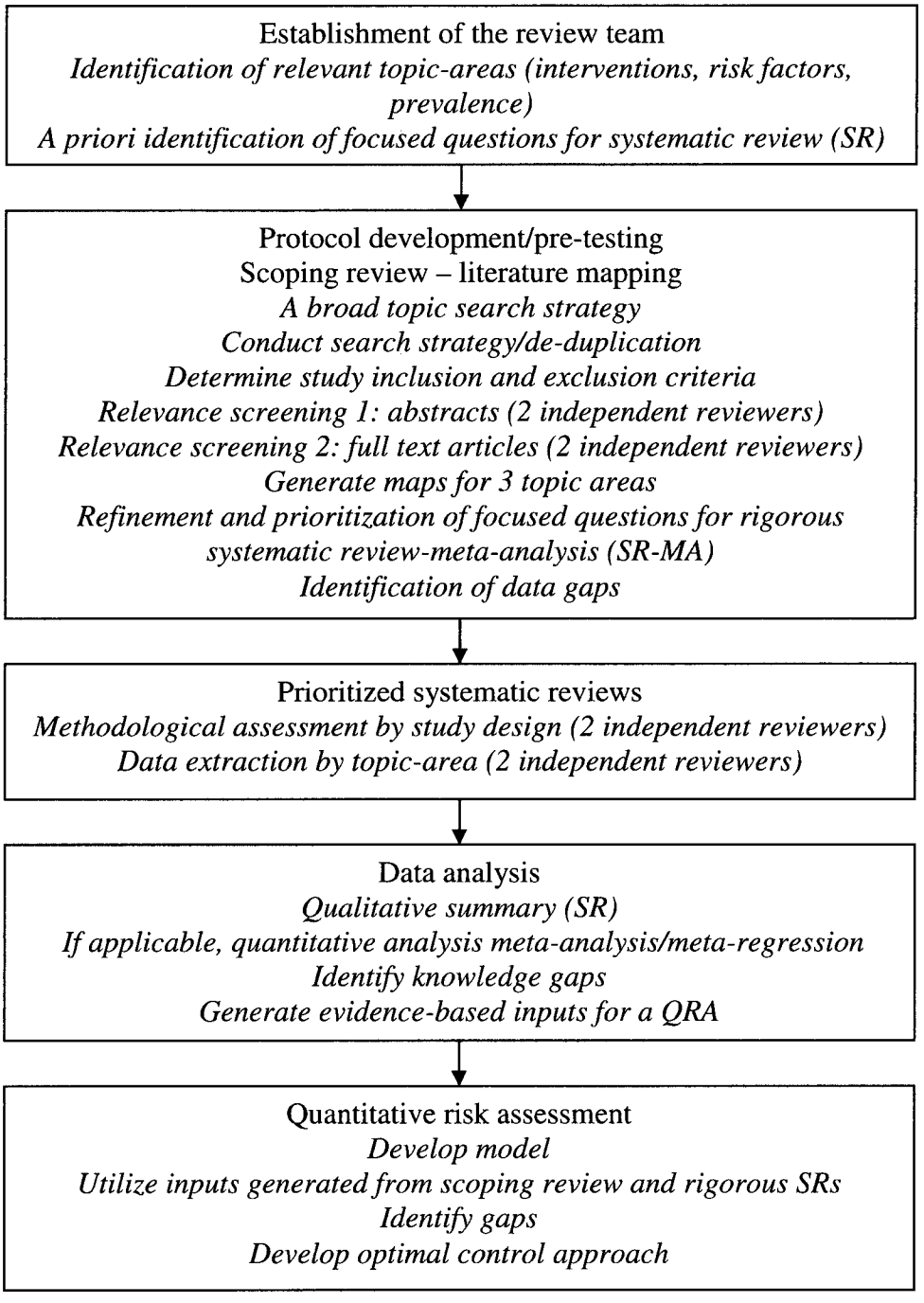

Figure 1.4.

Description of scoping review-systematic review-meta-analyses framework used in this thesis and the connection to a complementary project (Bucher et al., University of Guelph, in progress)

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CHAPTER TWO

A SCOPING REVIEW ON *SALMONELLA* IN BROILER CHICKENS: BENEFITS AND CHALLENGES

ABSTRACT

Scoping reviews are a relatively new research synthesis method primarily used in the health sector for characterizing broad topics in terms of the overall quantity, scope and distribution of the available primary research. In this paper, we propose a framework for conducting scoping reviews on broad microbial food safety (MFS) topics. The method was applied to the literature on *Salmonella* in broiler chickens from farm-to-secondary processing, in order to map the primary research on intervention, risk factor and prevalence studies, and to assess potential benefits and challenges for using this approach in MFS. An explicit literature search was conducted and *a priori* determined study inclusion criteria were applied. Each step of the electronic review was conducted by two independent reviewers. From 12,982 potentially relevant citations, 978 abstracts investigated interventions (n=748), risk factors (n=30), or prevalence (n=200). Among intervention abstracts, 79% (591/748) investigated an on-farm intervention, with competitive exclusion (CE, n=192) as the most frequently studied category. Among 200 prevalence studies, 73 were conducted in North America, of which 27 and 33 were conducted at the farm and processing levels, respectively. The research team selected, based on the results of the scoping review, five relevant on-farm interventions, and prevalence and risk factor studies conducted within North America for rigorous systematic reviews (SR). Within intervention categories, studies indicated large variability in terms of the types of intervention, type of products within intervention,

routes of administration, populations and outcome measurement. This was illustrated through an evidence map of a single CE product. The on-farm intervention evidence map comprised many poorly designed or reported studies of small sample size. A lack of large field studies was observed. The scoping review was useful for mapping out the primary research on our broad topic, prioritizing questions for rigorous SR, and identifying research gaps. This scoping review was time and resource demanding (10 months and 10 reviewers), as only part-time and inexperienced reviewers were available. However, with full-time and experienced reviewers, a scoping review could be completed within three to six months depending on the breadth of the topic and the availability of primary research. The advancement of existing electronic review formats and refinement of the existing methodological framework would increase the feasibility of this method in the future. Review groups should consider this approach for routine use in MFS on long-term issues for which there is the possibility of high impact decisions to be made and re-evaluated over time. This method allows structured mapping of the evidence and transparent evaluation of potential actions.

INTRODUCTION

Scoping review is a relatively new approach for identifying, evaluating and briefly summarizing research evidence on broad topics (Katz et al., 2003; Arksey and O'Malley, 2005; Anderson et al., 2008). This approach, also known as evidence mapping, has been applied primarily in nursing and health care sectors to evaluate the quantity, scope and characteristics of primary research underpinning broad topics of interest as a way to make

sense of diverse evidence prior to conducting several systematic reviews (SR) (Arksey and O'Malley, 2005; Anderson et al., 2008; Davis et al., 2009).

Scoping reviews follow some of the principles of SR methodology, such as explicit and reproducible search and study selection methods (Arksey and O'Malley, 2005; Sargeant et al., 2005). Systematic review is the most popular research synthesis method used to identify the effectiveness of interventions and to a lesser extent, the accuracy of diagnostic tests and consistency of risk factors or prevalence estimates (Sargeant et al., 2006; Centre for Reviews and Dissemination, 2009). This methodology, when used appropriately, reduces bias in interpretation of findings from a pool of studies by following a structured, transparent and replicable methodology to search for, select, appraise and extract information from primary research in which each step is conducted independently by at least two reviewers (Mulrow, 1994; Sargeant et al., 2006). Over the past five years, the use of SR and meta-analysis has gained momentum in microbial food safety (MFS) over the past five years (Sargeant et al., 2005; Sargeant et al., 2006; Sanchez et al., 2007; Sargeant et al., 2007; O'Connor et al., 2008; Waddell et al., 2008; Wilhelm et al., 2009; Young et al., 2009).

Although useful, SRs are mainly conducted on narrow, focused questions, resulting in very specific summaries of evidence, which on their own might not be sufficient to support decision-making on broad and complex issues which are often of greatest interest to decision makers (Katz et al., 2003; Arksey and O'Malley, 2005). Many microbial food safety topics are broad in nature and stakeholders require that scientific evidence is

evaluated within its broad context (Bero and Jadad, 1997). Moreover, a single, in-depth SR requires substantial manpower and time to address one focused question. For the above reasons, a scoping review offers an intuitively appealing framework for mapping out the breadth of evidence and research gaps on a broad topic that will help answer some questions about the availability of evidence and help prioritize the use of an in-depth, rigorous SR on specific, focused questions based on the overall quantity and distribution of primary research (Anderson et al., 2008).

The objectives of this study were to: apply a scoping review on a broad MFS topic and evaluate its utility using the topic of *Salmonella* in broiler chickens to map out the quantity, scope, characteristics and knowledge gaps pertaining to primary research on interventions, risk factors and prevalence estimates; prioritize from a list of *a priori* developed questions those that are relevant for rigorous SR; and provide direction for a complementary quantitative risk assessment (QRA). The benefits and challenges of the approach have been assessed throughout the text.

SCOPING REVIEW APPROACH

Topic refinement

A review team was established including professionals with expertise in veterinary epidemiology, microbiology, poultry production, SR, and literature search. The group refined the broad topic, *Salmonella* in broiler chickens, into three sub-topics: interventions, risk factors and prevalence and/or concentration studies conducted from farm-to-secondary processing (Table 2.1.). The scope was limited to these three areas

primarily to meet the needs of a complementary QRA currently being constructed, and due to manpower and time constraints.

Search strategy

A simple search strategy was implemented in December 2007 in PubMed (1860-2007), Agricola (1924-2007), Current Contents (1999-2007), Scopus (1960-2007), CAB (1913-2007) and CAB Global Health (1971-2007). The algorithm included one broad outcome "*Salmonella*" and four broad population "chicken" terms (Table 2.2.) to increase citation retrieval for all three sub-topics. No time period or language restrictions were used at this stage. All citations were imported into the reference manager Procite 5.0 (Thomson ResearchSoft, Philadelphia, PA) and de-duplicated.

Search verification included hand-searching reference lists of four broad literature reviews on *Salmonella* in poultry, and up to three reviews each, published after 1990, addressing various interventions, risk factor and prevalence data (Table 2.3.). For most categories, three specific reviews were not identified, in which case, only available reviews were hand-searched. The reference lists of selected chapters (1, 5-10, 13-18) of a recently published, relevant text book were also hand-searched (Table 2.4.) (Mead, 2005). All citations identified during search verification and not already captured in the electronic searches were obtained, if possible, and subjected to the outlined review process.

Study selection criteria

In order to be considered relevant, abstracts must have reported primary research in English that investigated interventions, risk factors and/or prevalence/concentration of *Salmonella* serovars of public health importance from farm-to-secondary processing. Studies that reported other aspects of the *Salmonella* issue (e.g., diagnostic tests for *Salmonella* or outbreaks in humans), in which only environmental samples, such as feed and litter, were collected or only measured the outcome through serology were beyond the scope of this review. Intervention studies conducted under *in vitro* conditions and prevalence studies reporting only proportional morbidity/mortality or that did not present a numerator, denominator and point in production chain of sampling were excluded. The initial relevance screening criteria was quite broad in scope and brief in order to allow quick progression through the abstracts and remove irrelevant citations.

Relevance screening and prioritizing questions for systematic review

Two levels of relevance screening (RS) were conducted. Relevance screening 1 (described in the previous section) was conducted on abstracts to identify the primary research relevant to the three areas of interest within the broad topic (Appendix 1). Relevance screening 2 was conducted on the full papers to confirm relevance and to categorize them by sub-topic (intervention, risk factor and/or prevalence), type of intervention, point in production chain, as well as type of outcome, continent and time period for prevalence and risk factor studies (Appendix 2). Based on the overall breadth and distribution of evidence at this level, considering the needs of a complementary QRA and available manpower, the research team prioritized, from a list of *a priori* identified

potential focused questions (Appendix 3) for rigorous SR, which includes the implementation of methodological assessment and data extraction tools.

Methodological assessment and data extraction

The methodological assessment was conducted using a form with 28 questions (Appendix 4), all applicable to intervention research (but not to all study designs) and 18 questions applicable to prevalence and risk factor research. Two questions were used as additional study exclusion criteria; lack of appropriate control group (intervention studies) and lack of sufficient raw/unadjusted or adjusted data for analysis. Only papers that met these criteria proceeded to data extraction. Data extraction was separately conducted for intervention and prevalence/risk factor studies. Both forms are shown in Appendices 5 and 6.

Review management

All previously described steps, except for the search strategy, were conducted by two independent reviewers, after adequate agreement (kappa ≥ 0.8) was achieved among reviewers at pre-testing. Pre-testing included 87 randomly selected abstracts for RS, 15 papers of various study design for methodological assessment and four for data extraction. A total of 19 reviewers contributed to different steps of the scoping review-SR project. During this process all disagreements were resolved by consensus or a senior team member resolved the conflict. The review was managed in the online program SRS 4.0 (TrialStat! Corporation, Ottawa, ON).

RESULTS

Scoping review

The search strategy resulted in 12,957 de-duplicated abstracts (Table 2.2.). The search verification resulted in 61 potentially relevant citations that were not captured through the electronic searches, of which only 25 were successfully procured (n=12,982) (Tables 2.3. and 2.4.). None of 25 passed RS 1 (n=1 was a review, n=24 either did not study *Salmonella* serovars of public health importance or did not study broiler chickens). After RS 1, 1,255 abstracts passed to RS 2; 277 studies were excluded at this level for reasons summarized in Figure 2.1. The quantity, scope and distribution of primary intervention, risk factor and prevalence research in English, the prioritization process and outcomes are shown in Figure 2.1. and Table 2.5.

From scoping review to prioritized systematic reviews

Of the 978 studies that passed RS 2, intervention research for *Salmonella* in broilers from farm-to-secondary processing was reported in 748 studies; of these, 591 (79%) investigated one or more farm level interventions. Competitive exclusion (CE, n=192) was the most frequently studied intervention, followed by other feed and water additives (n=114), antimicrobials (n=89), vaccination (n=62) and biosecurity (n=19). These five farm level interventions were prioritized for rigorous SRs. Vaccination and biosecurity studies are currently undergoing methodological assessment and data extraction, thus no results will be presented for these interventions. No studies evaluating interventions at the transport level were identified. At the processing level, 157 (21%) intervention studies were identified; most frequently treatment spraying or dipping (n=75), chilling

(n=37) and scalding (n=21). Due to considerably less studies at this level than at the farm level, all processing interventions were selected for SR.

Prevalence and risk factor data were reported in 200 and 30 papers, respectively, and 87 were studies conducted in North America (n=73 prevalence; n=14 risk factors). A total of 127 prevalence studies and 16 risk factor studies were conducted outside North America. Of the 73 North American studies that included prevalence estimates, 27 were farm level, 33 were processing level, and 13 included estimates from both the farm and processing levels. The majority of these 73 studies (58%) were conducted after 1990. In Tables 2.6. and 2.7., the quantity, distribution and characteristics of prevalence and risk factor studies are shown by continent, respectively. Only prevalence and risk factor studies conducted within North America were prioritized for SRs, as differences among broiler production and processing systems vary between countries and regions and the complementary QRA is focusing on North America.

Methodological assessment and data extraction applied to prioritized systematic review questions

Among 552, 73 and 14 prioritized intervention, prevalence and risk factor studies, 154 (28%), ten (14%) and five (36%) were excluded, respectively, for the reasons summarized in Table 2.5. In this paper, for brevity reasons, the results of the in–depth methodological assessment and data extraction are presented only for CE, to illustrate the main highlights that were commonly observed in the other intervention SRs. Many studies reported multiple treatment comparisons or replicates, which were extracted as

unique trials for analytical purposes. The CE evidence map included 2,789 unique trials from 149 studies (range 1-214 trials/per study; mean=19 trials/study). The *Salmonella* outcome was measured either as prevalence (1,794 trials), concentration (300 trials) or infection/protection factor values (695 trials), which are geometric means (Pivnick et al., 1985).

Several consistent methodological soundness or reporting issues were observed for primary research investigating CE and other prioritized interventions. None of the CE studies provided justification for the sample size and in 55% of the 2,789 trials a sample size of 30 broilers or less was used. The majority of CE trials (98%) were conducted in laboratory facilities that were not representative of commercial broiler chicken production. Random assignment of broiler populations to treatment groups was adequately described in only 3% of trials, and another 28% of trials reported random assignment, but did not provide an explicit definition of the allocation process. Additionally, the use of blinding was almost never reported in the trials. Forty-two percent of the 2,789 trials did not report conducting a statistical analysis, and 13% of trials that had clustering (grouping of chicks resulting in a lack of statistical independence) did not properly address it. Intervention protocols, including challenge protocols and laboratory methods, were described in sufficient detail for replication in the majority of the trials. More detailed information about the methodological assessment of CE is shown in Table 2.8.

Overall, the CE evidence map indicated that 18% of 2,789 trials were published between 2000 and 2008, 69% were conducted in either Canada or the US and 96% used day old chicks as the study population. Fifteen unique CE products were examined; an undefined, chicken related source was the most common (62%). The most common route of CE administration was oral gavage (65%). A variety of sample types were collected to measure the *Salmonella* outcome, with cecal contents as the most frequent (28%). Sampling was conducted at various ages of the broiler population, ranging from 0-7 days (21%) to more than 84 days (<1%). The variability of studies evaluating the effectiveness of CE is mapped out in Figure 2.2. using a single CE product (FM-B11) as an example.

Review management

It took six one-hour meetings of either the core research team (authors of this paper) or reviewers to determine the project scope, the search strategy and a list of *a priori* focused questions for potential rigorous SR. The broader and more complex the question, the larger the review core team needs to be to accommodate more topic area expertise. The RS 1 and 2 forms were developed, discussed and pre-tested with the core research team and reviewers at each level. Prioritization of questions for rigorous SR, after the scoping review was completed, was discussed at two meetings. Methodological assessment and data extraction forms were developed, discussed and pre-tested in three and four meetings with the core research team and reviewer team, respectively.

Regular bi-weekly meetings were held throughout the duration of the review to address any questions or concerns brought up by individual reviewers. All meetings were organized and facilitated by the review manager (primary author), who revised and maintained up-dated review forms and specific guidelines for consistent review according to the decisions made by the core team. This individual also oversaw allocation of resources to various tasks, monitored the review progress, and handled regular communication between the core research and reviewer teams through meetings and e-mails. All changes and up-dates in review approaches were presented at the bi-weekly meetings and captured in review guideline documents that evolved during the review process (Appendices 7-9). The review manager spent approximately seven to 15 hours per week training reviewers, mostly veterinary epidemiologists, some of whom were not familiar with SR methodology, and addressing their questions and concerns. The time it took to complete each step of the review is shown in Table 2.9. A total of 7, 3, 13 and 11 reviewers contributed to the completion of RS 1 (12,982 abstracts), RS 2 (1,255 papers), methodological assessment (639 papers) and data extraction (470 papers), respectively. The abstracts or papers were assigned to reviewers on a bi-weekly basis by the project manager to ensure that reviewers were kept on task. The scoping part of the review took approximately 10 months, from establishing the research team to summarizing RS 2 results and prioritizing the questions for rigorous SRs.

DISCUSSION

The main purposes of scoping reviews are to map and assess the quantity, scope and characteristics of available primary research (evidence mapping), to determine if one or

more full SRs are feasible and necessary, to summarize and distribute research findings, and to identify knowledge gaps (Arksey and O'Malley, 2005; Anderson et al., 2008; Davis et al., 2009). In this study we accomplished these objectives by conducting a scoping review on the broad microbial food safety topic, *Salmonella* in broiler chickens. First, we determined and evaluated the quantity and distribution of primary research for three sub-topics, interventions, risk factors and prevalence. This required two levels of RS; a very short abstract-based RS 1 tool that was essential for reducing the number of abstracts from 12,982 to 1,255 by removing irrelevant abstracts. A longer, paper-based RS 2 tool (~12 questions) was useful for mapping additional characteristics of the primary research such as type of intervention, point in production chain, outcome measurement, publication year and geographical location. These data were necessary to create fairly informative and complex evidence maps for each sub-topic. The research team used the maps to prioritize the questions for rigorous SRs, based on the overall quantity of primary research, its biological relevance, and the needs of a complementary QRA, as well as to identify major knowledge gaps and future research needs.

Among relevant abstracts, substantially more primary research studies investigated the effectiveness of interventions (n=748) than prevalence (n=200) and risk factors (n=30) combined. The intervention studies were primarily conducted at the farm level within North America. This is somewhat surprising because in North America, *Salmonella* in broiler chickens is primarily controlled at the processing level (Cox et al., 2005; Rajić et al., 2007), but it might indicate that the industry stakeholders in this region are considering on-farm control options. It is somewhat less surprising that CE was the most

frequently studied intervention because although researchers disagree about its effectiveness, it has been successfully implemented in Sweden. By contrast, the use of various biosecurity practices, which are frequently recommended in both government and industry *Salmonella*-control guidelines, was evaluated in very few (n=19 intervention; n=5 risk factor) studies. Better understanding and an increased quantity of valid evidence demonstrating the effectiveness of various biosecurity practices for controlling *Salmonella* is necessary for wider adoption of such practices on-farm. It is possible that large integrated poultry companies might have this type of data, but currently these are not publicly available. This is an important gap in knowledge that should be addressed by encouraging large poultry corporations to share such data, if available, and the journal editors to promote the submission of experiments or field trials regardless of the significance or lack of significance of the results. Although evaluating biosecurity practices or other interventions in a field setting is challenging for financial and logistic reasons, funding agencies should support these larger trials conducted under commercial conditions. For a wide range of interventions at the farm (four types of interventions) and processing level (six types of interventions), a sufficient amount of primary research studies was identified allowing multiple rigorous SR to be prioritized and are currently in progress. This has provided direction for a complementary QRA.

Ideally, the inputs used for QRA should be generated and reported using transparent and valid evidence from the literature and where appropriate literature is not found, expert opinions. Expert opinions are frequently biased (Fazil et al., 2008) and SR is recommended as a structured, transparent and replicable way of generating evidence-

based inputs for QRA. Transparency was achieved through the scoping review because the review team was able to evaluate if a sufficient amount of utilizable primary research existed for various interventions along with some characteristics of interest. Whenever possible, the distribution of prevalence or concentration data were summarized for various interventions across various stages of broiler chicken production, allowing the research team to select the most appropriate outcomes for a complementary QRA.

Substantial methodological soundness issues observed in most studies included in the review (e.g., poor study conduct or reporting) severely limits our ability to analyze and generalize the findings to commercial conditions in North America, which is the target of the QRA. For example, a lack of field studies conducted under commercial conditions was observed, and in fact, the majority of CE studies were of small sample sizes and not representative of field conditions. The significant heterogeneity observed within each intervention type, or products within the same intervention, substantially reduced the number of studies eligible for meta-analysis, as only sufficiently similar studies may be pooled. Our analysis was restricted to particular outcomes, authors who reported in a manner that could not be reconciled further limited opportunities for increased power in the meta-analysis. Infection/protection factor values, which were primarily reported in older publications, were not considered as useful for meta-analysis, as they could not be transformed into an outcome useful for a QRA. The most preferable type of outcome, concentration of *Salmonella*, was reported in 56 trials covering five defined CE products, both limiting the use of meta-analysis. Although these limitations compromise the precision and validity of the effect estimates generated through our meta-analysis for

QRA, they still might be the more transparent and valid option when compared to a single study estimate or expert opinion.

The prevalence evidence map allowed us to evaluate the quantity of prevalence data versus concentration data across varying time periods and to limit rigorous SR to data generated within North America. These data were fairly evenly spread across the broiler production chain, except for the transport level where no data were found. This is not surprising given that the scoping review was limited to studies collecting samples directly from broilers, while at the transport level, samples are frequently collected from crates. In all continents, except Australia and Asia, there was an overall increase in the number of prevalence studies across time (more from 1990 to present). However, this might be a result of inclusion criteria that required reporting of the numerator, denominator and the point in production chain of sampling. It was observed that publications before 1960 often did not provide the denominator or the location of sampling and more recent prevalence estimates likely contain more relevant data.

The utility of scoping reviews to identify knowledge gaps and future research needs varies depending on the process used. This scoping review successfully identified areas where no substantial research existed, for example, on the effectiveness of biosecurity practices, *Salmonella* prevalence at the transport level or the distribution of prevalence versus concentration data. Many scoping reviews do not include methodological assessment of included studies at all or this aspect is conducted on a fairly superficial level (Arksey and O'Malley, 2005). In this scoping review, the prioritization of questions

for rigorous SRs was conducted after RS 2 was completed. Many aspects of the usefulness and soundness of identified relevant primary research were assessed post-scoping review, at the methodological assessment level, using a long and complex tool. For this reason, this stage took a long time and some resources were wasted because 149 studies that did not meet some basic criteria, such as sufficient reporting of results, were evaluated fully for methodological soundness. This number might be higher once all individual SRs are completed. In retrospect, knowledge gap identification and prioritization of questions for in-depth SRs would be more effective if a brief methodological assessment, consisting of a couple pertinent questions (2-3), was added to RS 2 to further exclude studies that do not contain useable data. These could include sufficient reporting of results (raw/unadjusted or adjusted estimates with measures of variability), the use of an appropriate control group for intervention studies, and sufficient reporting of intervention, challenge (if applicable) and laboratory protocols. This would improve the utility of the evidence maps produced by the scoping review. Additionally, to improve the efficiency of methodological assessment and data extraction, these levels should be implemented simultaneously to save time and resources as the paper would have to be read once instead of twice.

Scoping review-SR tools are more complex than those used in a narrow, focused SR because they deal with a broad topic. Thorough definitions and clear communication among reviewers was necessary to ensure all papers relevant to each sub-topic of the review were identified during the scoping review, at RS. By prioritizing several specific questions to conduct in-depth SRs on, complexity of the methodological assessment was

due to the many different study designs captured under the three sub-topics. Common intervention study designs included challenge trials, whereas a common risk factor and prevalence study design was the cross-sectional observational type. Data extraction was also organized by study design because of the differences between sub-topics, and data extraction was completed separately for each unique trial within the study, requiring extra data clean-up and manipulation. Each of these issues should be considered when developing tools for future scoping review-SRs.

A challenge of both scoping reviews and SRs are the substantial study methodological conduct or reporting limitations, which we identified in the SR on on-farm use of CE, although it appeared that these aspects improved over time, particularly for studies published after 2000. These are encouraging findings and suggest that further improvement might be possible. Guidelines have recently been published for authors, specific to human health research, for randomized controlled trials (CONSORT statement) (Equator network, 2009), observational studies (STROBE) (Equator network, 2009) and other types of studies, including SRs and meta-analyses (PRISMA) (Moher et al., 2009), in order to improve the reporting and overall utility of research. Other researchers have recently advocated for similar initiatives in animal health (Sargeant et al., 2009a; Sargeant et al., 2009b). The implementation of such initiatives in practice would also improve the strength of future SR-meta-analyses conducted in this field. Before such guidelines become available specifically for animal health, the journal editors and researchers in this field should be encouraged to apply modified versions of the Equator guidelines.

The complexity of the scoping review-SR increased the time to completion of each step of the review, and as a result, the review took longer than expected. A large number of reviewers were required to complete the scoping steps, RS 1 and 2, in a timely manner, due to the large number of abstracts/papers that entered these levels, 12,982 and 1,255, respectively. Similarly, a large number of trained epidemiologists were required for the methodological assessment of relevant papers included in the rigorous SRs. In our study, most reviewers were graduate students, including the review manager, and worked on the project in a part-time capacity. Many of these students required basic training in SR methodology in addition to training on specific review tools. At each stage of the review, new reviewers were generally slower than more experienced reviewers, which had a significant impact on the speed of each stage of our review. A full-time review manager and experienced reviewers would significantly decrease the time it takes to complete both a scoping review (three to six months) and the prioritized SRs, depending on the breadth of the topic and available of primary research. Manpower availability should be addressed prior to initiating a scoping review-SR. When evaluating how much manpower is available, time should be allocated to each reviewer for pre-testing, reviewing and resolving conflicts at each level of the review, and to the review manager for maintaining communication among the team, as these aspects can each take a significant amount of time.

The scoping review-SR approach is appealing for broad microbial food safety topics. In order for decision-makers to confidently develop guidelines or control programs addressing complex microbial food safety topics, the best available evidence are needed

for further evaluation within a specific context. An enormous amount of primary research typically exists within a single topic area, and research synthesis methodology frameworks provide a logical way for utilizing these data and translating scientific evidence into practice and/or policy. This approach should also be considered as a standard tool for generating evidence-based inputs for QRAs, in which a variety of control options spanning across the food production chain could be evaluated. The main stakeholders in microbial food safety likely spend substantial resources on commissioning literature reviews on broad microbial food safety topics. While an initial scoping review and individual SRs require substantial time and resources, the advantage of the approach is that it can be updated in a replicable manner summarizing new effect estimates and updating knowledge gaps as new primary research becomes available. Concurrently, this would allow the update of existing risk assessments and/or re-evaluation of decisions based on updated evidence. In the EU, Australia and US, government-based research synthesis and translation infrastructures have already been developed in support of transparent evidence-based policy making in health care and public health sectors. There is a need for government stakeholders responsible for microbial food safety and veterinary public health in Canada and elsewhere to consider the same. Researchers and funding agencies will also benefit from this approach as transparently identified knowledge gaps and future research needs will guide funding organizations on spending their dollars effectively for the most efficient advancement of scientific knowledge.

When to consider doing a scoping review is a question that requires careful consideration of the issue to be scoped (long-term questions may need to be updated etc.), available manpower, who the stakeholders are and if the scoping outputs are going to answer their question(s). Some topics, such as *Salmonella* in broilers, is of international interest, thus international teams may be the most appropriate and effective use of resources for many complex and difficult microbial food safety issues. It is likely that national governments or international organizations are good candidates to execute this type of work because the products will be publically accessible, the data will remain in the organization and can be utilized or updated by any individual at any point in the future. This type of methodology is ill-suited for the current structure of academia as the sheer number of people required to conduct these reviews is prohibitive.

The use of existing electronic review formats is very helpful in managing large reviews; however, their further advancement would make both scoping reviews and SRs more feasible in the future. Currently, citations and abstracts can be loaded into electronic SR programs for reviewing. Logic statements can be developed at each stage of the review, and programs will automatically exclude or pass citations into the next level of the review. However, at this time, the software does not handle broad scoping reviews well at the later stages when the project needs to branch into specific SRs. This currently is largely managed manually by the review manager which takes a great deal of time and can increase the risk of error. In addition, there would be less emphasis on the reviewer to remember which questions do or do not apply to the paper being reviewed.

CONCLUSION

Scoping reviews are a relatively new research synthesis method that has been used primarily in the nursing and other health care sectors (Katz et al., 2003; Arksey and O'Malley, 2005; Anderson et al., 2008; Davis et al., 2009). In this study, we applied a scoping review on the broad microbial food safety topic, *Salmonella* in broiler chickens. The primary research characteristics underpinning intervention, risk factor and prevalence studies were summarized and mapped out using a replicable review process. Evidence maps were evaluated by the research team to prioritize the questions for rigorous SRs, to assess the availability of potential inputs for a complementary risk assessment, and to identify the main knowledge gaps and future research needs. Our experience suggests that the utility of scoping reviews will be higher if brief and basic methodological assessment are included in the scoping review process as part of or immediately following RS 2, so improved maps are produced with information as to the data available for rigorous SRs. Currently, the scoping review is used in a number of different ways and a good deal of discussion centers around the level of detail that can be or should be extracted. Some proposed methods mirror the equivalent of what a full SR would extract, while others border on not being useful because not enough information is extracted (Arksey and O'Malley, 2005). The methodological framework for scoping reviews is still being developed and there is debate over whether they are part of an on-going process or a stand-alone activity. The first evaluates the range, extent and nature of the research under the broader question and helping to inform the value of undertaking one or more SRs, essentially making scoping reviews part of an on-going process rather than a stand-alone activity where the level of detail extracted borders on a full SR

(Arksey and O'Malley, 2005). Nevertheless, this approach is appealing for addressing the broader microbial food safety topics with ambiguous or contradictory evidence, and when consensus among multiple stakeholders is necessary for action development.

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Table 2.1. Three topic-related areas selected for a scoping review

Broad topic (* identifies terms with specific definitions)	
*Salmonella** in broilers* from farm-to-secondary processing*	
Refined questions (* identifies terms with specific definitions)	
1) Which interventions* have been shown to be effective at reducing *Salmonella** in broilers* from farm-to-secondary processing*? 2) What is the prevalence* of *Salmonella** in broilers* from farm-to-secondary processing*? 3) What are the risk factors* for *Salmonella** colonization and infection in broilers* from farm-to-secondary processing*?	
Definitions	
Salmonella	All serovars of public health importance should be included. *S.* Pullorum and *S.* Gallinarum are not of public health importance
Broiler	All conventional chicken or broiler eggs intended for meat production or examination of raw chicken products. Includes general terms such as poultry. Organic chicken products (free range, all natural, antibiotic free, antimicrobial free) are not included.
Farm-to-secondary processing	Includes studies performed at breeding farms, hatcheries, grow-out farms, catching and transport to slaughter, live-bird supply to the slaughterhouse (lairage), all slaughter, evisceration, wash, and chilling activities up to secondary processing. Secondary processing includes cut-up, de-boning, partitioning and grinding of raw chicken carcasses.

Intervention	Most often studied as controlled trials or challenge trials. In some cases, cohort study designs may be used to evaluate an intervention. Some will be conducted under field conditions and others under lab or unnatural conditions (such as a research farm).
Prevalence	Often follow cross-sectional designs, but there are several types: one measure at one stage in the processing chain, multiple measures (longitudinal) at one stage in the processing chain, single measure at more than one stage in the processing chain (multi-stage prevalence), and multiple measures (longitudinal) at more than one stage in the processing chain (multi-stage prevalence).
Risk factors	Most likely refer to observational studies (cross-sectional, cohort, case-control). Some studies may model a number of risk factors.

Table 2.2. The number of electronic hits before and after de-duplication in a scoping review of *Salmonella* in broiler chickens

Population	**Outcome**	**Database**	**# of hits**
Chick* OR	Salmonell*	CAB	9,656
Poultry* OR		Scopus	4,974
Broiler* OR		PubMed	4,239
Gallus*		Agricola	3,086
		Current Contents	2,467
		CAB Global Health	2,489
		TOTAL	26,911
		DE-DUPLICATED	12,957

Table 2.3. Number of new hits, obtained citations and relevant abstracts from selected literature reviews included in the search verification of a scoping review of *Salmonella* in broiler chickens

Item searched	# hits[1]	# obtained[2]	# relevant[3]
General review articles			
Burnham, V.E., 2007	0	0	0
Bolder, N.M., 2007	7	2	0
Maijala, R., et al., 2005	1	0	0
Nash, W.A., 2004	0	0	0
Competitive exclusion			
Schneitz, C., 2005	8	0	0
Revolledo, L., et al., 2006	1	1	0
Competitive exclusion and antimicrobials			
Fowler, N.G., 1992	0	0	0
Antimicrobials			
Ricke, S.C., et al., 2005	3	2	0
Naidu, A.S., et al., 2003	0	0	0
Other feed and water additives			
Van Immerseel, F., et al., 2006	3	2	0
Bacteriophage/bacteriocins			
Greer, G.G., 2005	0	0	0
Treatment spraying/dipping			
Capita, R., et al., 2002	1	0	0

General processing articles			
Rasekh, J., et al., 2005	4	2	0
Mead, G.C., 2004	1	0	0
Fries, R., 2002	7	4	0
Total	36	13	0

[1] The number of citations that were located in the item, that were not found in the original search conducted in December, 2007
[2] The number of citations for which abstracts were found
[3] The number of abstracts that passed relevance screening levels one and two, of the citations for which abstracts were found

Table 2.4. Number of new hits, obtained citations and relevant abstracts from selected chapters of a text book included in the search verification of a scoping review of *Salmonella* in broiler chickens (Mead, 2005)

Item searched	**# hits**[1]	**# obtained**[2]	**# relevant**[3]
Chapter 1	7	3	0
Chapter 5	0	0	0
Chapter 6	0	0	0
Chapter 7	0	0	0
Chapter 8	0	0	0
Chapter 9	1	0	0
Chapter 10	0	0	0
Chapter 13	2	2	0
Chapter 14	7	3	0
Chapter 15	1	1	0
Chapter 16	0	0	0
Chapter 17	7	3	0
Chapter 18	0	0	0
TOTAL	25	12	0

[1] The number of citations that were located in the item, that were not found in the original search conducted in December, 2007

[2] The number of citations for which abstracts were found

[3] The number of abstracts that passed relevance screening levels one and two, of the citations for which abstracts were found

Table 2.5. Scoping review-systematic review summary of intervention, risk factor and prevalence research for *Salmonella* in broilers from farm-to-secondary processing

Category	**Stage of review**		
	RS2[1]	**MA**[2]	**DE**[3]
CE	192	192	149[4]
Other additives	114	112[5]	94[6]
Antimicrobials	89	87[7]	59[8]
Vaccination	62	62	--[9]
Biosecurity	19	19	--[9]
Feed withdrawal	17	--[10]	--[10]
Bacteriophage/bacteriocins	8	--[10]	--[10]
Other[11]	90	--[10]	--[10]
TOTAL FARM INTERVENTIONS	591	472	302
Treatment spraying/dipping	75	69[12]	39[13]
Chilling	37	33[14]	28[15]
Scalding	21	16[16]	11[17]
Final wash	7	6[18]	6
Reprocessing	5	5	5
Other[19]	12	12	7[20]
TOTAL PROCESSING INTERVENTIONS	157	141	96
TOTAL INTERVENTIONS	748	613	398
Prevalence inside NA[21]	73	73	63[22]

Prevalence outside NA[23]	127	--[10]	--[10]
TOTAL PREVALENCE	200	73	63
Risk factors inside NA[24]	14	14	9[25]
Risk factors outside NA[26]	16	--[10]	--[10]
TOTAL RISK FACTORS	30	14	9

[1] Relevance screening at level two
[2] Methodological assessment
[3] Data extraction
[4] 40 studies contained no data, 2 studies did not use a control group and 1 study was a duplicate
[5] 2 relevant studies could not be obtained
[6] 18 studies contained no data
[7] 2 relevant studies could not be obtained
[8] 28 studies contained no data
[9] Data extraction is on-going for this intervention
[10] This intervention was not prioritized for rigorous systematic review
[11] The other category at the farm level includes a variety of less popular interventions, such as genetic studies
[12] 6 relevant studies could not be obtained
[13] 26 studies contained no data and 4 studies did not use a control group
[14] 4 relevant studies could not be obtained
[15] 5 studies contained no data
[16] 5 relevant studies could not be obtained
[17] 5 studies contained no data
[18] 1 relevant study could not be obtained
[19] The other category at the processing level includes a variety of less popular interventions, such as a steam chamber
[20] 5 studies contained no data
[21] Prevalence inside of North America
[22] 6 studies contained no data and 4 studies contained environmental samples only
[23] Prevalence outside of North America
[24] Risk factors inside of North America
[25] 3 studies contained no data and 2 studies contained environmental samples only
[26] Risk factors outside of North America

Table 2.6. The quantity, distribution and characteristics of *Salmonella* prevalence studies in broiler chickens by continent

	North America	**Africa**	**Europe**	**South America**	**Australia**	**Asia**
Total studies	73	18	52	13	2	42
Point in chain						
Farm[1]	27	6	22	2	1	0
Transport[2]	0	0	0	0	0	2
Processing[3]	33	8	20	9	1	5
Multiple[4]	13	4	10	2	0	35
Time period						
<1960	3	0	2	0	0	21
1960-1979	17	1	9	1	1	0
1980-1989	11	5	6	1	1	11
1990-present	42	12	35	11	0	10

Outcome						
Group prevalence[5]	4	2	12	0	1	5
Within group prevalence[6]	19	9	17	6	0	19
Concentration	0	0	0	0	0	1
Multistage[7]	0	0	0	0	0	0
Environmental	1	0	0	0	0	0
Other	0	0	0	0	0	0
Multiple[8]	49	7	23	7	1	17

[1] Farm includes breeding, hatchery and grow-out farms
[2] Transport involves catching and transport to slaughter
[3] Processing includes both primary and secondary processing. Secondary processing includes cut-up, de-boning, partitioning and grinding of raw chicken carcasses up to the point of freezing
[4] Study spans over more than one point in chain
[5] The proportion of groups (e.g. flocks, batches) containing 1 or more *Salmonella* positive samples
[6] The number of samples within a group that are *Salmonella* positive
[7] A study that samples at more than one point in time in the farm to processing continuum to examine the level of *Salmonella* contamination; the study might be single point in time or longitudinal in design
[8] Includes two or more of the listed outcomes

Table 2.7. The quantity, distribution and characteristics of *Salmonella* risk factor studies in broiler chickens by continent

	North America	**Africa**	**Europe**	**South America**	**Australia**	**Asia**
Total studies	14	3	10	2	1	0
Point in chain						
Farm[1]	7	2	7	1	1	0
Transport[2]	0	0	0	0	0	0
Processing[3]	3	1	1	1	0	0
Multiple[4]	4	0	2	0	0	0
Time period						
<1960	1	0	0	0	0	0
1960-1979	0	0	0	0	1	0
1980-1989	2	0	1	0	0	0
1990-present	11	3	9	2	0	0

[1] Farm includes breeding, hatchery and grow-out farms
[2] Transport involves catching and transport to slaughter
[3] Processing includes both primary and secondary processing. Secondary processing includes cut-up, de-boning, partitioning and grinding of raw chicken carcasses up to the point of freezing
[4] Study spans over more than one point in chain

Table 2.8. Questions and responses from the methodological assessment of competitive exclusion studies included in a systematic review of *Salmonella* in broiler chickens

Criteria[1]	**Responses**		
		# trials	**%**
Was the sample size justified?	Yes	0	0
	No	2789	100
Was the representativeness of the sample population to the target population explained and sufficiently justified?	Yes	26	<1
	No	33	1
	N/A	2730	98
Were the birds housed, grouped or slaughtered in a way that is representative of field conditions?	Yes	55	2
	No	2734	98
How was the intervention assigned to the experimental unit?	Random	71	3
	Reported random	790	28
	Systematic	0	0
	Convenience	1926	69
	N/A	2	<1
Were the intervention protocols described in sufficient detail to allow reproduction of the experiment?	Yes	1698	61
	No	240	9
	Reference paper	849	30
	N/A	2	<1

Was the challenge protocol adequately described so that the challenge could be reproduced?	Yes	1856	67
	No	220	8
	Reference paper	646	23
	N/A	67	2
Were laboratory methods used to determine the outcome described sufficiently to allow replication of the study?	Yes	1345	48
	No	355	13
	Reference paper	1089	39
Did the author report that blinding was used?	Yes	9	<1
	No	2780	100
	N/A	0	0
Was the statistical analysis described adequately so it can be reproduced?	Yes	1471	53
	No	141	5
	Reference paper	8	<1
	No analysis	1169	42
Based on the study design, was clustering accounted for appropriately in the analysis?	Yes	162	6
	No	359	13
	N/A	2268	81
TOTAL TRIALS		2789	

[1] For detailed explanation see Appendix 4

Table 2.9. Estimated and actual time required to complete various steps of a scoping review-systematic review of *Salmonella* in broiler chickens

Scoping review step	**# of reviewers**	**# reviewed**	**Estimated time[1]**	**Actual time[2]**
Establishment of research team	N/A[3]	N/A	N/A	1 month
Protocol/Pre-testing	Core team	N/A	1 month	3 months
Search/De-duplication	1 librarian	N/A	3 days	6 days
Relevance tool 1	7 reviewers	12,982 abstracts	1 month	2 months
Article procurement	3 students	1,255 papers	1 month	3 months
Relevance tool 2	3 reviewers	1,255 papers	1 month	4 months
Descriptive summary of evidence/prioritization for rigorous systematic reviews	Core team	N/A	1-2 weeks	2 weeks

Systematic review step				
Methodological assessment	13 epidemiologists	639 papers	2 months	4 months
Data extraction	11 reviewers	470 papers	2 months	5 months
Data analysis	Project manager	N/A	2 months	2 months
Report writing	Core team	N/A	2 months	2 months

[1] Estimated time was based on previous experience with a single, focused question systematic review
[2] Actual time was the time it took to complete various stages of our scoping review-systematic review study
[3] Not applicable

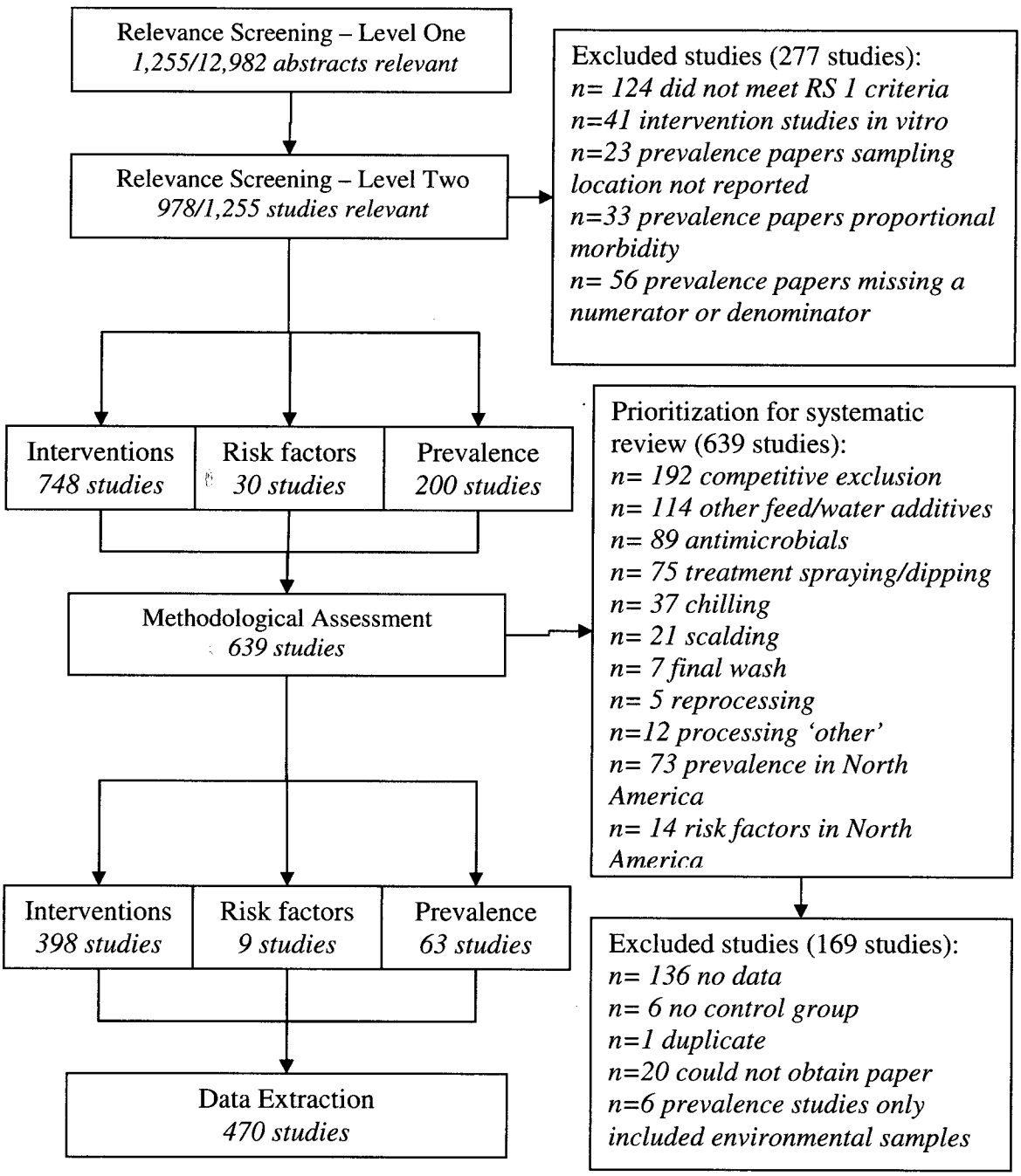

Figure 2.1.

Scoping review of *Salmonella* in broiler chickens results summary

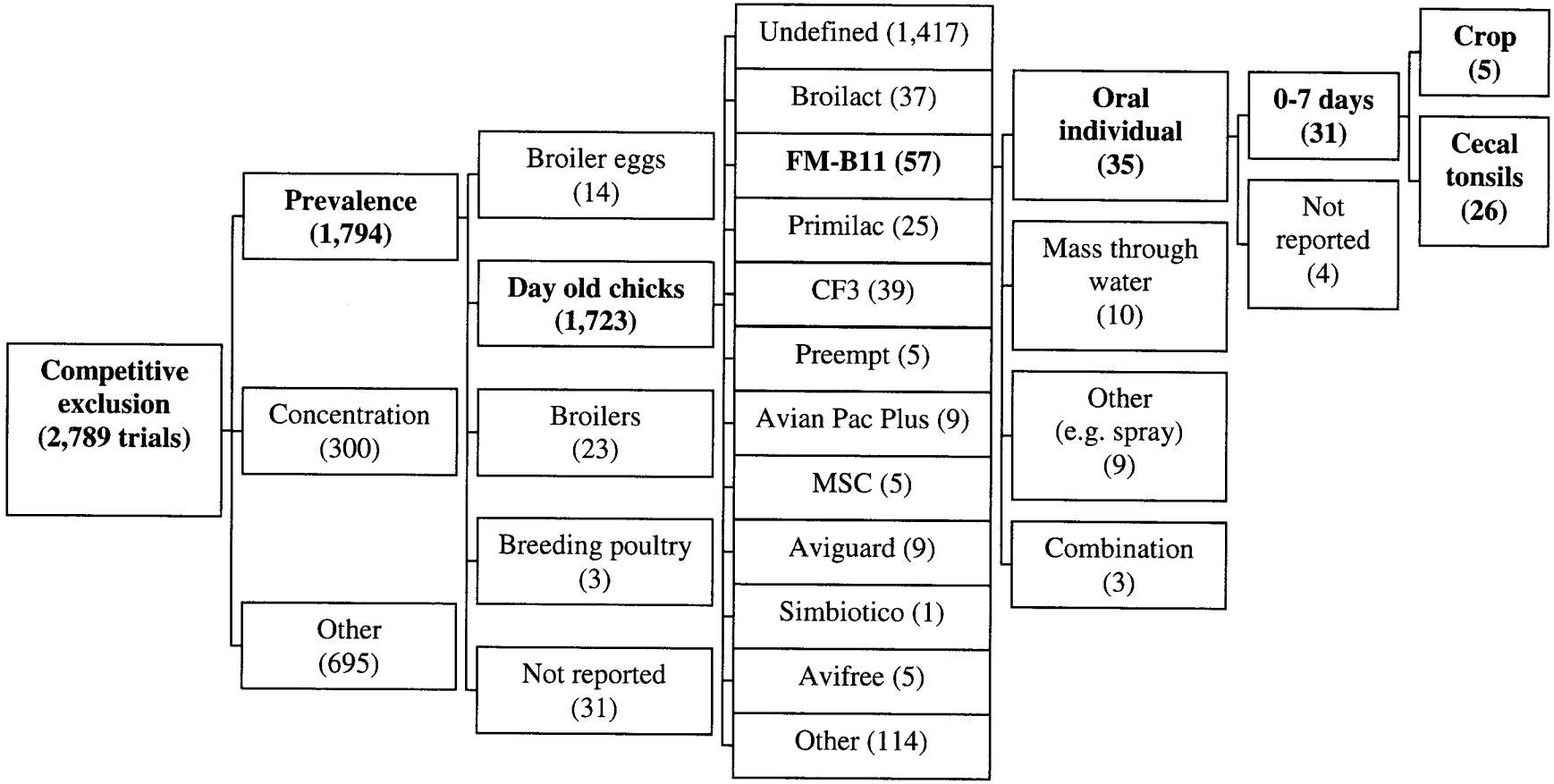

Figure 2.2.

Illustration of the variability of study design in primary research for a single competitive exclusion product (FM-B11)

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CHAPTER THREE

A SYSTEMATIC REVIEW-META-ANALYSIS OF ON-FARM USE OF COMPETITIVE EXCLUSION ON *SALMONELLA* PREVALENCE AND CONCENTRATION IN BROILER CHICKENS

ABSTRACT

The effectiveness of various competitive exclusion (CE) products for reducing *Salmonella* colonization in broiler chickens was evaluated using a systematic review-meta-analysis (SR-MA). Relevance screening identified 192 relevant studies that underwent methodological assessment. Of these studies, 149 were suitable for data extraction, 59 are presented in a number of MA's and 104 were examined in a meta-regression (MR). Fifteen different CE products were identified, and the most common route of administration was oral gavage (65% of trials). The MA indicated that a number of defined CE products reduce *Salmonella* colonization in broilers. Undefined CE products outperformed all defined products, except for a continuous-flow culture, CF3. Broilers treated with CF3 were 1.6 times less likely to become colonized with *Salmonella* than those treated with an undefined product. Administration of CE through feed and water was as effective as through oral gavage. Six study (study design, publication year, population type, CE product type, route of CE administration, *Salmonella* serovar administered/recovered) and three methodological soundness characteristics (treatment assignment, intervention and laboratory methods description) were included in the final MR model. The results from the MA might guide the on-farm use of CE products in broiler chickens, or act as inputs for risk assessments. The MR results improve overall

understanding of existing on-farm CE research and provide useful information for designing new intervention research in this area.

INTRODUCTION

Over the past two decades *Salmonella* has become a major global food safety challenge for the agricultural industry, particularly the poultry industry. The industrialization of the poultry industry has led to methods that focus on production efficiency and profitability. As a result, conditions, such as highly sanitized facilities and a lack of contact with breeder birds, slow the development of normal, mature cecal microflora (Nurmi and Rantala, 1973; Mead, 2005). This slow development increases the susceptibility of chicks to colonization with a variety of enteric bacteria, including *Salmonella* (Barnes et al., 1972; Blankenship et al., 1991; Schneitz and Mead, 2000). Thus, provision of a protective microflora is a conceptually appealing prospect for *Salmonella* control.

Originally, competitive exclusion (CE) products were cecal contents from healthy adult chickens suspended in an aqueous solution, then serially cultured under anaerobic conditions and consisting of a complex mixture of intestinal bacteria (Jeffrey, 1999; Mead, 2000). Since these products include most elements of the normal microflora, they are highly compatible with recipient birds (Mead, 2000). However, there is some concern with undefined products as their exact composition is unknown and harmful pathogens might be part of their composition, thus extensive testing must be conducted before a product is commercialized (Jeffrey, 1999; Mead, 2000; Mead, 2005). Screening can be costly, and it is questionable whether all pathogens can be identified by current

methods and it is difficult for the industry to keep up with an increasing list of possible pathogens (Jeffrey, 1999; Mead, 2000). In addition, testing may be unnecessary, for example, the use of undefined products was shown to be safe and effective in Sweden's comprehensive and mandatory *Salmonella* eradication control program between 1981 and 1990 (Blankenship et al., 1991; Wierup et al., 1992; Mead, 2000; Mead, 2005; Sternberg Lewerin et al., 2005).

For widespread commercial use, it is widely accepted that an equally effective, defined CE product, known to be free of human and avian pathogens and undergoing quality control during production, would be preferable to an undefined CE product (Jeffrey, 1999; Mead, 2000; Mead, 2005). In recent years, a variety of defined mixtures of bacteria for use as CE products has been developed and patented (Jeffrey, 1999). However, researchers have had difficulties in maintaining a stable culture of the strains required to confer protection to *Salmonella*. As a result, some research groups applied continuous culture techniques, but this can limit the number of bacterial strains that can be maintained in the culture, reducing the efficacy of the resulting CE product (Jeffrey, 1999).

Over the last two decades, a considerable amount of research has been conducted investigating the effectiveness of various CE products, resulting in different and often contradictory recommendations. Better understanding of the comparative effectiveness of the undefined and defined products based on the existing information is necessary to

ensure the on-farm use of the most effective products, and to identify the current gaps in knowledge and future research needs.

Systematic review-meta-analysis (SR-MA) is a transparent and replicable research synthesis method that is often used to determine the effectiveness of interventions (Sargeant et al., 2006; Centre for Reviews and Dissemination, 2009). When used appropriately, SRs reduce the bias in the interpretation of findings from a pool of studies by following a structured, transparent and replicable methodology in which each step is conducted independently by at least two reviewers (Mulrow, 1994; Sargeant et al., 2006). A MA allows the results from multiple, independent studies, identified and critically appraised in a SR, to be combined in homogeneous pools in order to generate a more precise overall estimate of the effectiveness of the intervention (Borenstein et al., 2009; Crombie and Davies, 2009). Although the effectiveness of CE at reducing *Salmonella* colonization in poultry has been explored in narrative, expert-based literature reviews or commentaries (Fowler, 1992; Stavric and D'Aoust, 1993; Mead, 2000; Schneitz, 2005; Revolledo et al., 2006), to the best of our knowledge, none has used a SR-MA approach.

The main objectives of this study were to: identify, appraise and summarize the findings of primary research investigating the on-farm use of CE and its effect on *Salmonella* prevalence and concentration in broiler chickens using a SR; when possible, quantitatively evaluate the effectiveness through MA of various types of CE at reducing *Salmonella* colonization in broiler chickens; and assess potential associations between

various individual study design and methodological soundness characteristics with the reported effectiveness of the treatment through meta-regression (MR).

METHODOLOGICAL APPROACH

The review team and question development

The review team consisted of professionals with extensive expertise in epidemiology, microbiology, poultry production and management, SR, and literature search. An *a priori* developed protocol was pre-tested at each step of the review addressing the following question: What is the effectiveness of the on-farm use of various CE products on *Salmonella* prevalence and concentration in broiler chickens from farm-to-secondary processing? This SR-MA was part of a scoping review addressing the broader topic of *Salmonella* in broiler chickens (Chapter 2).

Literature search strategy

A simple search strategy, implemented in December 2007, was conducted in PubMed (1860-2007), Agricola (1924-2007), Current Contents (1999-2007), Scopus (1960-2007), CAB (1913-2007) and CAB Global Health (1971-2007). The algorithm included one broad outcome and four broad population terms (Table 3.1.). No time period or language restrictions were used at this stage.

Search verification included hand-searching reference lists of three literature reviews, published after 1990, addressing CE use in poultry (Table 3.2.) (Fowler, 1992; Schneitz, 2005; Revolledo et al., 2006), as well as four general *Salmonella* in poultry reviews

(Nash, 2004; Maijala et al., 2005; Bolder, 2007; Burnham, 2007). The reference lists of selected chapters (1, 5-10, 13-18) of a recently published and relevant text book were also hand-searched (Table 3.3.) (Mead, 2005). All citations identified during search verification and not already captured in the electronic searches were obtained, if possible, and subjected to the outlined review process. Five topic experts were selected based on their publications related to the topic, or involvement in control programs for *Salmonella* in broiler chickens and contacted via email in June, 2009. They were informed of the study purpose and asked to share with the research team any information regarding on-going or unpublished research, or to name researchers with current projects.

Relevance screening

Two levels of relevance screening (RS) were conducted as part of a broad scoping review (Chapter 2). Relevance screening 1 was conducted on abstracts to identify primary research in English relevant to interventions, risk factors or prevalence of *Salmonella* of public health importance in broiler chickens from farm-to-secondary processing (Appendix 1). Studies were excluded from the SR if CE was investigated through an *in vitro* experiment.

Relevance screening 2 was conducted on the full papers to confirm relevance and to categorize captured research by topic area, point in production chain, type of intervention, and type of outcome, continent and time period for prevalence and risk factor studies (Appendix 2). Based on the overall breadth and distribution of evidence at this level and biological relevance, the research team prioritized specific interventions at

the farm and processing levels for rigorous SRs (Chapter 2). The focus of this review is the on-farm use of CE products.

Methodological assessment and data extraction

All relevant primary research articles were assessed for methodological soundness using study design-based criteria (Appendix 4). Two of 28 questions were used as exclusion criteria; if an appropriate control group was not used, or raw/unadjusted or adjusted data and measures of variability were not reported. Only studies that met these criteria proceeded to data extraction. The data extraction tool used in this SR is shown in Appendix 5.

Review management

Citations were imported into the reference manager Procite 5.0 (Thomson ResearchSoft, Philadelphia, PA) and de-duplicated. The SR was managed in the online program SRS 4.0 (TrialStat! Corporation, Ottawa, ON). Pre-testing included 87 abstracts (RS 1 and 2), 15 full papers (methodological assessment) and 4 full papers (data extraction). All previously described steps, except for the search strategy, were conducted by two independent reviewers, after adequate agreement (kappa ≥ 0.8) was achieved among reviewers at pre-testing. A total of 19 reviewers contributed to the various steps of the SR. During this process all disagreements were resolved by consensus or a senior team member resolved the conflict.

Data analysis

The extracted data were exported into MS Excel (Microsoft Corporation, Redmond, WA) spreadsheets and cleaned, and all the analyses were performed in the statistical package Stata 10 (Stata Corporation, College Station, TX). Many studies reported multiple treatment comparisons or replicates (trials), which were extracted as unique trials for analytical purposes. Crude odds ratios (OR) and standard errors were calculated for trials that provided complete, raw prevalence data for both the control and treatment groups and concentration data were transformed to log base 10 values.

The descriptive statistics and evidence maps were evaluated for identifying biologically sensible data subsets for MA. All CE trials were descriptively summarized and the dataset was stratified by type of outcome (prevalence or concentration), study design (challenge or controlled trial) and type of CE product (undefined or defined). Each stratum was further sub-grouped based on the time the *Salmonella* outcome was measured after treatment administration (0-42 days vs. >43 days post-treatment) to compare the effectiveness of the product over time. Studies were excluded from the MA if: a second intervention was measured in combination with CE; the study population that was administered CE was other than day old chicks; the sample type was not cecal contents, ceca or cecal tonsils; or the reported outcome was an infection/protection factor. Samples such as liver and spleen were excluded as they are biologically different than the included samples.

A random effects MA was conducted within each biologically sensible stratum (data subset) if estimates were available from two or more studies. It was decided *a priori* that random effects was the most appropriate approach based on the assumption that heterogeneity existed in the trials. A fixed effects model was also explored; however, only the random effects model is reported in this paper, as it was the more conservative approach. For each analysis, a pooled estimate (OR, mean difference) and forest plot was calculated using the DerSimonian and Laird (D-L) method. Cochran's Q statistic and I^2 (the percentage of total variation between studies due to heterogeneity) were used to evaluate heterogeneity (Higgins et al., 2003). Pooled estimates were reported if heterogeneity was considered acceptable (p-value for Q statistic >0.1) (Sutton et al., 2000). If the 95% confidence interval of the pooled estimate excluded the null value, the estimate was considered statistically significant. The results were not reported for a pool of trials investigating the use of undefined CE products and reporting a prevalence outcome because such practice is not reproducible in farm settings. Forest plots were presented primarily for illustrative purposes. The possibility of publication bias was evaluated in both the prevalence and concentration datasets using Begg's rank correlation test and Egger's regression test.

A random effects MR was conducted on the trials reporting a prevalence outcome (n=1,794 trials), using the restricted maximum likelihood (REML) method (Metareg, Stata 10, Stata Corporation, College Station, TX). No MR was conducted on the trials reporting concentration as the outcome, due to a small number of trials.

Study design and methodological characteristics (Table 3.4.) were evaluated to determine potential associations between these variables and the reported effectiveness of CE treatment (OR), in all CE trials where broiler chickens were the unit of analysis. These variables were selected based on biological relevance, completeness of records (i.e., no missing values) and variability of responses between studies. Variables with missing data, or homogeneous responses across trials were excluded from the MR. Unconditional associations between each predictor variable and the effectiveness of CE treatment were screened for significance ($p<0.15$) in univariable regression models. Statistically significant variables ($p<0.05$) were included in a multivariable model. A main effects model was developed using a backwards stepwise selection process. Currently, no valid sensitivity analyses exist for this type of model, and thus, none were conducted.

RESULTS

Literature search and relevance screening

The SR process is shown within the context of a comprehensive scoping review (Chapter 2) in Figure 3.1. The search strategy, after de-duplication, resulted in 12,957 citations. The search verification resulted in 61 potentially relevant citations that were not captured through the electronic searches, of which, only 25 were successfully procured (n=12,982). Abstract-based RS 1 was performed on all 12,982 abstracts, and RS 2 on the 1,255 full papers that passed RS 1. Among 748 papers that reported various farm-to-processing interventions, 192 evaluated the effectiveness of the on-farm use of CE in broiler chickens. All 192 citations are reported in Appendices 10 and 11. Two topic experts provided seven relevant, unpublished studies. None of these studies reported

enough data to evaluate the study methodological soundness and none were included in the analysis.

Descriptive data summary

Of the 2,789 trials, 18% were published in the year 2000 or later, 69% were conducted in either Canada or the US and 96% used day old chicks as the study population. A total of 22% of the trials used a laying breed as the experimental population, and these are identified in Appendix 11. Fifteen unique CE products were examined, with an undefined, chicken related source, as the most common (63%) (Table 3.6.). The most common route of CE administration was oral gavage (65%) (Table 3.7.). A variety of sample types were collected to measure the *Salmonella* outcome, with cecal contents as the most frequent (28%), followed by ceca (26%) and cloaca (23%) (Table 3.8.). Sampling was conducted at various times, ranging from 0-7 days post-treatment (21%) to more than 84 days (<1%) (Table 3.9.). The variability of studies evaluating the effectiveness of CE is mapped out in Figure 3.2. using a single CE product (FM-B11) as an example.

In 78% of the trials, CE was administered to the chicks one time only, in the first 24 hours of life; and, in 72% of trials, chicks only received the CE treatment. The other 28% also received an additional intervention during the study period, most often other feed and water additives (66% of combination interventions), vaccination (15%), antimicrobials (11%), feed withdrawal (7%), and bacteriophages (1%). Within each combination intervention, a variety of products were used. For example, of 253 trials that

evaluated CE in combination with another feed or water additive, 10 additives were evaluated. The majority of these combination interventions evaluated an undefined CE product with lactose (n=119 trials). Figure 3.3. displays the heterogeneity of the combination interventions. Among all trials, the most commonly recovered serovars were *S.* Typhimurium (47%), *S.* Enteritidis (29%) and *S.* Infantis (13%). Other recovered serovars each comprised less than 5% of the total. Among challenge trials, the two most common administered serovars were *S.* Typhimurium (43%) and *S.* Enteritidis (22%).

Methodological assessment

Of 192 relevant CE studies that were assessed for methodological soundness, 149 were suitable for data extraction (n=40 no data, n=2 no control group, n=1 duplicate that was missed at the de-duplication stage) (Appendix 10). The 149 papers reported 2,789 unique trials (range 1-214 trials/per study; mean=19 trials/study) (Chapter 2). The *Salmonella* outcome was measured either as prevalence (1,794 trials), concentration (300 trials) or infection/protection factors (695 trials), which are geometric means.

Several consistent methodological soundness issues were observed for primary research investigating the effectiveness of CE. None of the CE trials (n=2,789) provided justification for the sample size and in 55% of the trials a sample size of 30 chicks or less was used. The majority of CE trials (98%) were conducted in laboratory facilities that were not representative of commercial broiler chicken production. Broiler populations were randomly assigned to treatment groups in only 3% of trials, and another 28% of trials reported random treatment assignment, but did not provide an explicit definition of

the treatment allocation process. Additionally, the use of blinding was almost never reported in the studies. Forty-two percent of the 2,789 trials did not report any statistical analysis, and clustering was present and not controlled for statistically in 13% of the trials. Intervention protocols, challenge protocols and laboratory methods were described in sufficient detail for replication in the majority of the trials. More detailed information about the methodological assessment of CE trials is shown in Table 3.5.

Meta-analysis

Of the 149 CE studies subjected to data extraction, 54 and 20 reporting prevalence and concentration outcomes, respectively, were analyzed separately. Fifteen studies reported both prevalence and concentration outcomes and are included in the above numbers, for a total of 59 studies. Reasons for the exclusion of 90 studies are indicated in Appendix 11 and Figure 3.1. Challenge trials accounted for 51 of the 59 studies. Meta-analyses were separately reported for Broilact, FM-B11, CF3 and Aviguard products using challenge trials reporting prevalence outcomes. For FM-B11, CF3 and Aviguard, the treatment effectiveness was evaluated at two time periods (0-42 days post-treatment; >43 days post-treatment), whereas Broilact was only investigated between 0 and 42 days post-treatment, as there was no primary research evaluating its effectiveness beyond this time period. Within the time period from 0 to 42 days post-treatment, significant heterogeneity ($Q < 0.1$) was observed for the datasets reporting the on-farm use of Broilact, FM-B11 and Aviguard, and thus, pooled MA estimates were not reported. After 42 days post-treatment, pooled estimates were not reported for Aviguard for the same reason. Among challenge trials reporting a concentration outcome, only one analysis was conducted on

the commercial product CF3, which investigated its effectiveness at both time periods, due to a lack of trials evaluating other CE products. This analysis revealed significant heterogeneity and thus, a pooled MA estimate was not reported. Tables 3.10. and 3.11. show the number of trials per product in the MA for prevalence and concentration data, respectively.

The commercial product CF3 significantly decreased the prevalence of *Salmonella* colonization in treated chicks compared to non-treated chicks at both time periods, and FM-B11 at >42 days post-treatment. Meta-analysis results are reported in Tables 3.10. (prevalence) and 3.11. (concentration) for strata (data subsets) in which Cochrane's Q statistic was not significant ($p>0.1$). Forest plots for all products, including undefined, are shown in Figures 3.4. to 3.12., primarily for visual observation of trends. However, pooled effect estimates are shown only for data subsets for which statistically significant heterogeneity was not observed (Figure 3.6.). Begg's rank correlation test and Egger's regression test detected publication bias in the concentration outcome dataset (Figure 3.13.), where studies with large sample size reporting less effectiveness might have been missed. Publication bias was not detected in the prevalence outcome dataset (Figure 3.14.).

Meta-regression

From the initial 149 studies considered for MR, 104 studies (1,794 unique trials) were included and are described in Table 3.4. The 45 studies were excluded because no prevalence outcome was reported, either as raw data or adjusted data with measures of

variability (Appendix 11). Study design, publication year, population type, type of CE product, route of CE administration, serovar administered/recovered, method of treatment assignment and reproducibility of intervention protocols and laboratory methods were the significant predictors (<0.05) in the final multivariable model (Table 3.12.). The MR showed no significant difference between trials that used a laying breed versus broiler breed as the experimental population.

DISCUSSION

Overall, the MA indicated that a variety of defined CE products significantly decrease the odds of *Salmonella* colonization in treated chicks as compared to non-treated chicks across the entire life span. Our approach to stratify a very heterogeneous dataset into biologically sensible subsets for various defined CE products and to assess their effectiveness over time frequently resulted in significant heterogeneity remaining in the subsets and prevented reporting of more precise pooled effect estimates. A variety of factors were tested through stratification and exclusion, for example serovar and route of CE administration; however, this only resulted in substantial reduction in the number of trials without changing Cochrane's Q statistic for heterogeneity. We suspect that more homogeneous strata could not be created for all of the products because of other factors heavily influencing heterogeneity. One possibility is the dose of CE administration; however, there was a lack of uniformity in this variable and it could not be categorized for stratified MA or examined in the MR.

The MR indicated that undefined CE products originating from a chicken source are more effective than the commercial, defined products. The only defined product that outperformed the undefined product was CF3, where chicks treated with CF3 were 1.6 times less likely than chicks treated with an undefined product to become colonized with *Salmonella.* The inability of most defined products to outperform undefined products may be a result of the difficulty that researchers had maintaining a stable culture of the defined strains over time that confer resistance to *Salmonella* (Jeffrey, 1999) or defined products may be missing an important component from the start. An important benefit of defined CE commercial products is the quality control aspect of their manufacturing process, which ensures that harmful human and avian pathogens are not present (Mead, 2005). As a result, products like CF3, which are both defined and as effective or more than undefined products, are preferable for use by producers.

The methods of CE administration available for field use are very different from those that are usually employed in laboratory settings, where accurate dosing of chicks is necessary (Mead, 2000). Oral gavage was the most popular route of CE administration identified in this study. However, results obtained using some of the more frequently employed methods of administration in the field, for example, as additives in water or feed, were not significantly different from those using oral gavage. One method, however, was more effective at protecting chicks from colonization; chicks sprayed at the hatchery and given oral gavage in the first 48 hours of life were 18.1 times less likely to become colonized with *Salmonella* than the chicks only administered oral gavage in the first 48 hours of life. However, this is a result of a couple very significant trials, and thus,

this result should be interpreted cautiously. In addition, the MR showed that treatment of broiler eggs was 2.4 times less effective than treatment of day old chicks. This indicates that the use of oral gavage is not necessary in the field to gain the *Salmonella* protection offered by CE treatment, but that treatment should be administered to chicks, as opposed to spraying eggs, to maximize effectiveness.

The effectiveness of CE treatment was shown to vary depending on the type of *Salmonella* isolated or used in the challenge. The MR showed a greater difference between treatment groups when *S.* Enteritidis or *S.* Infantis was used in the study as opposed to *S.* Typhimurium. The majority of research evaluated the effectiveness of CE when applied to day old chicks that had been challenged with an antimicrobial resistant strain of *S.* Typhimurium. Perhaps these resistant strains may be more difficult to treat successfully with CE than non-resistant strains of *S.* Enteritidis or *S.* Infantis; however, more research needs to be conducted to test this hypothesis and other characteristics of *S.* Typhimurium may have an impact.

A large quantity of the primary research examining interventions to reduce *Salmonella* in broilers were evaluating the effectiveness of various CE products in broiler chickens. This is not surprising, given that contradictory recommendations regarding their use currently exist, and it has been challenging to develop defined products that remain effective over time (Jeffrey, 1999). Prior to 1990, the effectiveness of undefined CE products was studied in Canada, the US, Europe and Australia. This coincides with the use of undefined CE products as part of the mandatory *Salmonella* eradication program in

Sweden (Sternberg Lewerin et al., 2005). Since 1990, the use of CE has not been permitted in Sweden, mainly because the products are not well-defined or sufficiently validated to be licensed (Sternberg Lewerin et al., 2005). This probably contributed to the shift in research from undefined to defined CE products. Simultaneously, an increase in CE research was observed in other regions, including South America, Southeast Asia and the Middle East, although these studies made up less than 10% of the total studies identified.

Consistent issues with the methodological soundness of studies was observed, as a result of both study conduct and poor reporting. For example, a lack of field studies conducted under commercial conditions was observed, and in fact, the majority of CE studies were conducted in research facility settings with small sample sizes that were not representative of field conditions. The majority of trials were challenge trials, with a small number of controlled trials. Controlled trials tend to be more representative of field conditions, and offer stronger support to the reported effectiveness of the treatment. Members of the industry indicated that work carried out in the field, often as controlled trials, is kept confidential by large integrated poultry companies (Sue Reynolds, Microbial Developments Ltd., Worcestershire, UK, personal communication), and therefore, is not captured in this study. In the future, these integrators should be encouraged to share their knowledge with the research community. The lack of large experiments and field studies was also indicated by Begg's rank correlation and Egger's regression test suggesting publication bias for the prevalence outcome dataset. Conducting such studies and publishing the results irrespective of their significance

would result in stronger MA and better understanding of the effect of on-farm CE on *Salmonella* prevalence and concentration in broiler chickens.

These methodological issues may compromise the external validity of the overall effect estimates obtained through the MA. In order to strengthen external validity, more field studies should be conducted with large populations of chicks under natural exposure to *Salmonella*, preferably with a wide range of *Salmonella* serovars. Ideally, a variety of study designs, including experimental and observational designs, particularly cohort studies, would support the conclusions. This is particularly important given that the MR indicated significant differences in the effectiveness of CE products based on study design. Broilers were 2.5 times less likely to become colonized with *Salmonella* after CE treatment in challenge trials (artificial infection) than natural infection in controlled trials. This may be a result of the small sample sizes included in the challenge trials, or the sanitized laboratory settings these studies were conducted in.

Where available, variables assessing the methodological soundness of trials were examined in the MR, but this was often not possible due to lack of variation in data. Variables with enough variation in responses between trials were included in the MR (trial sample size, method of treatment assignment, intervention protocol and laboratory methods descriptions) and the latter three were significant in the final model. The effectiveness of CE treatment was associated with method of assignment of chicks to treatment groups in trials, where trials that reported and described random assignment to treatment groups showed treatment to be more effective. Broilers in trials that reported

assignment to treatment groups was random but did not describe the allocation process or where a convenience approach was used were 5 to 6 times more likely to become colonized with *Salmonella* than chicks in trials that adequately described the random allocation process. This may be a result of different baseline prevalence in groups where random assignment was not used, particularly since baseline prevalence was not measured in chicks after challenge but prior to assignment in 36% of the trials. In trials that described intervention protocols and laboratory methods in sufficient detail to be replicated, broilers were more likely to become colonized with *Salmonella* than in trials that did not do so. It is possible that studies that reported laboratory test protocols in detail had used more sensitive culture for the detection of *Salmonella*. The overall methodological soundness results show the importance of methodological assessment of studies included in literature reviews as the study conduct or reporting aspects might be associated with the reported effectiveness of CE products.

Due to financial and time constraints, studies not published in English were excluded from this SR-MA. A total of 242 non-English citations, titles or abstracts, were identified during RS 1 as primary research investigating various types of interventions for *Salmonella* in broilers from farm-to-secondary processing, of which, 32 potentially evaluated CE and were not evaluated as full papers. Although the effect of excluding non-English language studies on the overall effect estimates of this study is unknown, other research showed that, if anything, it can lead to more conservative estimates of treatment effects (Juni et al., 2002). In an up-dated SR-MA, securing funding for

translation of non-English language studies evaluating the effectiveness of CE products would strengthen the results reported in this paper.

Estimates reported from our MA should be interpreted cautiously, as calculations were based on crude ORs calculated *post hoc* in the prevalence outcome dataset, and although a random effects model was used, heterogeneity was present in some of the strata. In addition, some, but not all, studies reported more than one trial. As a result, statistical clustering may exist in the data because the results from multiple trials reported in the same study may not be statistically independent. This relationship was not quantified in the MR to avoid partitioning too much variance to the study level, since the number of trials was not consistent across studies (range 1-214 trials/study; mean=19 trials/study). When the clustering was accounted for in univariable analyses and compared to the reported MR, only a small change was observed in the coefficients, suggesting that clustering did not affect our results.

CONCLUSION

The SR captured a large quantity of data evaluating the effectiveness of CE in broiler chickens. Overall, the MA-MR indicated that commercial CE products were effective at protecting broilers from *Salmonella* colonization over time, by a variety of administration routes, however, only CF3 was significantly more effective than undefined products originating from chicken sources. Six study characteristics (study design, publication year, population type, type of CE product, route of CE administration, *Salmonella* serovar administered/recovered) and three study methodological soundness characteristics

(method of treatment assignment, replicable description of intervention protocol and laboratory methods) were included in the final model.

The methodology used in this study was useful for obtaining more precise estimates of the effectiveness of certain CE products at reducing *Salmonella* colonization in chicks, and for exploring the factors associated with their variability. The study results may support future guidelines on the on-farm use of CE in broiler chickens, or act as input parameters in a risk assessment. The results from the MR provide useful information for the interpretation and future design of primary research evaluating the effectiveness of CE products.

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Table 3.1. The number of electronic hits before and after de-duplication in a scoping review of *Salmonella* in broiler chickens

Population	**Outcome**	**Database**	**# of hits**
Chick* OR	Salmonell*	Current Contents	2467
Poultry* OR		Agricola	3086
Broiler* OR		PubMed	4239
Gallus*		Scopus	4974
		CAB	9656
		CAB Global Health	2489
		TOTAL	26, 911
		DE-DUPLICATED	12, 957

Table 3.2. Number of new hits, obtained citations and relevant abstracts from selected literature reviews included in the search verification of a scoping review of *Salmonella* in broiler chickens

Item searched	# hits[1]	# obtained[2]	# relevant[3]
General review articles			
Burnham, V.E., 2007	0	0	0
Bolder, N.M., 2007	7	2	0
Maijala, R., et al., 2005	1	0	0
Nash, W.A., 2004	0	0	0
Competitive exclusion			
Schneitz, C., 2005	8	0	0
Revolledo, L., et al., 2006	1	1	0
Competitive exclusion and antimicrobials			
Fowler, N.G., 1992	0	0	0
Antimicrobials			
Ricke, S.C., et al., 2005	3	2	0
Naidu, A.S., et al., 2003	0	0	0
Other feed and water additives			
Van Immerseel, F., et al., 2006	3	2	0
Bacteriophage/bacteriocins			
Greer, G.G., 2005	0	0	0
Treatment spraying/dipping			
Capita, R., et al., 2002	1	0	0

General processing articles			
Rasekh, J., et al., 2005	4	2	0
Mead, G.C., 2004	1	0	0
Fries, R., 2002	7	4	0
Total	36	13	0

[1] The number of citations that were located in the item, that were not found in the original search conducted in December, 2007
[2] The number of citations for which abstracts were found
[3] The number of abstracts that passed relevance screening levels one and two, of the citations for which abstracts were found

Table 3.3. Number of new hits, obtained citations and relevant abstracts from selected chapters of a text book included in the search verification of a scoping review of *Salmonella* in broiler chickens (Mead, 2005)

Item searched	**# hits**[1]	**# obtained**[2]	**# relevant**[3]
Chapter 1	7	3	0
Chapter 5	0	0	0
Chapter 6	0	0	0
Chapter 7	0	0	0
Chapter 8	0	0	0
Chapter 9	1	0	0
Chapter 10	0	0	0
Chapter 13	2	2	0
Chapter 14	7	3	0
Chapter 15	1	1	0
Chapter 16	0	0	0
Chapter 17	7	3	0
Chapter 18	0	0	0
TOTAL	25	12	0

[1] The number of citations that were located in the item, that were not found in the original search conducted in December, 2007
[2] The number of citations for which abstract s were located
[3] The number of abstracts that passed relevance screening levels one and two

Table 3.4. Study design and methodological soundness or reporting characteristics and their frequency in 1794 trials from 104 studies included in a meta-regression analysis evaluating the effectiveness of competitive exclusion at reducing *Salmonella* colonization in broiler chickens

Variable	**Categories**	**# of trials**	**% of 1794 trials**
Study design used	ChT[1]	1732	97
	CT[2]	56	3
	QE[3]	6	<1
Date of study publication	<1979	470	26
	1980-1989	671	37
	1990-1999	391	22
	>2000	262	15

Type of CE used	Undefined chicken related source	1297	72
	Undefined unknown source	74	4
	Partially defined, contains lactobacillus	77	4
	Simbiotico	1	<1
	Avian Pac Plus	9	<1
	Broilact	37	2
	Aviguard	9	<1
	FM-B11	57	3
	MSC	9	<1
	Avifree	5	<1
	Preempt	5	<1
	CF3	39	2
	Primilac	25	1
	Other	150	8

Route CE administered	Oral individual	1168	65
	Mass through feed	61	3
	Mass through water	242	13
	Other (e.g. spray)	159	9
	Oral individual, mass through feed	9	<1
	Oral individual, mass through water	124	7
	Oral individual, other	3	<1
	Mass through water, other	21	1
	Not reported	7	<1
Population CE was evaluated	Day old chicks	1723	96
	Broiler eggs	14	<1
	Broilers	23	1
	Day old chicks and breeding poultry	31	2
	Not reported	3	<1

Serovars recovered	Typhimurium	768	43
	Enteritidis	579	32
	Infantis	284	16
	Other	135	7
	Multiple	28	2
How were broilers assigned to treatment groups?	Random[4]	59	3
	Reported random[4]	455	25
	Convenience[4]	1278	71
	Not applicable[4]	2	<1
Was the intervention protocol described in sufficient detail?	Yes[4]	982	55
	No[4]	153	9
	Reference paper[4]	657	37
	Not applicable[4]	2	<1

Were the laboratory methods described in sufficient detail?	Yes[4]	796	45
	No[4]	206	11
	Reference paper[4]	792	44

[1] Challenge trial
[2] Controlled trial
[3] Quasi experiment
[4] For detailed explanation see Appendix 4

Table 3.5. Questions and responses summarizing the methodological assessment of competitive exclusion studies included in this systematic review

Criteria	**Responses**[1]		
		# trials	*%*
Was the sample size justified?	Yes	0	0
	No	2789	100
Was the representativeness of the sample population to the target population explained and sufficiently justified?	Yes	26	<1
	No	33	1
	N/A	2730	98
Were the birds housed, grouped or slaughtered in a way that is representative of field conditions?	Yes	55	2
	No	2734	98
How was the intervention assigned to the experimental unit?	Random	71	3
	Reported random	790	28
	Systematic	0	0
	Convenience	1926	69
	N/A	2	<1
Were the intervention protocols described in sufficient detail to allow reproduction of the experiment?	Yes	1698	61
	No	240	9
	Reference paper	849	30
	N/A	2	<1

Was the challenge protocol adequately described so that the challenge could be reproduced?	Yes	1856	67
	No	220	8
	Reference paper	646	23
	N/A	67	2
Were laboratory methods used to determine the outcome described sufficiently to allow replication of the study?	Yes	1345	48
	No	355	13
	Reference paper	1089	39
Did the author report that blinding was used?	Yes	9	<1
	No	2780	100
	N/A	0	0
Was the statistical analysis described adequately so it can be reproduced?	Yes	1471	53
	No	141	5
	Reference paper	8	<1
	No analysis	1169	42
Based on the study design, was clustering accounted for appropriately in the analysis?	Yes	162	6
	No	359	13
	N/A	2268	81
TOTAL TRIALS		2789	

[1] For detailed explanation see Appendix 4

Table 3.6. Frequency of competitive exclusion (CE) products evaluated in the 2,789 trials captured in the systematic review

CE product	**Frequency**	%
Undefined, chicken source	1,743	62.5
Other	304	10.9
Containing lactobacillus	200	7.17
Undefined, unknown source	95	3.41
Aviguard	76	2.72
Vermicompost	69	2.47
FM-B11	67	2.40
Broilact	66	2.37
Primilac	47	1.69
CF3	43	1.54
Avian Pac Plus	30	1.08
Preempt	24	0.86
MSC	11	0.39
Simbiotico	8	0.29
Avifree	6	0.22
TOTAL	2,789	100

Table 3.7. Frequency of routes used to administer competitive exclusion (CE) products in the 2,789 trials captured in the systematic review

CE route	**Frequency**	**%**
Oral individual	1,804	64.7
Mass through water	344	12.3
Other (e.g. spray)	197	7.06
Oral individual + Mass through water	193	6.92
Mass through feed	181	6.49
Mass through water, Other	46	1.65
Oral individual + Mass through feed	11	0.39
Oral individual + Other	3	0.11
Oral individual + Mass through feed and water	2	0.07
Not reported[1]	8	0.29
TOTAL	2,789	100

[1] Studies that did not report the route of administration were included in the meta-analysis-meta-regression

Table 3.8. Frequency of sample types evaluated for *Salmonella* in the 2,789 trials captured in the systematic review

Sample type	**Frequency**	**%**
Cecal contents[1]	791	28.4
Ceca[1]	735	26.4
Cloaca[2]	643	23.1
Other (e.g., bile, heart blood)[2]	169	6.06
Cecal tonsils[1]	102	3.66
Crop[2]	79	2.83
Liver[2]	73	2.62
Liver and Spleen[2]	42	1.51
Spleen[2]	28	1.00
Duodenum[2]	24	0.86
Multiple organs[2]	19	0.68
Whole carcass rinse[2]	14	0.50
Ileum[2]	11	0.39
Gizzard[2]	10	0.36
Jejunum[2]	5	0.18
Colon[2]	5	0.18
Not reported[2]	39	1.40
TOTAL	2,789	100

[1] These sample types were included in the meta-analysis
[2] These sample types were excluded from the meta-analysis

Table 3.9. Frequency of time periods post-competitive exclusion treatment samples were evaluated for *Salmonella* in the 2,789 trials captured in the systematic review

Time measured	**Frequency**	**%**
0-7 days of age	579	20.8
8-14 days of age	787	28.2
15-21 days of age	228	8.17
22-28 days of age	146	5.23
29-35 days of age	56	2.01
36-42 days of age	103	3.69
43-49 days of age	63	2.26
50-56 days of age	59	2.12
57-63 days of age	4	0.14
64-70 days of age	14	0.50
71-77 days of age	5	0.18
78-84 days of age	10	0.36
>84 days of age	21	0.75
Multiple days	81	22.7
Not reported[1]	633	2.90
TOTAL	2,789	100

[1] Studies that did not report the time *Salmonella* was measured post-treatment were not included in the meta-analysis

Table 3.10. Meta-analysis results from studies evaluating the effect of competitive exclusion on *Salmonella* in broiler chickens using a challenge trial study design and reporting a prevalence outcome by time measured for each competitive exclusion type

Time measured	# trials[1]	OR (95% CI)	P-value	Q-statistic	I (%)[2]
Broilact					
0-42 days	14	NR[3]	NR	0.003	59.2
>42 days	0	--[4]	--	--	--
Overall	14	NR	NR	0.003	59.2
FM-B11					
0-42 days	42	NR	NR	0.000	68.2
>42 days	4	0.047 (0.021, 0.104)	0.000	0.784	0.0
Overall	46	NR	NR	0.000	68.5

CF3					
0-42 days	16	0.044 (0.027, 0.071)	0.000	0.328	11.0
>42 days	11	0.080 (0.044, 0.145)	0.000	0.817	0.0
Overall	27	0.051 (0.036, 0.073)	0.000	0.468	0.0
Aviguard					
0-42 days	4	NR	NR	0.001	82.2
>42 days	5	NR	NR	0.003	75.2
Overall	9	NR	NR	0.000	75.9

[1] Number of trials in analysis
[2] Percent of total variation due to heterogeneity
[3] OR is not reported if the Q statistic is significant ($p<1.0$)
[4] No available data from the literature to report

Table 3.11. Meta-analysis results from studies evaluating the effect of competitive exclusion on *Salmonella* in broiler chickens using a challenge trial study design and reporting a concentration outcome by time measured for each competitive exclusion type

Time measured	**# trials**[1]	**OR (95% CI)**	**P-value**	**Q-statistic**	**I (%)**[2]
CF3					
0-42 days	15	NR[3]	NR	0.00	99.2
>42 days	8	NR	NR	0.00	93.1
Overall	23	NR	NR	0.00	98.9

[1] Number of trials in analysis
[2] Percent of total variation due to heterogeneity
[3] OR is not reported if the Q statistic is significant ($p<1.0$)

Table 3.12. Odds ratios and P-values from the final meta-regression model (n=104 studies, 1794 trials)

Variable	OR	P-value	Overall P-value
Study design			<0.01
Controlled trial	Referent		
Challenge trial	0.39	<0.01	
Quasi experiment	2.00	<0.01	
Date published			<0.01
>2000	Referent		
1990-1999	1.71	<0.01	
1980-1989	1.83	<0.01	
<1979	1.70	<0.01	
Population[1]			<0.01
Day old chicks	Referent		
Broiler eggs	2.44	0.04	
Broilers	1.23	0.47	
Day old chicks, breeding poultry	1.13	0.69	
Not reported	0.03	<0.01	
CE type			<0.01
Undefined, chicken source	Referent		
Undefined, unknown source	1.31	0.11	
Simbiotico	0.73	0.87	
Avian Pac Plus	0.70	0.37	

Broilact	1.48	0.03	
Aviguard	3.71	<0.01	
FM-B11	2.77	<0.01	
MSC	2.18	0.04	
Avifree	34.5	<0.01	
Preempt	1.51	0.36	
CF3	0.64	0.02	
Primilac	7.92	<0.01	
Containing lactobacillus	6.75	<0.01	
Other	3.29	<0.01	
CE route			<0.01
Oral gavage	Referent		
Mass through feed	1.35	0.15	
Mass through water	1.08	0.42	
Other (e.g. spray)	0.90	0.28	
Oral gavage + Mass through feed	0.75	0.62	
Oral gavage + Mass through water	0.83	0.25	
Oral gavage + Other	0.06	<0.01	
Mass through water + Other	0.84	0.42	
Not reported	0.91	0.75	

Serovar recovered			<0.01
S. Typhimurium	Referent		
S. Enteritidis	0.76	<0.01	
S. Infantis	0.42	<0.01	
Other	1.28	0.13	
Multiple serotypes	0.90	0.72	
Assignment to treatment groups[2]			<0.01
Random	Referent		
Reported random	4.71	<0.01	
Convenience	5.99	<0.01	
Not applicable	34.12	<0.01	
Intervention protocol description[1]			<0.01
Yes	Referent		
No	1.40	0.04	
Reference paper	1.01	0.93	
Lab methods description[1]			<0.01
Yes	Referent		
No	2.34	<0.01	
Reference paper	1.35	0.05	

[1] For detailed description see Appendix 5
[2] For detailed description see Appendix 4

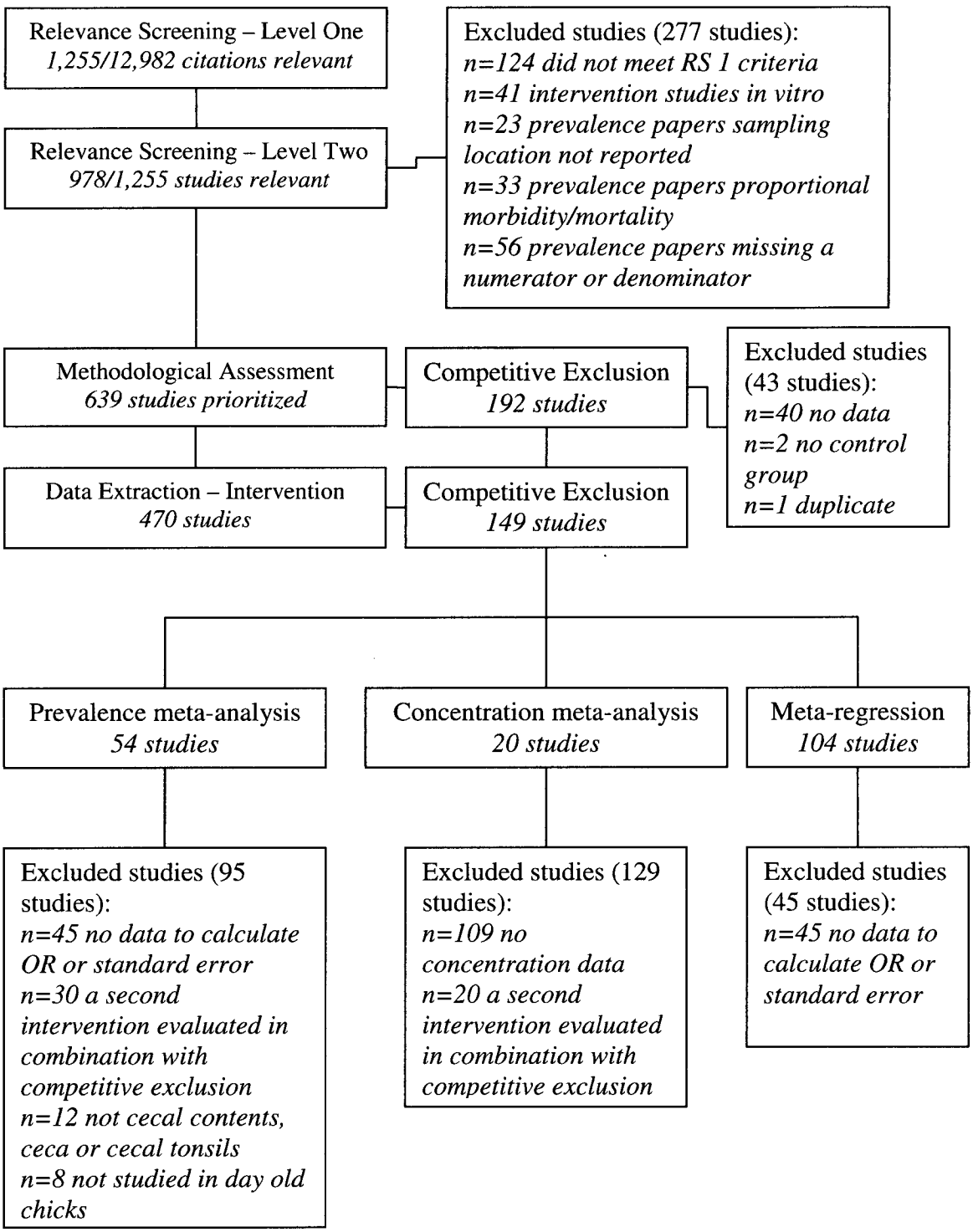

Figure 3.1.

The prioritization of competitive exclusion for rigorous systematic review-meta-analysis from a scoping review on *Salmonella* in broiler chickens

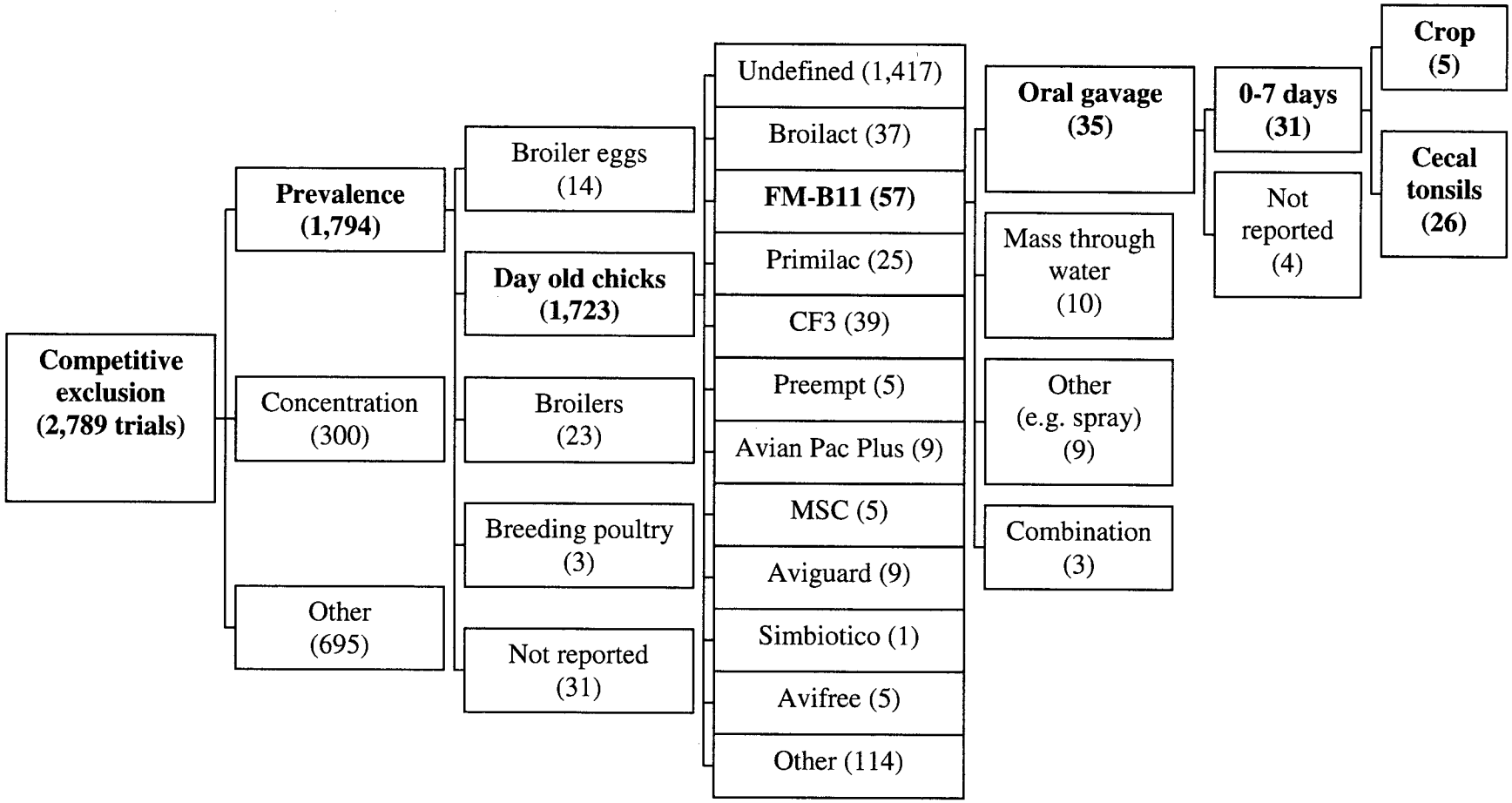

Figure 3.2.

Illustration of the variability of study design in primary research for a single competitive exclusion product (FM-B11)

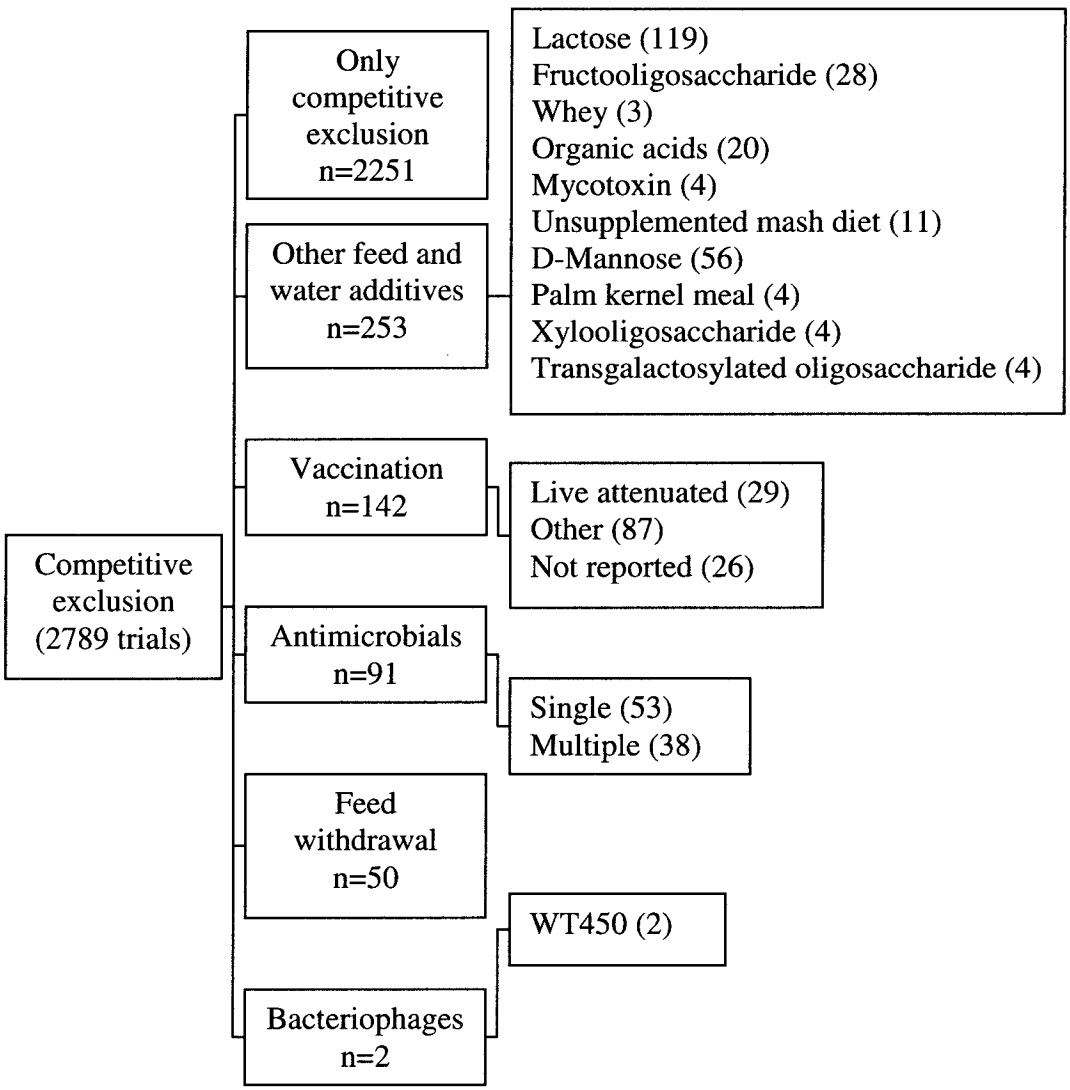

Figure 3.3.

Illustration of the variability of primary research evaluating competitive exclusion in combination with a second intervention on *Salmonella* in broiler chickens

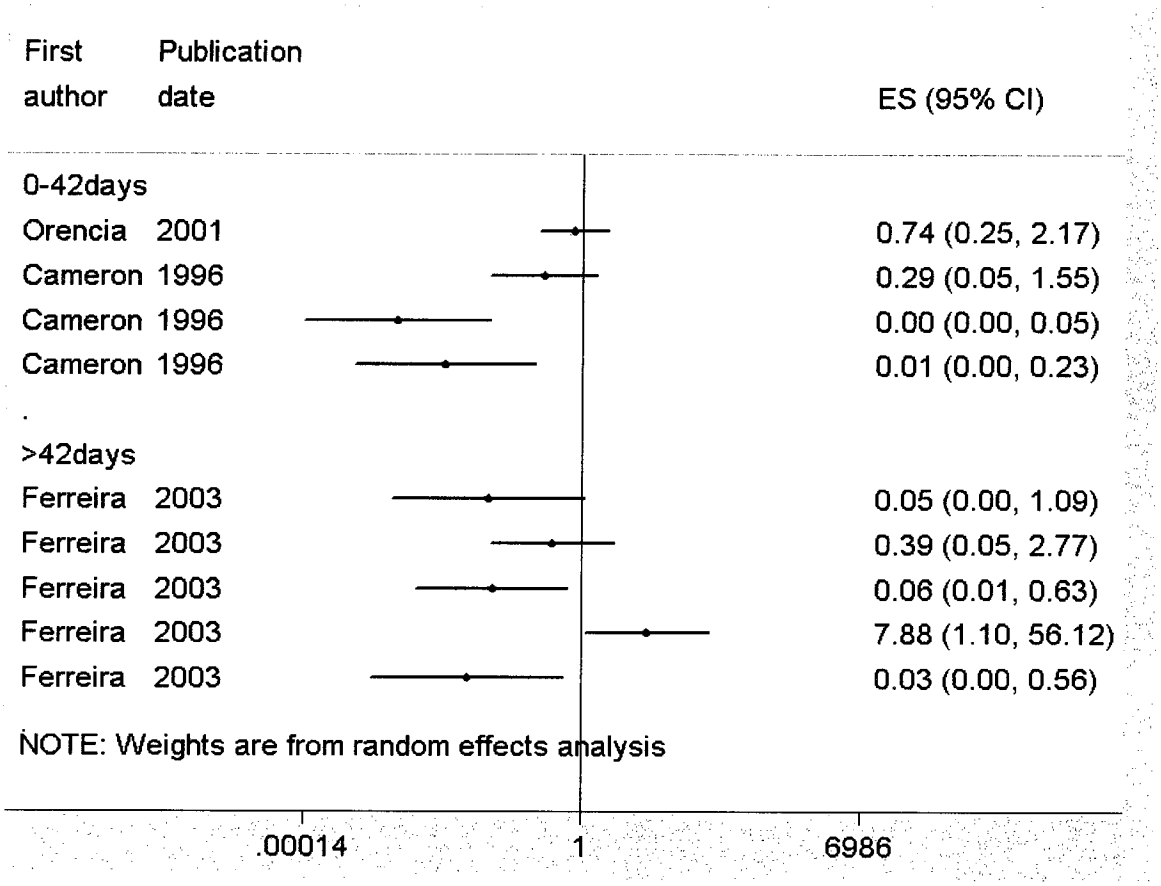

Figure 3.4.

Random effects meta-analysis of challenge trials reporting a prevalence outcome evaluating the effectiveness of Aviguard at reducing the odds of *Salmonella* colonization in broilers. Studies are stratified by time period and estimates of effect are presented as odds ratios (ORs).

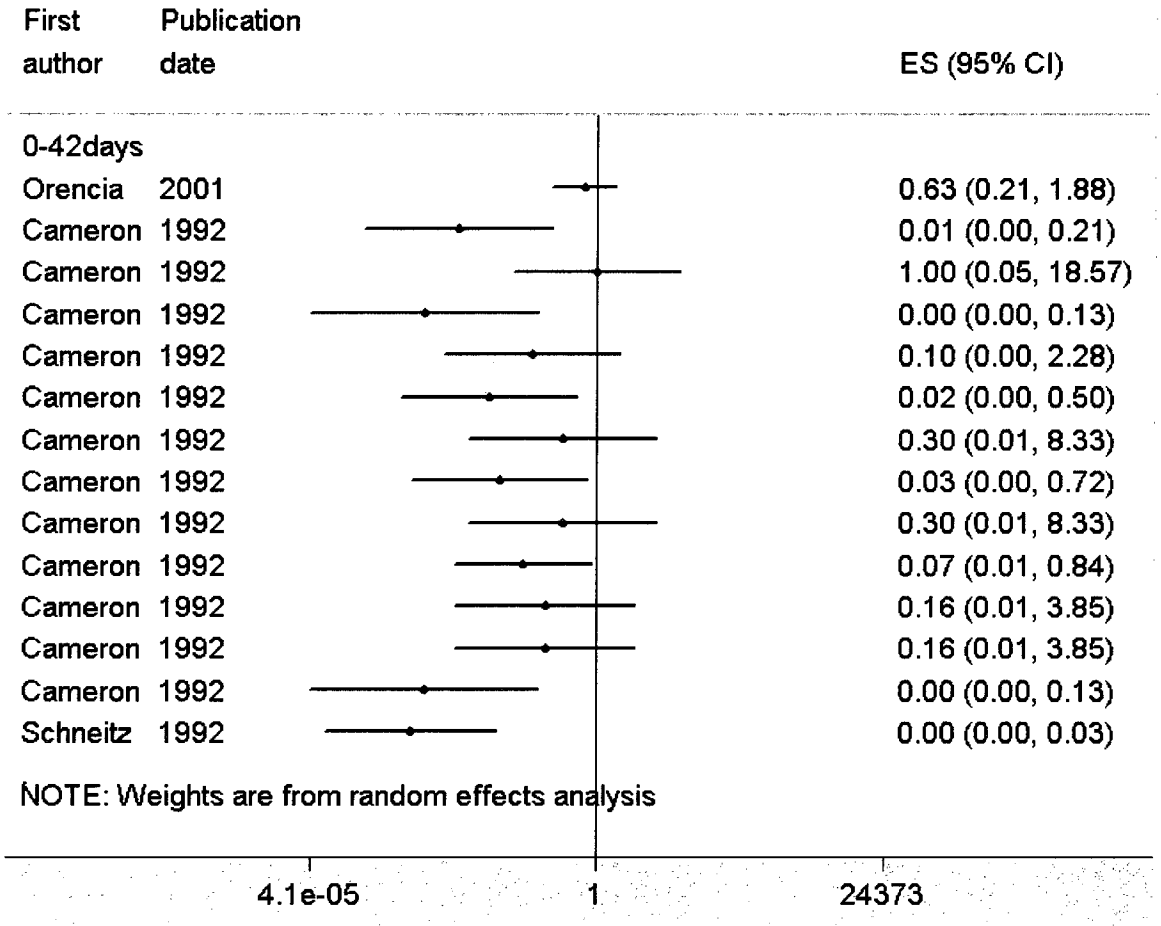

Figure 3.5.

Random effects meta-analysis of challenge trials reporting a prevalence outcome evaluating the effectiveness of Broilact at reducing the odds of *Salmonella* colonization in broilers. Studies are stratified by time period and estimates of effect are presented as odds ratios (ORs).

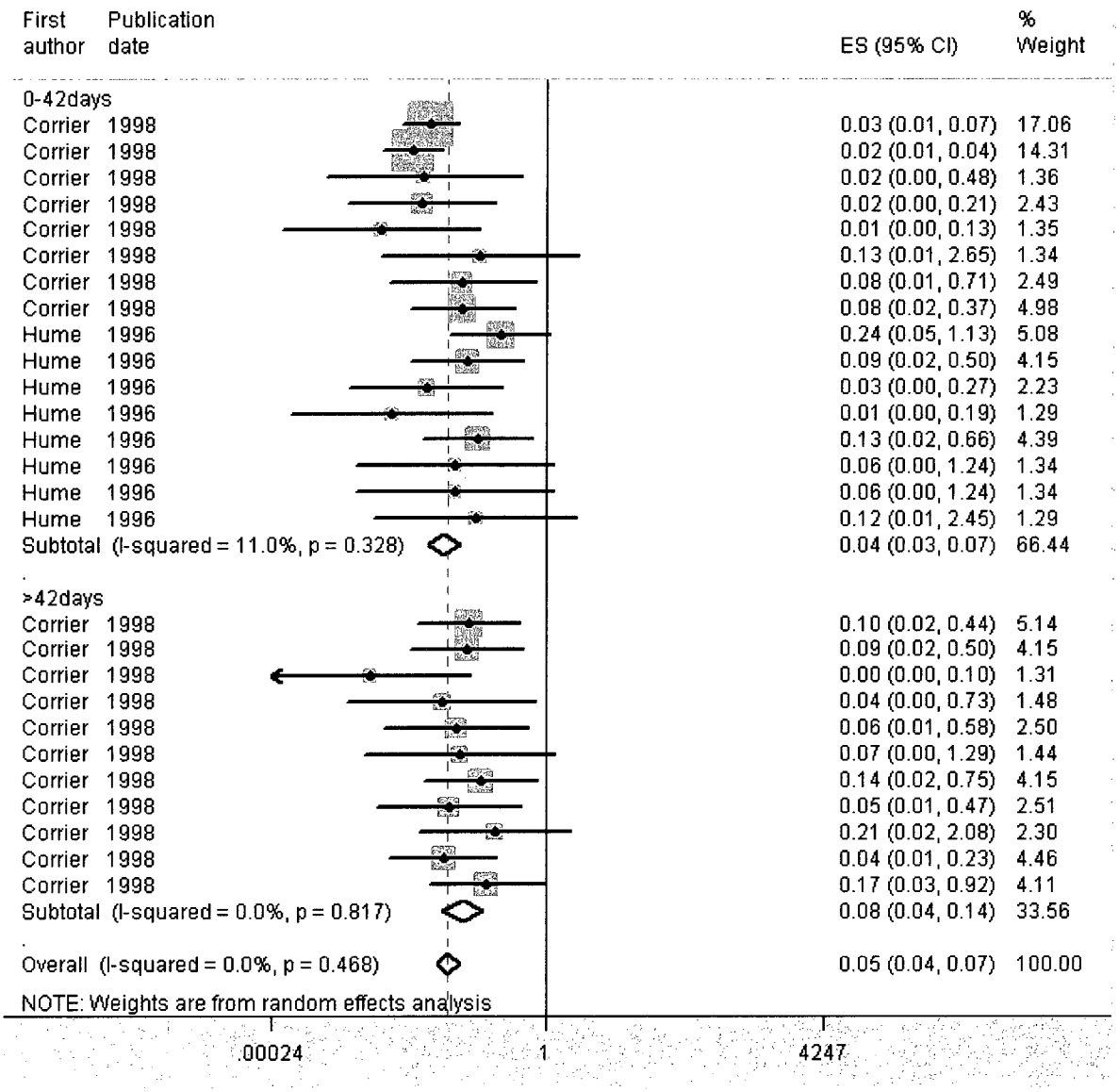

Figure 3.6.

Random effects meta-analysis of challenge trials reporting a prevalence outcome evaluating the effectiveness of CF3 at reducing the odds of *Salmonella* colonization in broilers. Studies are stratified by time period and estimates of effect are presented as odds ratios (ORs). The P value refers to the Q statistic test for heterogeneity.

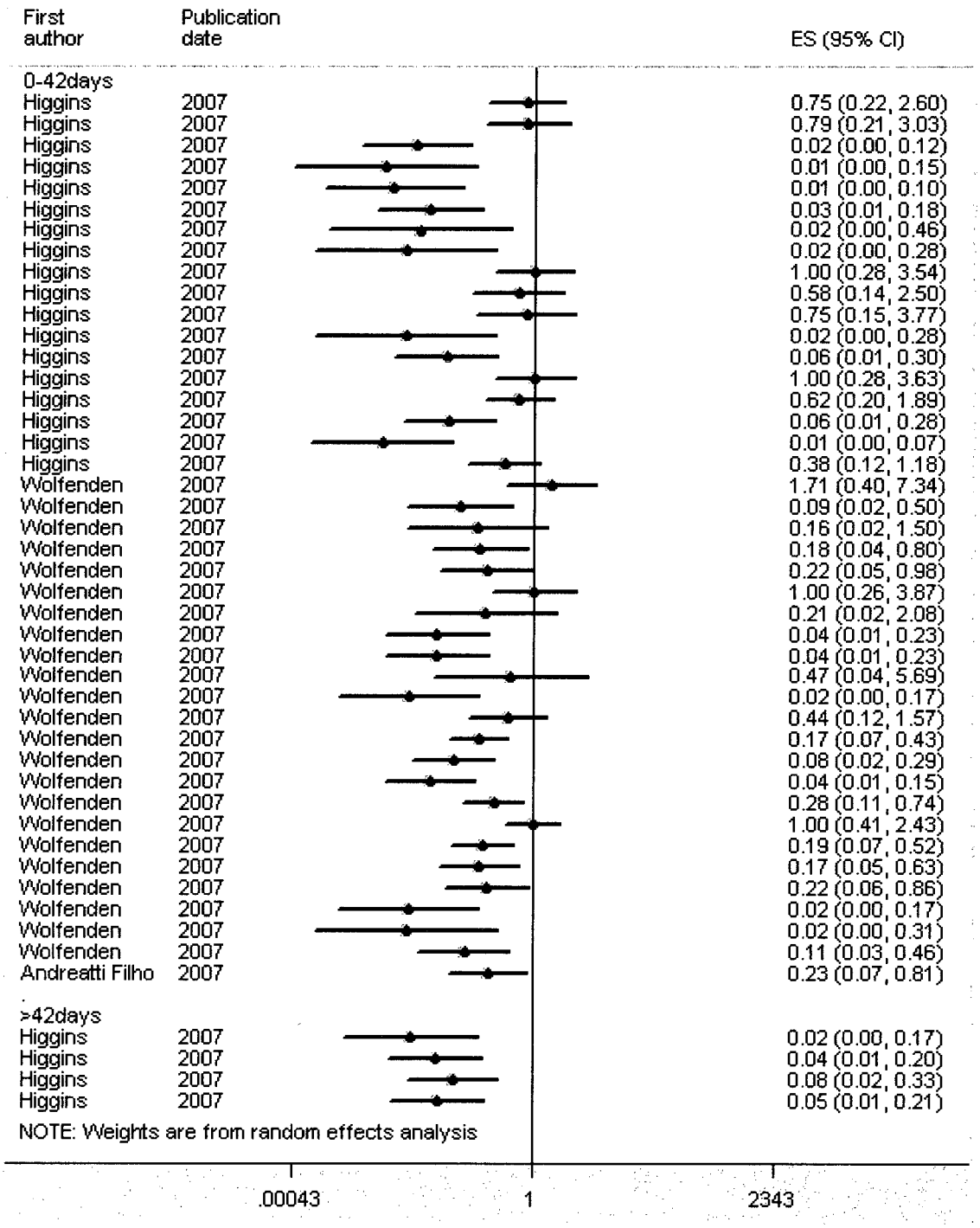

Figure 3.7.

Random effects meta-analysis of challenge trials reporting a prevalence outcome evaluating the effectiveness of FM-B11 at reducing the odds of *Salmonella* colonization in broilers. Studies are stratified by time period and estimates of effect are presented as odds ratios (ORs).

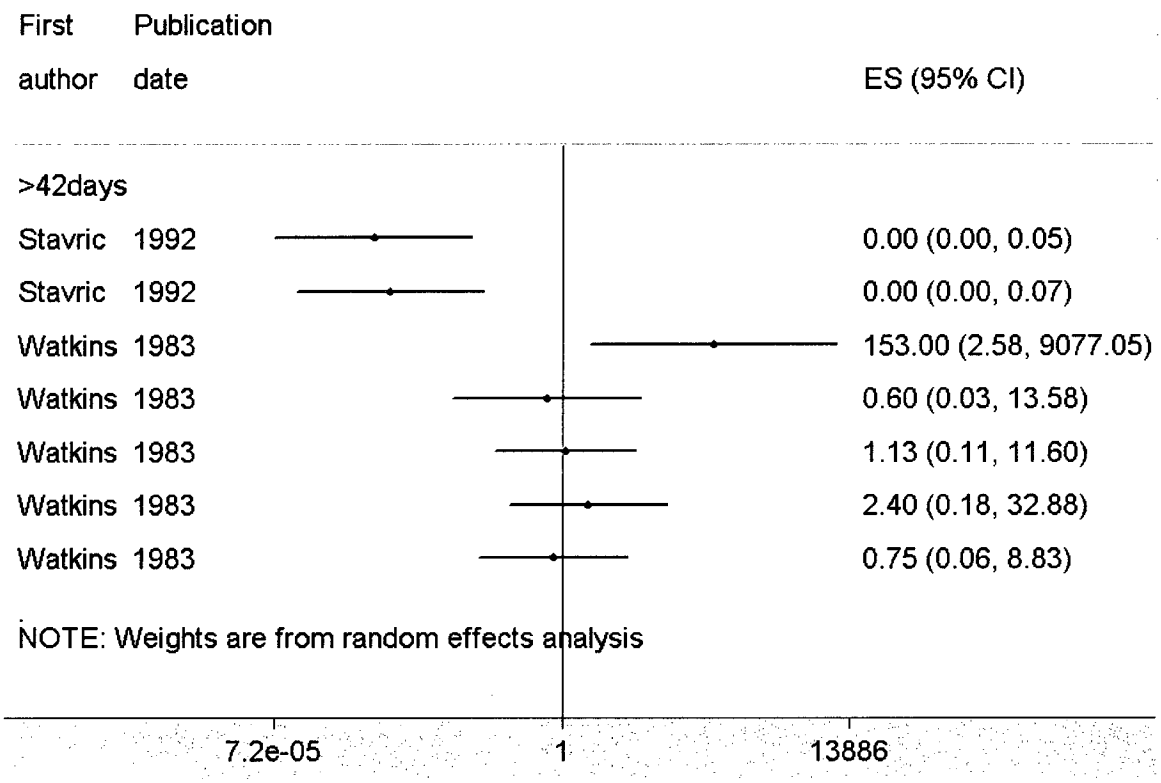

Figure 3.8.

Random effects meta-analysis of challenge trials reporting a prevalence outcome evaluating the effectiveness of partially defined competitive exclusion products containing lactobacillus at reducing the odds of *Salmonella* colonization in broilers. Studies are stratified by time period and estimates of effect are presented as odds ratios (ORs).

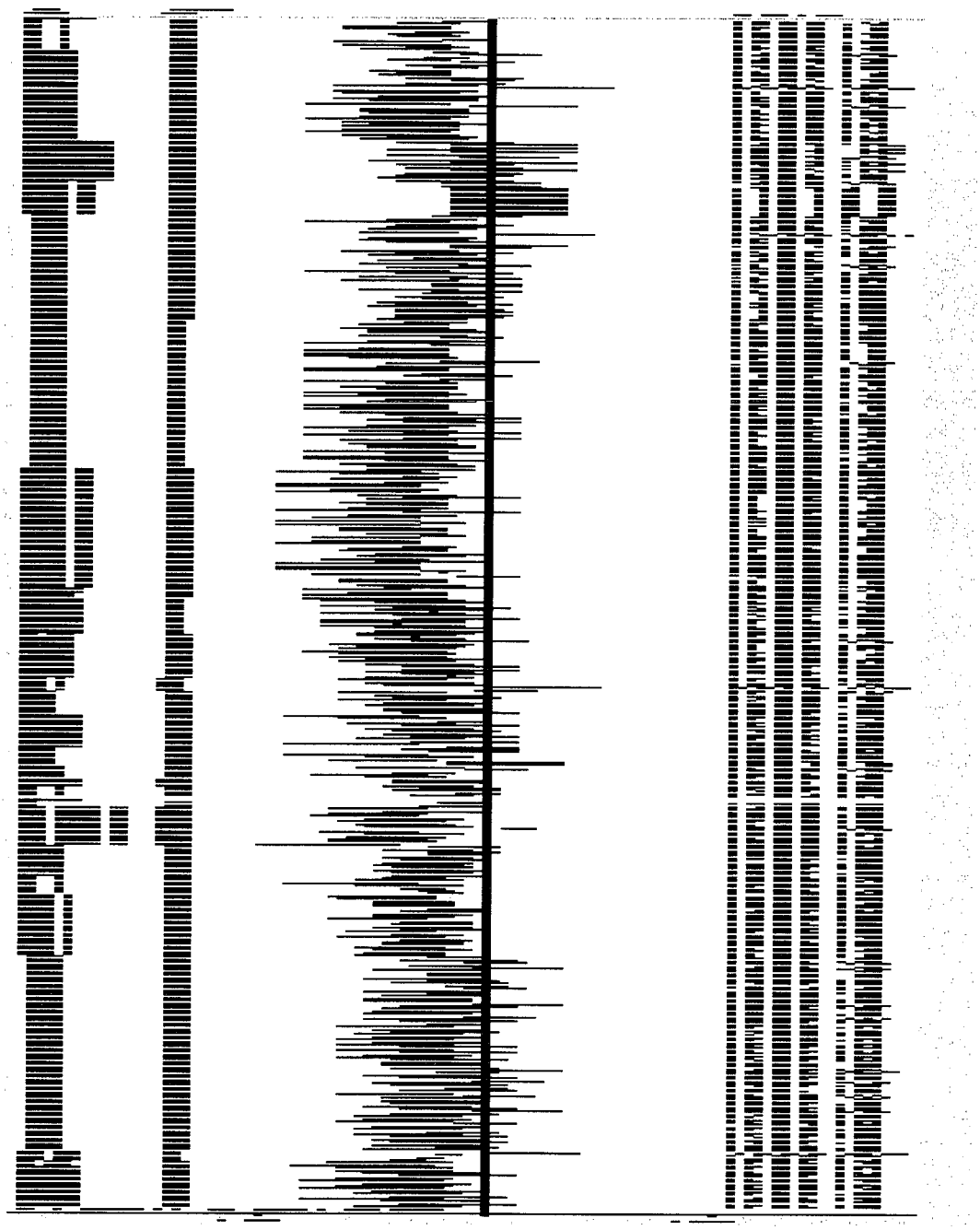

Figure 3.9.

Random effects meta-analysis of challenge trials reporting a prevalence outcome evaluating the effectiveness of undefined competitive exclusion products originating from a chicken source at reducing the odds of *Salmonella* colonization in broilers. Studies are stratified by time period and estimates of effect are presented as odds ratios (ORs).

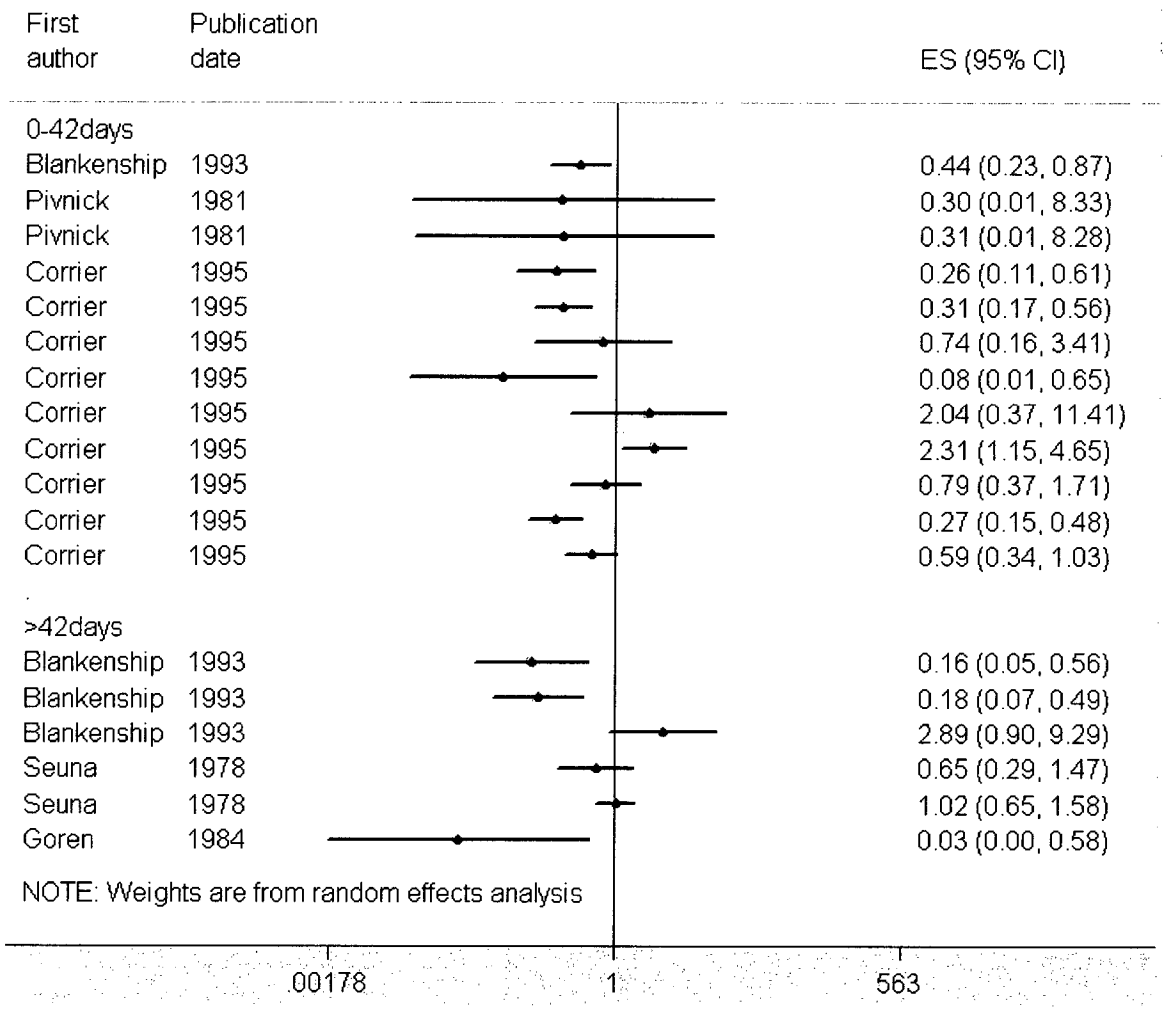

Figure 3.10.

Random effects meta-analysis of controlled trials reporting a prevalence outcome evaluating the effectiveness of undefined competitive exclusion products originating from a chicken source at reducing the odds of *Salmonella* colonization in broilers. Studies are stratified by time period and estimates of effect are presented as odds ratios (ORs).

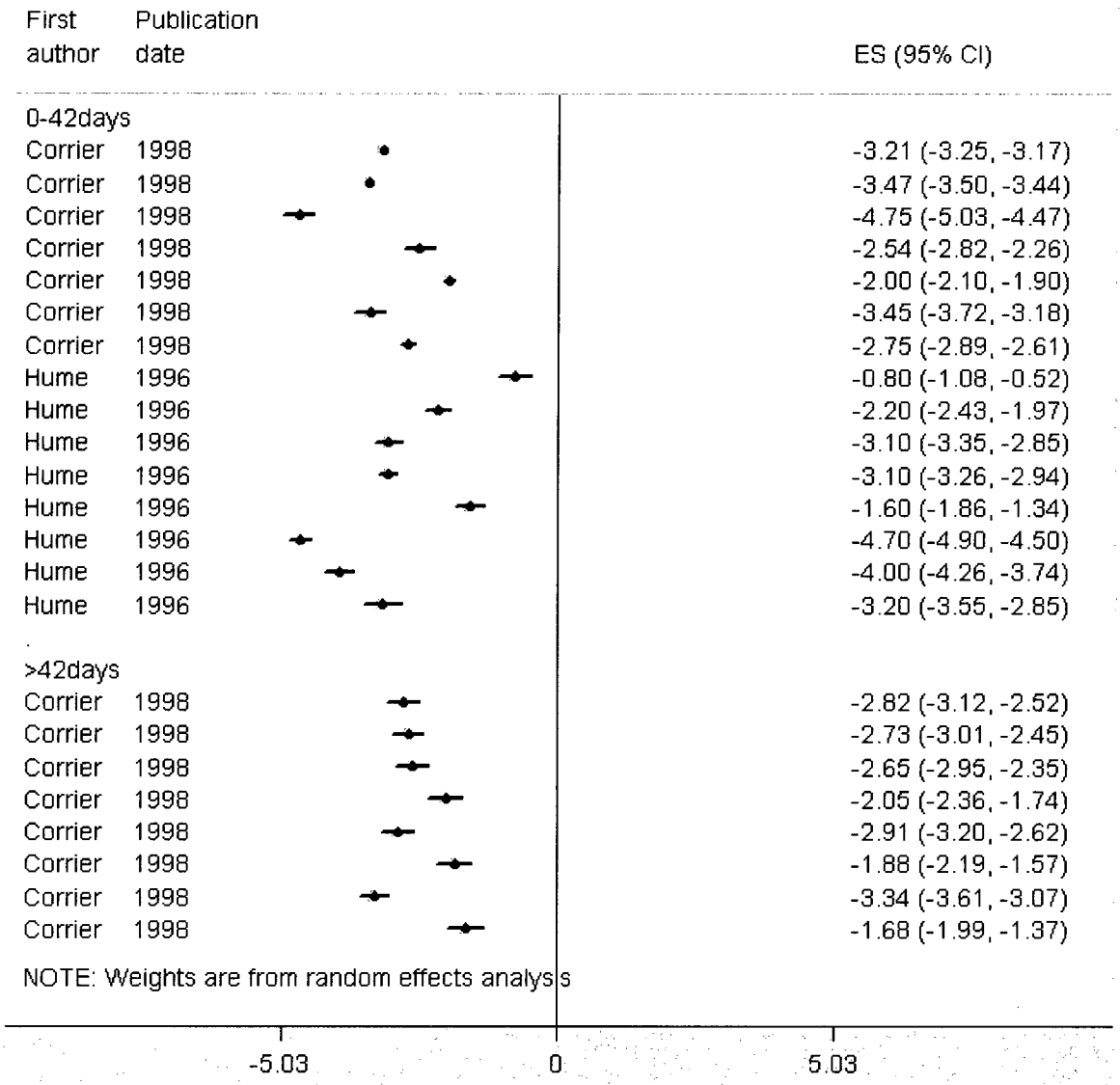

Figure 3.11.

Random effects meta-analysis of challenge trials reporting a concentration outcome evaluating the effectiveness of CF3 at reducing the concentration of *Salmonella* colonization in broilers. Studies are stratified by time period and estimates of effect are presented as the mean difference between the treated and untreated groups.

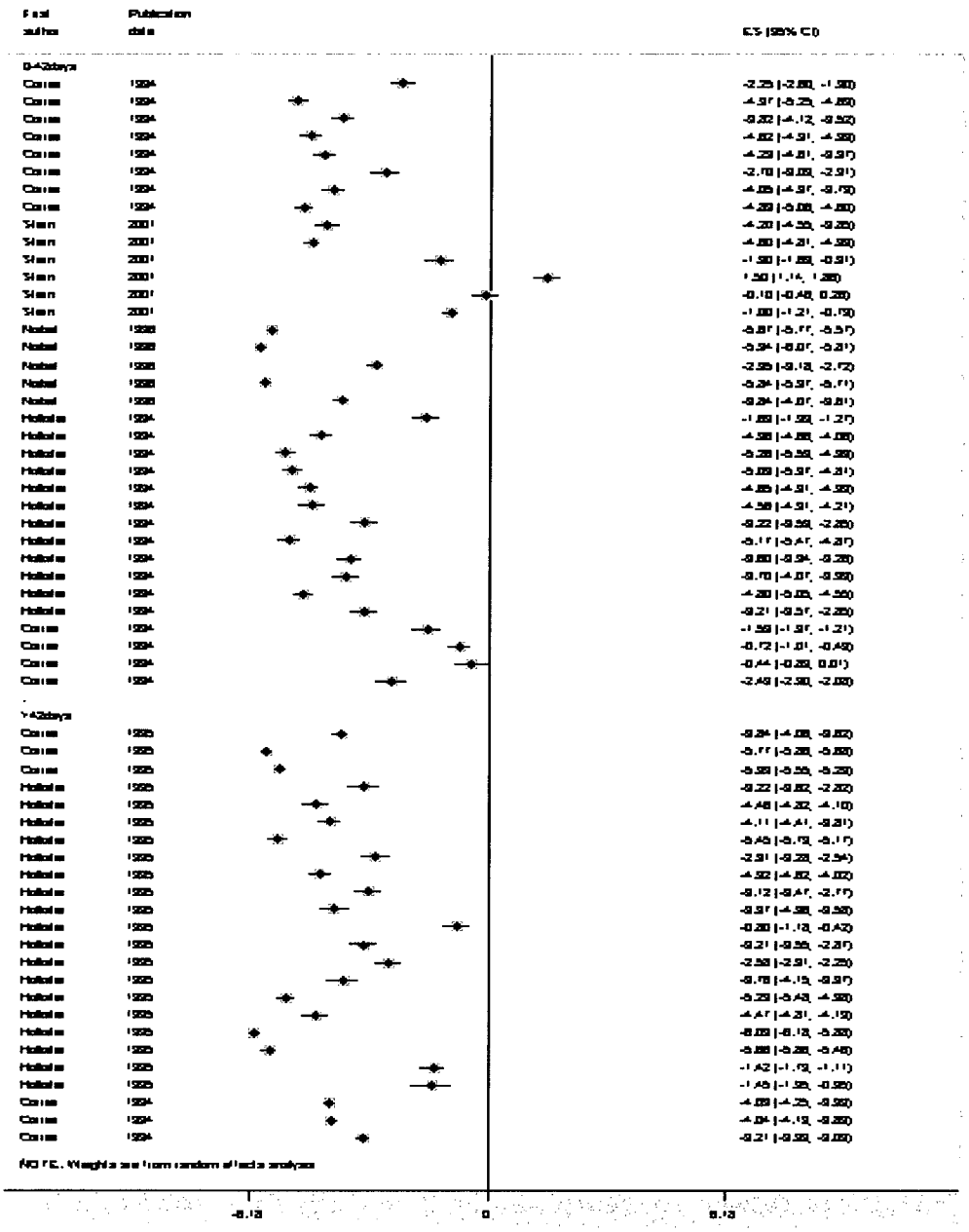

Figure 3.12.

Random effects meta-analysis of challenge trials reporting a concentration outcome evaluating the effectiveness of undefined competitive exclusion products originating from a chicken source at reducing the concentration of *Salmonella* colonization in broilers. Studies are stratified by time period and estimates of effect are presented as the mean difference between the treated and untreated groups.

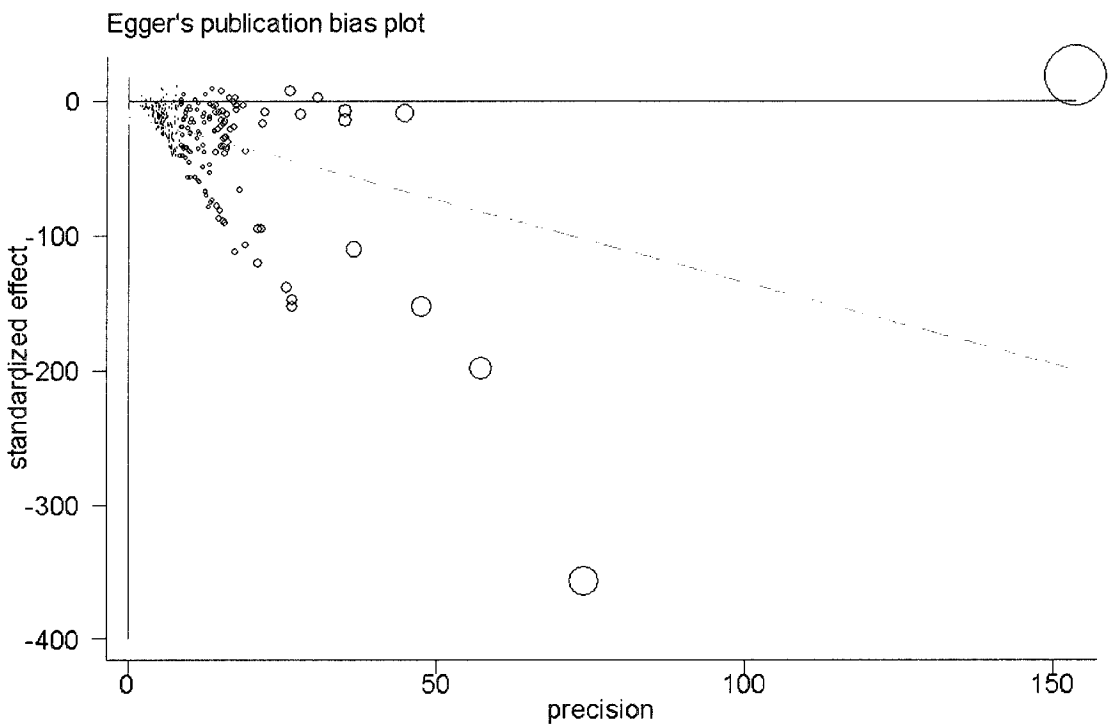

Figure 3.13.

Egger's test of publication bias plot for challenge trials evaluating the effectiveness of all competitive exclusion products at reducing the concentration of *Salmonella* colonization in broilers included in a systematic review of *Salmonella* in broiler chickens.

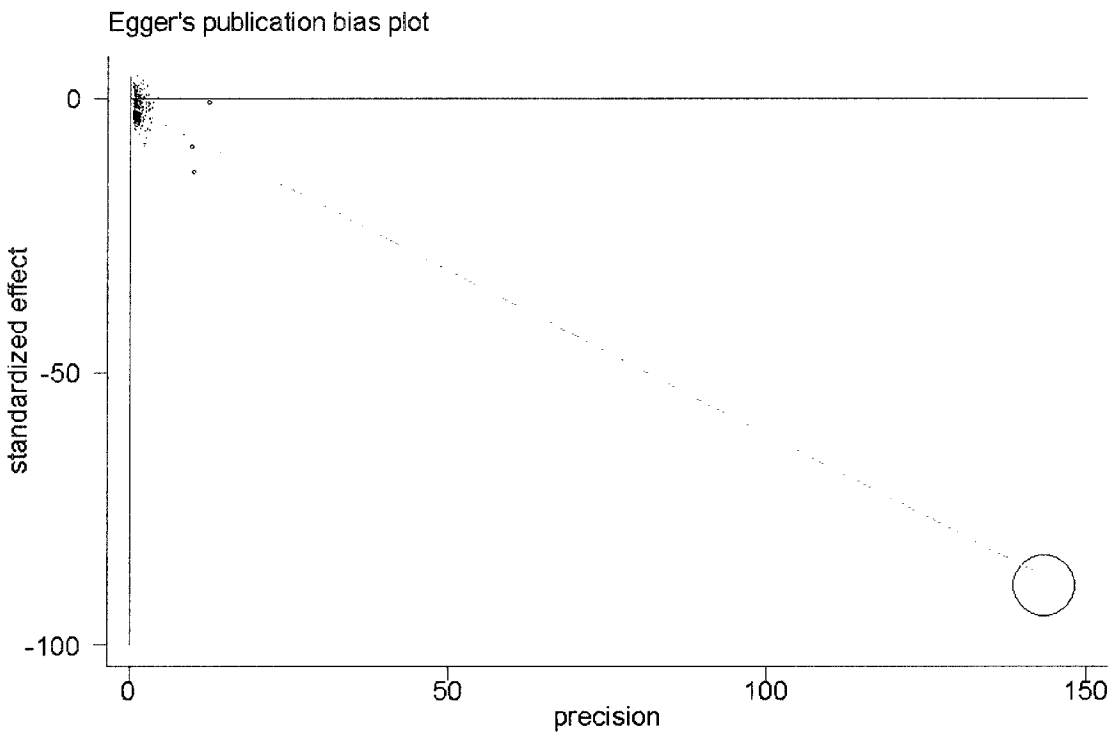

Figure 3.14.

Egger's test of publication bias plot for challenge trials evaluating the effectiveness of all competitive exclusion products at reducing the prevalence of *Salmonella* colonization in broilers included in a systematic review of *Salmonella* in broiler chickens.

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CHAPTER FOUR

SUMMARY DISCUSSION AND CONCLUSIONS

Worldwide, *Salmonella* is recognized as one of the most common causes of foodborne illness in humans (Mead et al., 1999; Wegener et al., 2003; Anonymous, 2007) and is often associated with the consumption of contaminated foods of animal origin (White et al., 1997). In Western countries, the most frequent cause of human salmonellosis is thought to be the result of inadequately processed, handled or cooked poultry products (Mead et al., 1999). Each year in Canada, approximately 6,000-12,000 cases of human salmonellosis are reported (Anonymous, 2006), however, these data have to be interpreted with caution because of high levels of underreporting (Todd, 1992; Todd, 1997; Mead et al., 1999; Campbell et al., 2003). The approaches used to control *Salmonella* in broiler chickens vary among regions and countries, and there is currently no mandated control program for broiler production in Ontario or Canada. Before various single or integrated control options are considered, it is important that the existing primary research for *Salmonella* in broilers is identified, critically evaluated and well-understood.

The use of research synthesis methods, such as systematic review (SR) and meta-analysis (MA) have gained momentum in microbial food safety (Sanchez et al., 2007; Sargeant et al., 2007; O'Connor et al., 2008; Waddell et al., 2008; Wilhelm et al., 2009; Young et al., 2009). Through a transparent and replicable review of all the available primary research, where each step is conducted by two independent reviewers, the most effective interventions could be identified along with gaps in knowledge and future research needs

(Sargeant et al., 2005; Sargeant et al., 2006; Borenstein et al., 2009). SR-MA is traditionally applied on a narrow and focused question. Scoping reviews, also known as evidence mapping, have been recently proposed as a potential framework for reviewing broad topics and characterizing the scope and characteristics of the diverse research underpinning such topics (Katz et al., 2003; Arksey and O'Malley, 2005; Anderson et al., 2008; Davis et al., 2009). This method, primarily used in health care and nursing sectors, offers appealing concepts for evaluating the broader microbial food safety topics, such as *Salmonella* in broiler chickens, within the context of evidence-based policy and decision-making.

The main objectives of this thesis were to apply these two research synthesis methods on *Salmonella* in broiler chickens. First, to identify, evaluate and summarize or synthesize the existing primary research on three sub-topics: interventions, risk factors, and prevalence of *Salmonella* in broiler chickens from farm-to-secondary processing using a scoping review, and to prioritize specific questions for rigorous SRs. Second, to select, based on the results of the scoping review and biological relevance, a single on-farm intervention and evaluate if the intervention is effective at reducing *Salmonella* colonization in broiler chickens using a SR-MA approach.

Through a scoping review (Chapter 2), the quantity, scope and characteristics of primary research on *Salmonella* in broiler chickens was determined and evidence maps were developed for three sub-topics (Chapter 2). The majority of primary research studied an intervention (748 studies), followed by prevalence (200) and risk factors (30). The

evidence maps clearly illustrated the scope and distribution of primary research between and within each sub-topic as well as the current knowledge gaps for researchers and funding agencies. These maps allowed the research team to successfully prioritize narrow and focused questions for rigorous SR, from an initial *a priori* developed list of potential questions (Appendix 3). For example, competitive exclusion (CE) was the most common farm intervention studied (192 studies), and was prioritized for rigorous SR. By contrast, a lack of primary research investigating the effectiveness of biosecurity practices, which are frequently recommended to producers, was observed. The review results were also evaluated to determine which data inputs might be useful for a complementary quantitative risk assessment (QRA). For example, four on-farm interventions, including CE, feed and water additives, vaccination and biosecurity were selected for rigorous SRs that should result in specific inputs for a QRA.

An in-depth SR-MA evaluating the effectiveness of on-farm use of CE resulted in a transparent evidence map describing various types of CE products (Chapters 2 and 3). Three main outcome types were reported in the CE studies; prevalence, concentration and infection/protection factor (IF/PF) values. While both prevalence and particularly, concentration are useful outcomes for MA, it was not possible to transform IF/PF values into anything useful for QRA so these studies were excluded from the review. As a result, the effectiveness of various types of CE products for reducing the prevalence and concentration of *Salmonella* in broiler chickens was quantitatively estimated using a MA approach. Overall, CE was effective at reducing *Salmonella* prevalence and concentration, throughout the lifespan of broiler chickens.

Potential associations between various individual study design and methodological soundness characteristics with the reported effectiveness of the treatment were assessed in a meta-regression (MR). Overall, the MR indicated that undefined CE products are more effective at reducing *Salmonella* colonization in broilers, except for a defined, continuous-flow culture, CF3. In addition, a variety of routes, including as an additive to feed and water were as effective at conferring protection as the most popular route studied, oral gavage.

Policy and decision-makers could use the results of this SR-MA to develop transparent and evidence-based guidelines for the on-farm use of CE for reducing *Salmonella* in broiler chickens and the industry may use this information to regulate the use of specific CE products in broiler chickens in North America. Broiler chicken producers may choose the methods of administration that are suitable to large populations. Researchers should further refine the development of defined products that are at least as effective as undefined products, and the specific characteristics that make CF3 more effective than other defined CE products should be better understood. The effect estimates generated for CE through SR-MA should be epidemiologically evaluated and transformed into useful evidence-based inputs that would allow risk assessors to compare various interventions within a specific context.

The evidence maps developed through the scoping review were beneficial for the review team because the areas with sufficient primary research could be identified for rigorous SRs. If existing primary research is large and sufficiently diverse, the SR could be

limited to certain study designs, periods or geographic locations, depending on the type of question. This may substantially reduce time for implementing the review or prevent significant waste of time associated with the conduct of a SR where little to no primary research exists.

The SR process involved methodological assessment of studies included in the review, to discriminate between acceptable and unacceptable primary research. Overall, the assessment of studies evaluating the effectiveness of CE products has shown poor conduct or reporting and supported recent initiatives advocating for the development and use of more stringent primary research reporting guidelines in animal health research (Sargeant et al., 2009a; Sargeant et al., 2009b). For example, in 28% of CE studies that reported use of random treatment allocation to broiler chicken populations, the actual method of treatment allocation was not reported. In addition, a lack of studies conducted in commercially representative settings was observed, with the majority of studies being challenge trials with small sample sizes. Large sample size controlled trials are needed for evaluating on-farm interventions for *Salmonella* in broilers under commercially representative conditions.

The scoping review-SR approach was fully reported in this thesis and each step, except the search strategy, was conducted by two independent reviewers. As a result, this study is transparent and may be replicated if necessary. Researchers in the veterinary public health arena interested in conducting a scoping review can learn and improve upon our experiences. For example, two levels of relevance screening in the scoping review and a

study design approach to methodological assessment and data extraction in in-depth SRs were found useful in the study. The review implementation timeline may also be used as a guide for future scoping reviews.

There are some limitations that pertain to this study. Due to time and funding restrictions, only English language papers were included in the scoping review. However, all non-English articles (n=242) were captured during the review, so if funding becomes available, it will be possible to translate these articles and up-date the review. Although the effect of excluding the non-English language studies is unknown, some research shows that, if anything, it leads to more conservative estimates of treatment effects (Juni et al., 2002). Despite extensive efforts to uncover all existing primary research with thorough search verification, we observed a statistical indication of publication bias in the MA assessing the effectiveness of CE with studies reporting a concentration outcome. This might suggest a lack of large studies reporting lower effectiveness of the CE products. It has been indicated that large, integrated poultry operations might have this data but keep it confidential (Sue Reynolds, Microbial Developments Ltd., Worcestershire, UK, personal communication) as they do not have incentive to publish trials, especially those with negative results.

This study has contributed to better understanding of the current state of evidence of *Salmonella* in broiler chickens. Government and industry stakeholders could use this approach for developing evidence-based guidelines for various on-farm interventions, particularly when the scientific recommendations are contradictory. Funding agencies

could use this information to support new primary research that would address knowledge gaps identified through both scoping review and SR. Results from this study might be used as inputs for a complementary QRA, which will compare various interventions within the Canadian (Ontario) context. Future research should evaluate the cost-effectiveness of various interventions prioritized in this thesis.

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Appendix 1. Relevance tool 1 (RS 1) for screening abstracts in a scoping review on *Salmonella* in broiler chickens

Relevance Criteria

1) Does this abstract describe primary research[1] in English[2]?
Y N (English lit review)[3] N Can't tell[4]

2) Does this abstract investigate *Salmonella* serovars of public health importance[5] in broilers[6] from farm to secondary processing[7]? Y N Can't tell[4]

3) Does this abstract investigate any of the following in broilers[6] from the farm to secondary processing[7]? (Check all that apply).

- ☐ The effectiveness of interventions[8] for reducing *Salmonella*
- ☐ Risk factors[9] for *Salmonella*
- ☐ *Salmonella* prevalence[10], contamination or concentration **inside of North America**[11]
- ☐ *Salmonella* prevalence[10], contamination or concentration **outside of North America**[11]
- ☐ Can't tell _____ (text box)
- ☐ None of the above

The goal of Relevance Tool 1 is to IDENTIFY potentially relevant studies on *Salmonella* in broilers from farm to secondary processing. This is generally evaluated by investigations into the effectiveness of interventions, association of risk factors, or measurement of prevalence, contamination or concentration levels.

Reviewer Decision

The following will be incorporated into the SRS system and will happen automatically: If the reviewer answers yes to questions 1 and 2 and any of 3 is checked (except none of the above or can't tell) the article will be included in RII for further screening and appraisal. If the reviewer answers can't tell to any of the questions 1 - 3, the full article will be obtained for further appraisal and decision making on RS 2.

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[1] Primary research represents a study where the authors collected and analyzed their own data.
[2] If the citation states that the article is in a language other than English, No should be selected.
[3] This should only be selected for abstracts that are English literature reviews.
[4] Reviewers should only use the "can't tell" option if the article may be relevant. If the article is obviously not-relevant, no should be selected. Full articles must be obtained for any can't tell responses.
[5] All *Salmonellas* are of public health importance unless the study only examined *S.* Pullorum and/or *S.* Gallinarum. If a study is measuring *Salmonella* spp. it should be included for further screening.
[6] If abstracts use a general term such as poultry, the answer should be yes. All abstracts that may include conventional chicken or broiler eggs intended for meat production or examination of raw chicken products should be answered yes. This question should be answered no if it is only about turkey, duck, retail eggs, or laying hens. Organic chicken products (free range, all natural, antibiotic free, antimicrobial free) will be answered yes only for prevalence studies.
[7] The farm to secondary processing level includes studies performed at breeding farms, hatcheries, grow-out farms, catching and transport to slaughter, live-bird supply to the slaughterhouse (lairage), all slaughter,

evisceration, wash, and chilling activities up to secondary processing. Secondary processing includes cut-up, de-boning, partitioning and grinding of raw chicken carcasses. Third processing is out of scope of this project, and includes marination, coating, frying, smoking, grill marking and formulations (heat and eat). Freezing, packing and storage of raw chicken products and retail is out of scope of this project. Samples such as faecal, cloacal, crop and skin swabs should be included. Environmental samples such as litter, feed and water should not be included. Serology samples should not be included.

[8] Studies that use a controlled/challenge trial are considered intervention studies. Occasionally studies may use a cohort study to evaluate an intervention. Studies that use a cohort study to evaluate an intervention should be classified as an intervention.

[9] Studies that use observational studies should be classified as risk factor studies, not intervention studies.

[10] This selection is only for studies whose objective is to evaluate prevalence in broilers from farm-to-secondary processing. This includes group prevalence and within group prevalence. Studies that only evaluate *Salmonella* prevalence before and after an intervention should be placed in the intervention selection, and not the prevalence selection. Exclude studies that only evaluate the persistence or prevalence after artificial infection.

[11] If the origin of the research is unknown, please choose can't tell.

Appendix 2. Relevance tool 2 (RS 2) for classifying papers in a scoping review on *Salmonella* in broiler chickens

Relevance Criteria

1) Does this abstract investigate the **effectiveness of an intervention(s)** that reduces *Salmonella*[1] in broilers? Y N

If yes, please categorize by point in chain and intervention. Check all that apply.

	Farm [2]	Transport [3]	Processing [4]	Beyond Processing [5]	Other (Specify below)	Can't tell
Vaccination[6]						
Antimicrobials[7]						
Other feed and water additives[8]						
Competitive exclusion[9]						
Bacteriophage and bacteriocins[10]						
Feed withdrawal[11]						
Biosecurity[12] (Specify below)						
Scalding/defeathering [13]						
Reprocessing[14]						
Treatment spraying or dipping carcasses[15]						
Chilling[16]						
Final wash[17]						
Other (Specify below)						
Can't tell						

Other: _____

Biosecurity: _____

2) Does this abstract investigate **risk factors** or **prevalence[18], contamination, or concentration** of *Salmonella*[1] in broilers? Y N

If yes, which segment of the production chain are **risk factors, prevalence, contamination or concentration** measured? Check all that apply.

	Risk Factors	Prevalence[18]	Can't tell
Farm[2]			
Transport[3]			
Processing[4]			
Beyond Processing[5]			
Can't tell			

3) If the answer to question 2 is yes, what time period[19] does the study represent?
- ☐ 1990 - present
- ☐ 1980 - 1989
- ☐ 1960 - 1979
- ☐ Before 1960
- ☐ Not reported[20]

4) If the answer to question 2 is yes, what type of publication is it?
- ☐ This is a prevalence study[21]
- ☐ This is an active surveillance report[22]
- ☐ This is a passive surveillance report[23]
- ☐ None of the above

5) If the answer to question 2 is yes, what outcome(s) are measured? Check all that apply.
- ☐ Group prevalence[24]
- ☐ Within group prevalence[25]
- ☐ Concentration
- ☐ Multi-stage prevalence[26]
- ☐ Environmental or water contamination
- ☐ This is only a risk factor study (no prevalence data reported)
- ☐ Other (Specify below)
- ☐ Can't tell

6) If the answer to question 2 is yes, what continent(s) is the estimate from?
- ☐ Africa
- ☐ Antarctica
- ☐ Asia
- ☐ Australia
- ☐ Europe
- ☐ North America
- ☐ South America

7) If the answer to question 2 is yes, are some or all of the results from organic animal production?

☐ Yes

☐ No

The goal of Relevance Tool 2 is to CATEGORIZE potentially relevant studies that study *Salmonella* interventions, risk factors, or prevalence, contamination or concentration levels in broilers.

Reviewer Decision

The following will be incorporated into the SRS system and will happen automatically: If the reviewer answers yes to questions 1 or 2 the article will be included for further screening and appraisal.

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[1] This should be answered yes if a study measures *Salmonella* (outcome) infection, colonization, prevalence, contamination or concentration. Outcomes must be measured in live chicken, or raw chicken products (including egg shell, fluff, feather and faecal samples). Faecal samples were only included if they could be linked to the flock/bird being sampled. The following types of studies should be excluded: studies that only measure environmental samples (such as litter) without measuring the outcome in the broiler, studies that only measure the outcome using serology/antibody detection, and studies that measure featherless birds as an intervention.

[2] Farm includes breeding, hatchery and grow-out farms.

[3] Transport involves catching and transport to slaughter.

[4] Processing includes both primary and secondary processing. Secondary processing includes cut-up, de-boning, partitioning and grinding of raw chicken carcasses up to the point of freezing.

[5] This should be selected for studies that are in third processing (including marination, coating, frying, smoking, grill marking and formulations (heat and eat) and retail studies.

[6] May be a killed or live vaccine. May be oral or injectable.

[7] Examples include: Flouroquinolones, cephalosporins, gentamicin, ampicillin, tetracyclines, spectinomycin, ciprofloxacin, ceftriaxone. These are often administered via feed.

[8] May include organic acids, sodium chlorate, sodium nitrate, formaldehyde, and propionic acid. Water supplies may be treated with acidic oxidizing agents (such as hydrogen peroxide, peracetic acid, lactic acid, volatile fatty acids (formic, acetic, propionic, butyric), sodium chlorate, sodium nitrate).

[9] May also be referred to as probiotics, prebiotics, synbiotics or the 'Nurmi concept'. May include Lactobacillus spp. (L. salivarius, L. acidophilus, L. casei, L. reuteri, L. plantarum), bacteroides, Bifidobacterium spp., Enterococcus faecium, Aspergillu oryzae, and Saccharomyces spp. (S. cerevisiae, S. boulardii). May be cecal contents or other materials from birds or the environment that contain many different or unknown bacterial species.

[10] Bacteriophages are viruses that can infect, multiply, and kill susceptible bacteria. Bacteriocins are a heterogeneous group of peptides produced by certain types of bacteria that are active against other (often closely related) bacterial strains. May include E. faecium and nisin.

[11] Withholding feed (and sometimes water) on farm often occurs prior to transport to allow clearance of the GI tract and to reduce visible contamination. This is a common practice and should only be included if it is used differentially between comparison groups.

[12] Biosecurity includes, but is not limited to, sanitation, biosafety, disinfection (and other antimicrobials), hygiene and hygiene barriers, all-in-all-out production, depopulation, monitoring pathogen levels in animals, staff and the environment, litter testing and treatment, removal of contaminated material, mice/pest/rodent/insect control, reduce water pooling and increasing water drainage. Lime may be used in litter or entrances.

[13] Also includes defeathering, plucking and picking. May have a single or multiple scald tanks.

[14] Removal of visibly contaminated birds with faecal matter. The birds are hand washed, and re-added to the slaughter line.

[15] Spraying or washing carcasses with a treatment such as an antimicrobial solution (which may include chlorine or trisodium phosphate). Differs from final wash in that the addition of this type of intervention is to treat the carcass as opposed to washing the carcass which is common practice within poultry production. Steam pasteurization may also be used.

[16] Includes studies on chillers and chill water. May be air or immersion chilling. Interventions such as chlorine or trisodium phosphate may be added to immersion water.

[17] A routine poultry production practice that includes spraying or washing carcasses with water. It is often referred to as inside/outside bird washer. Often occurs before chilling, but in some instances it is done before and/or after chilling. Common interventions include temperature, time, or chlorine levels.

[18] In order to be classified as a prevalence study the paper must provide: a numerator, denominator, and the point in chain it was measured at. If a paper does not report one or more of these, it is not a prevalence study.

[19] If a study spans over more than one category (for example, from 1985-1995), both categories should be selected.

[20] Not reported should only be selected after thorough review of the paper.

[21] An observational study that samples within defined sampling and time frames to capture prevalence estimates for a target population using a defined sampling protocol.

[22] An on-going program designed to purposively sample a target population using a defined sampling protocol. If only serovar distribution is reported (without prevalence estimates), the study should be placed in none of the above.

[23] The use of routine (e.g. volunteer, good will, spontaneous or ad hoc) sample submission for diagnostic testing of *Salmonella*. If only clinical cases, such as disease outbreaks, are reported, the study should be placed in none of the above.

[24] The proportion of groups (e.g. flocks, batches) containing 1 or more *Salmonella* positive sample.

[25] The number of samples within a group that are *Salmonella* positive.

[26] A study that samples at more than one point in time in the farm to processing continuum to examine the level of Salmonella contamination. The study may be single point in time or longitudinal in design.

Appendix 3. List of *a priori* identified potential focused questions for rigorous systematic reviews

What interventions have been identified as effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?

i. Is competitive exclusion effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
ii. Is vaccination effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
iii. Are other feed and water additives effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
iv. Are antimicrobials effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
v. Is feed withdrawal effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
vi. Are biosecurity practices effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
vii. Are bacteriophage/bacteriocins effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
viii. Is scalding effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
ix. Is reprocessing effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
x. Is treatment spraying or dipping of carcasses effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
xi. Is chilling effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?
xii. Is the final wash effective at controlling *Salmonella* contamination in broilers and raw chicken products from farm-to-secondary processing?

What are the risk factors for *Salmonella* colonization in broilers?

i. What are the risk factors for *Salmonella* colonization in broilers inside of North America?
ii. What are the risk factors for *Salmonella* colonization in broilers outside of North America?

What are the prevalence and concentration levels of *Salmonella* of public health importance in commercial farm and abattoir settings?

i. What are the prevalence and concentration levels of *Salmonella* of public health importance in commercial farm and abattoir settings inside of North America?

ii. What are the prevalence and concentration levels of *Salmonella* of public health importance in commercial farm and abattoir settings side of North America?

Appendix 4. Methodological assessment of relevant papers in a scoping review on *Salmonella* in broiler chickens for rigorous systematic review

QUESTION	CODING	RESPONSE OPTIONS AND DEFINITIONS	APPLICABLE STUDY DESIGNS
RefID			Included in SRS
1) What is the study design specified by the author?	1 Controlled trial (CT) 2 Quasi-experiment (QE) 3 Challenge trial (ChT) 4 Cohort study 5 Case-control study (C-C) 6 Cross-sectional study (XS) 7 Repeated cross-sectional study 8 Prevalence survey 9 Longitudinal prevalence 10 Other (please specify) 11 Not reported	The exact study design should be specified in the article (title, keywords, abstract or methods). **CT**: A planned experiment with natural disease exposure. May or may not be randomized. Lab trial = executed under highly controlled conditions. Field trial = executed under less controlled more "real" conditions . **Quasi-experiment**: Before and after trials, including prevalence measures at various points (before and after one or more stages) in the farm to processing continuum. **Challenge trial**: A planned experiment where subjects are artificially challenged or exposed to the disease agent. Lab trial = executed under highly controlled conditions. Field trial = executed under less controlled more "real" conditions. **Cohort study**: A group of animals exposed to a hypothesized risk factor (exposure), and a group not exposed to the factor are selected and observed over the study period to record development of disease in each group. **Case-control study**: A group of diseased animals and a group of non-diseased animals are selected and compared with respect to the presence of the hypothesized risk factor (exposure). **Cross-sectional study**: A study done at a single point in time to investigate the prevalence and distribution of disease and hypothesized risk factors within the population. **Repeated cross-sectional study:** A study that measures prevalence and distribution of disease and hypothesized risk factors at multiple points in time.	All

		Prevalence survey: A study that measures outcome (prevalence and distribution of disease only) at a single point in time. **Longitudinal prevalence:** A study that measures outcome (prevalence and distribution of disease only) at multiple points in time on the same population. **Other:** Hybrid or other designs that are clearly stated in the article (title, keywords, abstract, methods). **Not reported:** For articles that did not report a specific study design (or used generic descriptors such as, "observational study" or "trial").	
2) What is the study design as identified by the reviewer?	1 Controlled trial (CT) 2 Quasi-experiment (QE) 3 Challenge trial (ChT) 4 Cohort study 5 Case-control study (C-C) 6 Cross-sectional study (XS) 7 Repeated cross-sectional study 8 Prevalence survey 9 Longitudinal prevalence 10 Other (please specify)	Study design choice determines the sections to be answered on this QA tool. **CT**: A planned experiment with natural disease exposure. May or may not be randomized. Lab trial = executed under highly controlled conditions. Field trial = executed under less controlled more "real" conditions. **Quasi-experiment**: Before and after trials, including prevalence measures at various points (before and after one or more stages) in the farm to processing continuum. **Challenge trial**: A planned experiment where subjects are artificially challenged or exposed to the disease agent. Lab trial = executed under highly controlled conditions. Field trial = executed under less controlled more "real" conditions. **Cohort study**: A group of animals exposed to a hypothesized risk factor (exposure), and a group not exposed to the factor are selected and observed over the study period to record development of disease in each group. **Case-control study**: A group of diseased animals and a group of non-diseased animals are selected and compared with respect to the presence of the hypothesized risk factor (exposure). **Cross-sectional study**: A study done at a single point in time to investigate the prevalence and distribution of disease and hypothesized risk factors within the	All

		population. **Repeated cross-sectional study:** A study that measures prevalence and distribution of disease and hypothesized risk factors at multiple points in time. **Prevalence survey:** A study that measures outcome (prevalence and distribution of disease only) at a single point in time. **Longitudinal prevalence:** A study that measures outcome (prevalence and distribution of disease only) at multiple points in time on the same population.	
3) Was the sample size justified?	0 No 1 Yes	**No**: No details in the text regarding how sample size was determined or the author describes informal guesses of sample size. **Yes**: Use of sample-size formulas, based on desired power or precision and estimate of expected variability to detect differences, or the author justified the sample population as the census population/maximum accessible.	All
4) How were operations (hatchery/farm/ processor) selected to participate in this study? ***(In the text box indicate which operation, if more than one were used in the study).***	1 Whole registry..... 2 Random..... 3 Reported random..... 4 Systematic..... 5 Convenience..... 6 Not applicable	**Whole registry:** Operations were chosen through a registry (such as farm or disease). **Random:** Computer or random numbers table, a priori, stratified random sample, cluster random sample. **Reported random:** Author indicates random, but randomization is not explained. **Systematic:** Taking n samples at interval of x. **Convenience:** Participants were identified by personal contacts or responded to a survey, or it was not described in the paper. **Not applicable:** Hatchery/farm/processor was not selected to participate in this study or this is a lab based CT or ChT.	Field trials (CT, ChT) Quasi-experimental Case-control Cohort Cross-sectional Repeated cross-sectional Prevalence survey Longitudinal prevalence
5) Within the operations, was batch, flock, pen or cage selection described and justified? *(In the text*	1 Random..... 2 Reported random..... 3 Systematic..... 4 Convenience..... 5 Not applicable	**Random:** Computer or random numbers table, a priori, stratified random sample, cluster random sample. **Reported random:** Author indicates random, but randomization is not explained. **Systematic:** Taking n samples at interval of x. **Convenience:** Participants were identified by personal contacts or responded to a survey, or it was not described	Field trials (CT, ChT) Quasi-experimental Case-control Cohort Cross-sectional Repeated cross-

box indicate which operation, if more than one were used in the study).		in the paper. **Not applicable:** Batch/flock/pen/cage was not selected to participate in this study or this is a lab based CT or ChT.	sectional Prevalence survey Longitudinal prevalence
6) Within the operations, was egg, broiler, or carcass selection described and justified? *(In the text box indicate which operation, if more than one were used in the study).*	1 Random..... 2 Reported random..... 3 Systematic..... 4 Convenience..... 5 Not applicable	**Random:** Computer or random numbers table, a priori, stratified random sample, cluster random sample. **Reported random:** Author indicates random, but randomization is not explained. **Systematic:** Taking n samples at interval of x. **Convenience:** Participants were identified by personal contacts or responded to a survey, or it was not described in the paper. **Not applicable:** Egg/broiler/carcass was not selected to participate in this study.	All
7) Was the representativeness of the sample population to the target population explained and sufficiently justified?	0 No 1 Yes 2 Not applicable	**No:** No explanation of external validity of results was given or only a selected group was used and thus, the sample may not be representative. **Yes:** At least some information provided to reflect the representativeness of the sampled population and the target population. **Not applicable:** This is not a Field CT/ChT, QE, C-C, cohort, XS, Repeated XS, Prevalence survey, or Longitudinal prevalence study.	Field trials (CT, ChT) Quasi-experimental Case-control Cohort Cross-sectional Repeated cross-sectional Prevalence survey Longitudinal prevalence
8) Were the birds housed, grouped or slaughtered in a way that is representative of field conditions?	0 No 1 Yes	**No:** Birds were housed or grouped in small densities, not similar to field conditions, or birds were housed or grouped individually (usually due to the design of the study: lab CT/ChT). **Yes:** Birds were housed in densities, cages and enclosures representative of field conditions.	Field trials (CT, ChT) Quasi-experimental Case-control Cohort Cross-sectional Repeated cross-sectional

			Prevalence survey Longitudinal prevalence
9) Was a clear case definition given and case eligibility properly assessed?	0 No 1 Yes 2 Not applicable	**No:** A case definition and/or an assessment of case eligibility were not described. **Yes:** A clear case definition was provided and the cases were assessed at the beginning or upon entry into the study to confirm their outcome or disease status. **Not applicable:** This is not a C-C study.	Case-control
10) Were the controls selected from the same source population as the cases?	0 No 1 Yes 2 Not applicable	**No**: Not described in text. **Yes**: Controls were selected from the same study base population. **Not applicable:** This is not a C-C study.	Case-control
11) Were the exposure and/or risk factors sufficiently described and measured appropriately? ***(Please indicate which answer is for Exposure vs. Risk factor if they are different)***	0 No…… 1 Measured by researcher…….. 2 Questionnaire…….. 3 Not applicable	**No:** The exposure and/or risk factors were not fully described and/or measured appropriately. **Measured by researcher:** The exposure and/or risk factors were described fully and measured by the researcher. May be examined retrospectively, upon entry into the study, or at the same time as the outcome. **Questionnaire:** The exposure and/or risk factors were described fully and measured through questionnaire administration. **Not applicable:** This is not a C-C, cohort, XS, or repeated XS study.	Case-control Cohort Cross-sectional Repeated cross-sectional
12) How was the intervention assigned to the experimental unit?	1 Random 2 Reported random 3 Systematic 4 Convenience 5 Not applicable	**Random:** Computer or random numbers table, a priori. **Reported random:** Author indicates random, but randomization is not explained. **Systematic:** Taking n samples at interval of x. **Convenience:** Assignment is not described in the paper. **Not applicable:** This is not a CT or ChT.	CT Challenge trial
13) Were the intervention	0 No 1 Yes	**No:** Necessary information is missing. **Yes:** Methods are thoroughly described and allow for replication.	CT Challenge trial

protocols described in sufficient detail to allow reproduction of the experiment?	2 Reference paper 3 Not applicable	**Need to find referenced paper:** Methods are referenced in another paper. **Not applicable:** This is not a CT, ChT or QE.	Quasi-experimental
14) Was an appropriate control group used?	0 No 1 Yes, concurrent control group 2 Yes, historical control group 3 Before and after trial 4 Not applicable	**No:** Controls are from a different sampling frame (different flock or group) or no control group was used in the study. **Yes, concurrent:** Controls are drawn from same sampling frame (same flock or group) and are measured in the same timeframe as treatment group. **Yes, historical:** Controls are drawn from same sampling frame (same flock or group), however, they were measured in an earlier timeframe than the treatment group, and are not the same broilers as treatment group. **Before and after trial:** The study uses the same broiler(s) as its own control. **Not applicable:** This is not a CT, ChT, QE, or cohort study.	CT Challenge trial Quasi-experimental Cohort
15) Was the challenge protocol adequately described so that the challenge could be reproduced?	0 No 1 Yes 2 Reference paper 3 Not applicable	**No**: Some details are missing or not reported. **Yes**: Reported challenge organism, route of administration, dosage, and frequency of administration. **Need to find referenced paper:** Protocol is referenced in another paper. **Not applicable:** This is not a ChT.	Challenge trial
16) Was the time from intervention administration to first measurement of outcome reasonable for the intervention to have	0 No 1 Yes 2 Not applicable	**No:** The study did not allow enough time between intervention and outcome measurement. **Yes**: Study allows enough time to observe the outcome of interest after the intervention is performed, or the intervention did not require the passage of time before the outcome could be measured. **Not applicable:** This is not a CT, ChT or QE.	CT Challenge trial Quasi-experimental

had an effect? *(See guidelines if unsure)*			
17) Was the sample population tested for *Salmonella* status/prevalence at the beginning of the study?	0 No 1 Yes 2 Not applicable	**No:** Baseline was not established, comparison groups were not equal, or it was not described in the paper. **Yes:** Baseline was established and comparison groups were equal prior to intervention administration. **Not applicable:** This is not a CT, ChT, QE, or Cohort study. For ChT, N/A applies if a uniquely detectable strain was used (e.g., using "lactose-negative, NA-resistant *Salmonella* colonies" since it is a very rare strain). Field strain challenges require testing prior to intervention administration, as these cannot be distinguished from naturally occurring infections.	CT Challenge trial Quasi-experimental Cohort
18) Were laboratory methods used to determine the outcome described sufficiently to allow replication of the study?	0 No 1 Yes 2 Reference paper	**No:** Not sufficiently reported to be able to reproduce the study without contacting the author. **Yes:** Methods are reported in sufficient detail (e.g., media, time, temperature) to allow for replication. **Need to find referenced paper:** Methods are referenced in another paper.	All
19) Did the author report that blinding was used?	0 No 1 Yes 2 Not applicable	**No:** No blinding was reported. **Yes:** The author reported that blinding was used. **Not applicable:** This is not a CT, ChT, QE, C-C, Cohort, or Longitudinal prevalence study.	CT Challenge trial Quasi-experimental Case-control Cohort Longitudinal prevalence
20) Were the reasons for, and the proportion of, prospective participants that	0 No 1 Yes 2 Not applicable	**No:** The proportion of non-response and/or reasons were not stated. **Yes:** The proportion of, and reasons for non-response are stated clearly. **Not applicable:** This is not a Field CT/ChT, QE, C-C, Cohort, XS, Repeated XS, Prevalence survey, or	Field trials (CT, ChT) Quasi-experimental Case-control Cohort Cross-sectional

declined participation reported?		Longitudinal prevalence study.	Repeated cross-sectional Prevalence survey Longitudinal prevalence
21) Were mortality, withdrawals and/or loss to follow-up <15%?	0 No 1 Yes	**No:** Only numbers or reasons were reported, or numbers were reported but were greater than 15%. **Yes**: Numbers stated or deducible from tables are less than 15% and/or reasons provided for losses. There was no attrition, or this was a XS, repeated XS, or prevalence survey. Neither numbers nor reasons were reported.	CT Challenge trial Quasi-experimental Case-control Cohort Longitudinal prevalence
22) Was the statistical analysis described adequately so it can be reproduced?	0 No 1 Yes 2 Reference paper 3 Statistical analysis not done	**No:** Methods and adjustments are not clear or some details are missing. **Yes**: The methods were reported in sufficient detail to understand the statistical approach and reasoning. **Need to find referenced paper:** Methods are referenced in another paper. **Statistical analysis not done:** No statistical analysis was done.	All
23) Were identified confounders controlled for or tested?	0 No 1 Yes, analysis 2 Yes, inclusion/exclusion 3 Yes, matching 4 Not applicable	**No:** No adjustment was made for confounders/effect modifiers etc. that were identified by the author. **Yes:** Confounders were identified by the author and tested in the analysis (for example, Mantel-Haenszel, logistic regression) for their impact on the outcome, or taken care of in inclusion/exclusion criteria or by matching. **Not applicable:** There were no confounders identified by the author in this study, or the study used randomization to control for confounders.	All
24) Based on the study design, was clustering accounted for	0 No 1 Yes 2 Not applicable	**No:** The data is clustered (group hierarchy, repeated measures or multiple replicates exist and data is pooled/collapsed), and clustering was not controlled for. **Yes:** Clustering may be as a result of *group hierarchy,*	All

appropriately in the analysis?		*repeated measures, or multiple replicates.* Clustering was present and was accounted for (e.g. fixed or random effect in the model, GEE, GLMMs, MCMC estimation, robust variance estimation, linear mixed model, overdispersion factor, ANOVA, Bayesian mixed models). *Group hierarchy:* Data is clustered by group, for example, farm or pen. *Repeated measures:* Several measurements of an outcome are taken on the same unit of observation over a period of time. *Multiple replicates:* The experiment was conducted more than once. **Not applicable:** There was no clustering present or group hierarchy, repeated measures or multiple replicates exist, but data is not pooled/collapsed.	
25) Was raw or unadjusted data provided?	0 No 1 Yes	**No:** Raw results were not provided or were not presented in an extractable format: median, range, graph with no measure of variability. **Yes**: Raw data is presented in an extractable format or unadjusted summary estimates (e.g. mean, OR, RR) and measure of variability (e.g. SE, SD, CI) were provided.	All
26) Were adjusted estimates and measures of variability presented?	0 No 1 Yes 2 Not applicable	**No:** A multivariable model was used, but results were not provided or the results are not presented in an extractable format (e.g. in a graph). **Yes**: A multivariable model was used and parameter estimates (e.g. OR, RR) and measure of variability (e.g. SE, SD, CI) are provided after adjusting for other variables, confounders and/or clustering. **Not applicable:** No multivariable model was used.	All
27) Were model diagnostics presented?	0 No 1 Yes 2 Not applicable	**No:** Model diagnostics or sensitivity was not presented. **Yes:** Model diagnostics or sensitivity or evaluation of model robustness was presented (e.g. Residuals, AIC/BIC, Deviance, ROC/AUC, Hosmer-Lemeshow test, likelihood ratio test, sensitivity analysis). **Not applicable:** No model was presented in the results.	All
28) Additional	Text box		

comments *(If the reviewer feels there is something that was not captured in the tool but should be acknowledged in QA)*			

Appendix 5. Data extraction form for prioritized intervention studies identified in a scoping review on *Salmonella* in broiler chickens for rigorous systematic review

GENERAL INFORMATION		
Variable	**Category**	**Explanation**
Ref ID from SRS	.....	
Journal name	.....	
Author(s) name	.....	
Publication year	.....	
Publication type	a. Peer reviewed b. Conference proceeding c. Thesis d. Government or research station report e. Other (please specify)	
Country/region/province/state where the study was carried out.	a. ..... b. Not reported	If the author does not indicate in the paper, please use author affiliation. If author affiliation indicates more than one country/region/province/ state, please record all.
In what year(s) was the data collected?	a. ..... b. Not reported	Please do not use publication date to answer this question.
Institution(s) that funded the study	a. ..... b. Not reported	PLEASE DO NOT USE AUTHOR AFFILIATION TO ANSWER THIS QUESTION.
What is the study design? ** *When more than one trial is reported in a paper indicate in the text box abbreviations for multiple trials (i.e. T1, T2...Tn) and follow these abbreviations throughout the data extraction process.*	a. Controlled trial..... b. Challenge trial..... c. This is not an experimental design. **Please stop reviewing and contact Ashley Farrar**	**Trial:** One of a number of repetitions of an experiment. **Controlled trial:** A planned experiment with natural disease exposure. May or may not be randomized. **Challenge trial:** A planned experiment where subjects are artificially challenged or exposed to the disease agent.
POPULATION – DESCRIBE THE POPULATION STUDIED		
Age-group(s) of the study population	a. Broiler eggs..... b. Day old chickens..... c. Broilers (indicate age/size)... d. Breeding poultry..... e. Carcass..... f. Other..... g. Not reported	**Broiler eggs:** are eggs intended for hatcheries that produce broiler chickens. **Day old chickens:** are from hatching to 72 hours old. **Broilers:** are chickens, usually 6-8 weeks old and 3-5 pounds, raised primarily for meat. **Breeding poultry:** include broiler egg laying hens and cocks. **Carcass:** include whole carcasses, part of the carcass or skin from carcasses. Please indicate in the text if these are not dead broiler chickens (e.g. spent hens). **Other:** Please specify

What is the breed(s) of broilers sampled in this study?	a..... b. Not reported	Please specify as reported by the author.
Farm (if applicable)		
If farm level, what was the setting?	a. Commercial farm b. Research farm c. Research facility	a. **Commercial farms** include operations rearing broilers in a commercial setting. b. **Research farms** include operations affiliated with Universities and/or research organizations. Usually they are smaller farms with high biosecurity standards and do not resemble commercial farms. c. **Research facility** is an artificial "lab" environment.
If farm level, what is the type of farm sampled? If research farm/facility, indicate type of farm birds originated from.	a. Broiler breeder farm (parent/ grandparent flock) b. Hatchery c. Grow-out farm (broiler farm) d. Other (specify)..... e. Not reported	a. **Broiler breeder farms** produce hatching eggs intended for hatcheries. b. **Hatcheries** produce day old broiler chickens. c. **Grow out farms** produce broilers to be marketed for meat production.
If commercial farm, indicate the size of farm.	a. ..... b. Not reported c. Not applicable	Please specify as reported. May include the number of broilers produced per production cycle year, the average, median or range of flock size.
Transport (if applicable)		
If transport, what was the number of crates/cages per truck?	a. ..... b. Not reported	Crates/cages are the containers used for transporting broilers.
If transport, what was the number of broilers per crate?	a. ..... b. Not reported	Please indicate as reported by the author.
If transport, how many transport vehicles were sampled? If more than one type of vehicle used, please specify.	a. ..... b. Not reported	Vehicles include trucks, cars, and rail.
If transport, what was the duration of transport from farm to the slaughter plant?	a. ..... b. Not reported	Please specify minutes/hours/days.
Processing (if applicable)		
If processing, what was the setting?	a. Commercial slaughter plant b. Pilot slaughter plant c. Laboratory	a. **Commercial slaughter plant** is a slaughter plant where broilers are slaughtered for sale and human consumption. b. **Pilot slaughter plant** is usually a smaller plant and associated with research organizations or universities. c. **Laboratory** is a building equipped for scientific experimentation or research.
If commercial processing, indicate the slaughter	a. ..... b. Not reported	Please specify as reported. May include the number of broilers

capacity.		slaughtered per hour/ day/week/month/year or the average, median or range of broilers slaughtered.
Number sampled		
Please indicate the total number of broilers included in the study.	a. Farms ..... b. Hatcheries..... c. Barns/House..... d. Flocks..... e. Transport vehicles..... f. Day old chickens..... g. Broiler chickens..... h. Breeding poultry..... i. Broiler eggs ..... j. Slaughter plants..... k. Batches/lots..... l. Carcasses/pieces..... m. Other (please specify)..... n. Not reported	Please provide data as reported. Examples include: T1=32x2 groups, T2=10x2 groups; T1=64, T2=20; T1=20x2 replicates; 50 over 2 trials. Note that this is <u>not</u> the number of broilers reported in the results, but the number of broilers included in the study. a. **Farms** are plots of land devoted to raising animals, for example, broilers. b. **Hatcheries** a place where broiler eggs are hatched. c. **Barns** are usually large buildings for the storage/housing of broiler chickens. d. **Flocks** are groups of broilers raised together. e. **Transport vehicles** include trucks, cars or trains used to transfer broiler chickens from the barn to processing facilities. f. **Day old chickens** are from hatching to 72 hours old. g. **Broilers** are chickens, usually 6-8 weeks old and 3-5 pounds, raised primarily for meat. h. **Breeding poultry** include broiler egg laying hens and cocks. i. **Broiler eggs** are eggs intended for hatcheries that produce broiler chickens. j. **Slaughter plants** are buildings where broiler chickens are slaughtered and processed. k. **Batches** are groups of chickens that come from the same farm or source. l. **Carcasses** include whole carcasses, part of the carcass or skin from carcasses. m. **Other:** if category is not specified above, indicate here and provide total sampled.
Inclusion/Exclusion criteria		
List the inclusion/exclusion criteria reported in the study.	a. Farms ..... b. Hatcheries..... c. Barns/House..... d. Flocks..... e. Transport vehicles..... f. Day old chickens.....	a. **Farms** are plots of land devoted to raising animals, for example, broilers. b. **Hatcheries** a place where broiler eggs are hatched. c. **Barns** are usually large buildings for the storage/housing of broiler

	g. Broiler chickens..... h. Breeding poultry..... i. Broiler eggs ..... j. Slaughter plants..... k. Batches/lots..... l. Carcasses/pieces..... m. Other (please specify)..... n. Not reported	chickens. d. **Flocks** are groups of broilers raised together. e. **Transport vehicles** include trucks, cars or trains used to transfer broiler chickens from the barn to processing facilities. f. **Day old chickens** are from hatching to 72 hours old. g. **Broilers** are chickens, usually 6-8 weeks old and 3-5 pounds, raised primarily for meat. h. **Breeding poultry** include broiler egg laying hens and cocks. i. **Broiler eggs** are eggs intended for hatcheries that produce broiler chickens. j. **Slaughter plants** are buildings where broiler chickens are slaughtered and processed. k. **Batches** are groups of chickens that come from the same farm or source. l. **Carcasses** include whole carcasses, part of the carcass or skin from carcasses. m. **Other:** if the category is not specified above, indicate here and provide total sampled.

Intervention(s) being studied?	a. Vaccination b. Antimicrobials c. Other feed and water additives d. Competitive exclusion e. Bacteriophage and bacteriocins f. Feed withdrawal g. Biosecurity..... h. Scalding/defeathering i. Reprocessing j. Treatment spraying/dipping carcasses k. Chilling l. Final wash m. Other.....	Please check all that apply. a. **Vaccination:** May be a killed or live vaccine. May be oral or injectable. b. **Antimicrobials:** Examples include flouroquinolones, cephalosporins, gentamicin, ampicillin, tetracyclines, spectinomycin, ciprofloxacin, ceftriaxone. These are often administered via feed. c. **Other feed and water additives:** May include organic acids, sodium chlorate, sodium nitrate, formaldehyde, and propionic acid. Water supplies may be treated with acidic oxidizing agents (such as hydrogen peroxide, paracetic acid, lactic acid, volatile fatty acids (formic, acetic, propionic, butyric), sodium chlorate, sodium nitrate). d. **Competitive exclusion:** May also be referred to as probiotics, prebiotics, synbiotics or the 'Nurmi concept'. May include Lactobacillus spp. (L. salivarius, L. acidophilus, L. casei, L. reuteri, L. plantarum), bacteroids, Bifidobacterium spp., Enterococcus faecium, Aspergillu oryzae, and Saccharomyces spp. (S. cerevisiae, S. boulardii). May be cecal contents or other materials from birds or the environment that contain many different or unknown bacterial species. e. **Bacteriophage/bacteriocins:** Viruses that can infect, multiply, and kill susceptible bacteria. Bacteriocins are a heterogeneous group of peptides produced by certain types of bacteria that are active against other (often closely related) bacterial strains. May include E. faecium and nisin. f. **Feed withdrawal:** Withholding feed (and sometimes water) on farm often occurs prior to transport to allow clearance of the GI tract and to reduce visible contamination. This is a common practice and should only be included if it is used differentially between comparison groups. g. **Biosecurity:** Includes, but is not limited to, sanitation, biosafety, disinfection (and other antimicrobials), hygiene and hygiene barriers, all-in-all-out production,

		Depopulation, monitoring pathogen levels in animals, staff and the environment, litter testing and treatment, removal of contaminated material, mice/pest/rodent/insect control, reduce water pooling and increasing water drainage. h. **Scalding/defeathering:** Also includes defeathering, plucking and picking. May have single or multiple scald tanks. i. **Reprocessing:** Removal of visibly contaminated birds with faecal matter. The birds are hand washed, and re-added to the slaughter line. j. **Treatment spraying/dipping:** Spraying or washing carcasses with a treatment such as an antimicrobial solution (which may include chlorine or trisodium phosphate). Differs from final wash in that the addition of this type of intervention is to treat the carcass as opposed to washing the carcass which is common practice within poultry production. Steam pasteurization may also be used. k. **Chilling:** Includes studies on chillers and chill water. May be air or immersion chilling. Interventions such as chlorine or trisodium phosphate may be added to immersion water. l. **Final wash:** A routine poultry production practice that includes spraying or washing carcasses with water. It is often referred to as inside/outside bird washer. Often occurs before chilling, but in some instances it is done before and/or after chilling. Common interventions include temperature, time, or chlorine levels. m. **Other:** If an intervention is not listed, please indicate here.

Point(s) in chain where the intervention(s) was implemented.	a. Farm..... b. Transport..... c. Processing (to end of 2° processing)... d. Farm + Transport + Processing..... e. Farm + Transport..... f. Farm + Processing..... g. Transport + Processing.....	a. **Farm** includes studies performed at breeding farms, hatcheries and grow-out farms. b. **Transport** includes catching and transport to slaughter. c. **Processing** includes live-bird supply to the slaughterhouse (lairage), all slaughter, evisceration, wash and chilling activities up to secondary processing. Secondary processing includes cut-up, de-boning, partitioning and grinding of raw chicken carcasses. *Third processing is out of scope of this project, and includes marination, coating, frying, smoking, grill marking and formulations (heat and eat). Freezing, packing and storage of raw chicken products and retail is out of scope of this project.*
Please find the corresponding intervention section and respond to the questions specific to the intervention(s) in this study.		
Vaccination (if applicable)		
What type of vaccine(s) was used?	a. Live attenuated..... b. Killed..... c. Avirulent..... d. Other (please specify)..... e. Not reported	Please specify the generic name and commercial product as reported. For example, live attenuated temperature-sensitive (T(s)) mutant E/1/3 of *Salmonella enteritidis*; avirulent delta cya delta crp *S. typhimurium.*
What *Salmonella* strain(s) was used?	a..... b. Not reported	Please specify as reported by the author.
What was the dose?	a..... b. Not reported	Please specify as reported by the author.
What was the route of application?	a. Oral – individual..... b. Oral – mass through feed ... c. Oral – mass through water... d. Aerosol..... e. Spray on eggs..... f. Injection..... g. Other (please specify)..... h. Not reported	
What was the frequency of application?	a. ..... b. Not reported	For example, 2x/day.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
Antimicrobials (if applicable)		
What type of antimicrobial(s) was used?	.....	Please specify the generic name and the commercial name as reported. For example, enrofloxacin/Baytril.
What was the dose?	a..... b. Not reported	Please specify as reported by the author.

What was the route of application?	a. Oral – individual..... b. Oral – mass through feed ... c. Oral – mass through water... d. Aerosol..... e. Spray on eggs..... f. Injection..... g. Other (please specify)..... h. Not reported	
What was the frequency of application?	a. ..... b. Regularly (if during the full study period) c. Not reported	For example, 2x/day.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
Other feed and water additives (if applicable)		
What feed and water additive(s) was used?	.....	Please specify as reported by the author.
What was the dose?	a..... b. Not reported	Please specify as reported by the author.
What was the route of application?	a. Oral – individual..... b. Oral – mass through feed ... c. Oral – mass through water... d. Aerosol..... e. Spray on eggs..... f. Injection..... g. Other (please specify)..... h. Not reported	
What was the frequency of application?	a. ..... b. Regularly (if during the full study period) c. Not reported	For example, 2x/day or 1x.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
Competitive exclusion (if applicable)		
What was the strain(s) used?	.....	Please specify the generic name and commercial product name as reported. For example, Broilact. If undefined culture please report as following: Undefined, source (e.g. cecal contents from *Salmonella* free broilers), incubation time(s) (e.g. 24h). Please specify if these differ between trials using T1, T2... Tn.
What was the dose?	a..... b. Not reported	Please specify as reported by the author. For example, 1mL x 10^4 g/mL.

What was the route of application?	a. Oral – individual..... b. Oral – mass through feed ... c. Oral – mass through water... d. Aerosol..... e. Spray on eggs..... f. Injection..... g. Other (please specify)..... h. Not reported	
What was the frequency of application?	a. ..... b. Regularly (if during the full study period) c. Not reported	For example, 2x/day.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
Bacteriophage and bacteriocins (if applicable)		
What was the strain used?	.....	Please specify as reported by the author.
What was the dose?	a..... b. Not reported	Please specify as reported by the author.
What was the route of application?	a. Oral – individual..... b. Oral – mass through feed ... c. Oral – mass through water... d. Aerosol..... e. Spray on eggs..... f. Injection..... g. Other (please specify)..... h. Not reported	
What was the frequency of application?	a. ..... b. Regularly (if during the full study period) c. Not reported	For example, 2x/day.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
Feed withdrawal (if applicable)		
What was the time period between withholding feed or water and transport of broilers?	a..... b. Not reported	Please specify in minutes/hours.
What was the duration of transport?	a..... b. Not reported	Please specify in minutes/hours.
Biosecurity (if applicable)		
What biosecurity method was used?	.....	Please provide the name of the intervention and a detailed description.
If applicable, list all chemicals that were used.	a..... b. Not applicable	Please specify as reported by the author.
If applicable, what was the chemical(s) concentration?	a..... b. Not reported c. Not applicable	Please specify as reported by the author.
What was the level of application?	a. Farm..... b. Cage/crate.....	a. **Farm** includes studies performed at breeding farms, hatcheries and grow-

	c. Transport vehicle..... d. Processing slaughter plant... e. Other (please specify)..... f. Not reported	out farms. b. **Cage/crate** are the containers used when catching and transporting the broilers to slaughter. c. **Transport vehicle** includes car, truck or rail. d. **Processing** includes live-bird supply to the slaughterhouse (lairage), all slaugher, evisceration, wash and chilling activities up to secondary processing. Secondary processing includes cut-up, de-boning, partitioning and grinding of raw chicken carcasses. Third processing is out of scope of this project, and includes marination, coating, frying, smoking, grill marking and formulations (heat and eat). Freezing, packing and storage of raw chicken products and retail is out of scope of this project.
What was the frequency of application?	a..... b. Not reported	Please specify as reported by the author.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
If chemicals/water was used, what was the temperature?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
If chemicals/water was used, what was the pressure?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
Scalding (if applicable)		
How many scald tanks were used?	a..... b. Not reported	Please specify as reported by the author.
What was the water temperature in the scald tank?	a..... b. Not reported	Please specify as reported by the author.
What was the water pH in the scald tank?	a..... b. Not reported	Please specify as reported by the author.
If chemicals were used, list them.	a..... b. Not applicable	Please specify as reported by the author. Examples include chlorine and trisodium phosphate.
If chemicals were used, what was the concentration?	a..... b. Not reported c Not applicable	Please specify as reported by the author.
What was the scalding time for one application?	a..... b. Not reported	Please specify as reported by the author.
What was the frequency of application?	a..... b. Not reported	Please specify as reported by the author.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.

Treatment spraying or dipping carcasses (if applicable)		
If chemicals were used, list them.	a..... b. Not applicable	Please specify as reported by the author. Examples include chlorine and trisodium phosphate.
If applicable, what was the chemical(s) concentration?	a..... b. Not reported c. Not applicable	Please specify as reported by the author.
What was the treatment time for one treatment of spraying/dipping?	a. Spraying..... b. Dipping..... c. Not reported	Please specify as reported by the author.
What was the water/chemical pressure?	a..... b. Not reported	Please specify as reported by the author.
What was the water/chemical temperature used for spraying/dipping?	a..... b. Not reported	Please specify as reported by the author.
What was the water/chemical pH?	a..... b. Not reported	Please specify as reported by the author.
What was the frequency of application?	a..... b. Not reported	Please specify as reported by the author.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
Chilling (if applicable)		
What types of chilling methods were used?	a. Counter current immersion chilling... b. Parallel flow immersion chilling..... c. Air chilling..... d. Other..... e. Not reported	a. **Counter current immersion chilling** is where the flow of the chilling water runs opposite to the flow of broiler carcasses. b. **Parallel flow immersion chilling** is where the flow of the chilling water runs in the same direction as the flow of broiler carcasses. c. **Air chilling** is where broiler carcasses are hung by shackles and moved through coolers with rapidly moving air. d. **Other:** If not listed above, please indicate here.
If chemicals were used in the chilling tank, list them.	a..... b. Not applicable	Please specify as reported by the author. Examples include chlorine and trisodium phosphate.
If applicable, what was the chemical(s) concentration?	a..... b. Not reported c. Not applicable	Please specify as reported by the author.
What was the temperature of the chilling tank?	a..... b. Not reported	Please specify as reported by the author.
What was the pH of the chilling tank?	a..... b. Not reported	Please specify as reported by the author.
What was the treatment time of chilling?	a..... b. Not reported	Please specify as reported by the author.
What was the frequency of application?	a..... b. Not reported	Please specify as reported by the author.
If applied more than once, what was the time period	a. ..... b. Not reported	Please specify as reported by the author.

between the applications?	c. Not applicable	
Final wash (if applicable)		
List any chemicals used in the final wash	a..... b. Not applicable	Please specify as reported by the author. Examples include chlorine and trisodium phosphate.
If applicable, what was the chemical(s) concentration?	a..... b. Not reported c. Not applicable	Please specify as reported by the author.
What was the treatment time for final wash?	a..... b. Not reported	Please specify as reported by the author.
What was the temperature of the final wash?	a..... b. Not reported	Please specify as reported by the author.
What was the pH of the final wash?	a..... b. Not reported	Please specify as reported by the author.
What was the pressure of the final wash?	a..... b. Not reported	Please specify as reported by the author.
What was the frequency of application?	a..... b. Not reported	Please specify as reported by the author.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
Other Interventions (if applicable)		
Describe the intervention as reported in the paper	.....	Please provide the name of the intervention and a detailed description as reported by the author.
Route of application	.....	Please specify as reported by the author.
Dose/concentration of intervention	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
What was the length of the application?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
Frequency of application?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
If applied more than once, what was the time period between the applications?	a. ..... b. Not reported c. Not applicable	Please specify as reported by the author.
CHALLENGE STRAIN INFORMATION (if applicable)		
What was the challenge strain?	a. Typhimurium ..... b. Enteritidis..... c. Other (please specify)..... d. Not reported	Please specify as reported by the author. For example, Typhimurium ATCC 14028, nalidixic acid resistant.
What was the route of application?	a. Oral – individual..... b. Oral – mass through diet..... c. Oral – mass through water... d. Applied to carcass or piece of carcass- individual..... e. Seeder birds..... f. Other (please specify)..... g. Not reported	
When were the birds/carcasses challenged	a. Before the intervention..... b. Between intervention	Please specify the time between challenge and intervention, as

with *Salmonella*?	applications..... c. Simultaneously with the intervention d. After the intervention..... e. Before and after the intervention..... f. Not reported	reported by the author.
What was the challenge dose/ contact time, if applicable?	a. Dose..... b. Contact time..... c. Not reported	Please specify as reported by the author. For example, $5*10^8$ cfu. For carcasses/pieces, record the contact time (i.e. the length of time the challenge was kept on the carcass).
What was the frequency of application?	a..... b. Not reported	Please specify as reported by the author.
OUTCOME – NOTE THAT *SALMONELLA* IS THE ONLY OUTCOME WE ARE INTERESTED IN		
What test(s) were used to monitor Salmonella?	a. Bacteriological culture b. PCR (Polymerase Chain Reaction) c. Other (please specify)..... d. Not reported	
If bacterial culture, specify the details.	a. Enrichment media..... b. Enrichment time..... c. Enrichment temperature..... d. Culture media..... e. Culture time..... f. Culture temperature..... g. Confirmation method..... h. Not reported i. Not applicable	Please specify as reported by the author. a. **Enrichment media**: Enrichment media refers to broth based media. Please specify, for each different enrichment media, their names and supplements with a description of the order in which they were used (e.g. phosphate buffered peptone water THEN Rappaport-Vassiliadis broth AND tetrathionate broth). b. **Enrichment time:** Please specify the enrichment time, <u>as indicated by the author</u>, used for each type of enrichment media (e.g. 18-24h THEN 24h AND 24h). c. **Enrichment temperature:** Please specify the enrichment temperature, <u>as indicated by author</u>, used for each type of enrichment media (e.g. 37°C THEN 37°C AND 42°C). d. **Culture media:** Culture media refers to media capable of growing *Salmonella* colonies. Please specify, for each different plating media, all the media names and supplements used for plating with a description of the order in which they were used (e.g. BG sulfa agar AND modified lysine iron agar THEN MacConkey agar). e. **Culture time:** Please specify the

		culture time, as indicated by the author, used for each type of enrichment media (e.g. 18-24h AND 24h THEN 24h). f. **Culture temperature:** Please specify the enrichment temperature, as indicated by author, used for each type of enrichment media (e.g. 37°C AND 37°C THEN 37°C). g. **Confirmation method:** Please indicate how *Salmonella* was confirmed including biochemical tests that were conducted and if sent to a reference laboratory (e.g. slide agglutination with Poly-O and Poly-H antisera, PCR, visual confirmation if counting resistant marker strains, reference lab, triple sugar iron agar, lysine iron agar, Urea agar). If referenced in another paper, please answer ref in the text box.
If PCR, specify the details.	a. PCR type..... b. Cycle conditions..... c. Primer name..... d. Target sequence..... e. Not reported f. Not applicable	a. **PCR type:** For example real-time, multiplex, reverse transcriptase. b. **Cycle conditions:** What DNA polymerase enzyme was used, denaturation time/temperature, annealing time/temperature and extension time/temperature. c. **Primer name:** If applicable, indicate the name of the primers used, often the same name as the target sequence with different word formatting. If not applicable, provide author reference name and target sequence (e.g. Oresnik et al. 2006 – *Eco*R1). d. **Target sequence:** Please report the gene targeted by PCR to be amplified, as reported by the author. If referenced in another paper, please answer ref in the text box.
Specify sensitivity (Sn) and specificity (Sp) of the test(s) used.	a. Sn..... b. Sp..... c. Not reported	If applicable please indicate the Sn and Sp of all tests used.
What unit of sampling/cut-off level was used to classify as *Salmonella* positive?	a. Farms..... b. Hatcheries..... c. Barns/House..... d. Flocks..... e. Broiler chickens/day old chickens..... f. Breeding poultry.....	If applicable, indicate the cut off level used for *Salmonella* positivity of all levels. a. **Farms** are plots of land devoted to raising animals, for example, broilers. b. **Hatcheries** a place where broiler

	g. Broiler eggs..... h. Slaughter plants..... i. Batches/lots..... j. Carcasses/pieces..... k. Other (please specify)..... l. Not reported	eggs are hatched. c. **Barns** are usually large buildings for the storage/housing of broiler chickens. d. **Flocks** are groups of broilers raised together. e. **Transport vehicles** include trucks, cars or trains used to transfer broiler chickens from the barn to processing facilities. f. **Day old chickens** are from hatching to 72 hours old. g. **Broilers** are chickens, usually 6-8 weeks old and 3-5 pounds, raised primarily for meat. h. **Breeding poultry** include broiler egg laying hens and cocks. i. **Broiler eggs** are eggs intended for hatcheries that produce broiler chickens. j. **Slaughter plants** are buildings where broiler chickens are slaughtered and processed. k. **Batches** are groups of chickens that come from the same farm or source. l. **Carcasses** include whole carcasses, part of the carcass or skin from carcasses. m. **Other:** if the category is not specified above, indicate here and provide total sampled.
What secondary outcomes, if any, were measured in this study	.....	Please list any outcome other than *Salmonella,* for example, mortality or weight gain. Please do not provide any data on these outcomes.

RESULTS – NOTE THAT *SALMONELLA* IS THE ONLY OUTCOME WE ARE INTERESTED IN

Please use a different column for each *Salmonella* outcome.
Empty cells indicate that information was not reported.

***Salmonella* status in the broilers before intervention (if applicable)**

Trial ID, if applicable	Please indicate the trial number. Please use the following coding system (if possible): **T1, T2, T3..... Tn.**
Sample type	**Text** Crate papers Feces Fecal swab Cecal Swab Intestinal contents Crop contents Lymph nodes Carcass rinse Carcass swab Skin sample Fluff

	Other (specify)
Sample weight	The measure of weight, volume (of rinse) or area swabbed. Please indicate units as reported.
Samples per pool	Please indicate the number of samples pooled together prior to laboratory analysis.
Pool weight	The sum of the pooled sample weight. Please indicate units as reported (e.g. grams).
Serotype(s) targeted or recovered	Please specify as reported by the author (e.g. S. Typhimurium, S. Enteritidis).
Total samples	The total number of samples analyzed.
Total samples *Salmonella* positive	The total number of samples analyzed that were positive.
Prevalence	Number of positive units divided by the total number of units.
Prevalence 95% CI low	95% confidence interval.
Prevalence 95% CI high	95% confidence interval.
Prevalence SE/SD	Standard error or standard deviation (please indicate which).
Concentration	The mean concentration (e.g. cfu) of *Salmonella* in a sample.
Concentration 95% CI low	95% confidence interval.
Concentration 95% CI high	95% confidence interval.
Concentration SE/SD	Standard error or standard deviation (please indicate which).
Other	Open textbox for additional information.

Please use a different column for each *Salmonella* outcome.
Empty cells indicate that information was not reported.
Please try to input the data following the provided table in text from left to right/top to bottom.
****Please remember to record the units****

***Salmonella* status in the broilers after intervention**	
Trial ID, if applicable	Please indicate the trial number. Please use the following coding system (if possible): **T1, T2, T3..... Tn.**
Level of analysis	Examples include hatchery, day old chicks, broiler, farm, carcasses, processing plants etc.
Point(s) in chain	Please indicate where sampling took place. For example, farm or processing. Include as much detail as the author provides, for instance, processing, after evisceration before final wash.
Frequency of measurement	Please indicate the frequency of measurement of the outcome of interest after applying the intervention.
Specify comparison groups	Please indicate the treatment each group (including the control) received, or specify the parameter that makes the groups different using the following coding system (if possible): **g0, g1, g2... gn.** For example, a trial that compares three different levels of CE doses: g0=0 CE, g1=10^3, g2=10^4, g3=10^5 g/mL.
n	The number of subjects per treatment group (including the controls).
Sample type	**Text** Feces Fecal swab Cecal Swab Intestinal contents Crop contents Lymph nodes Carcass rinse Carcass swab

	Skin sample Fluff Other (specify)
Sample weight	The measure of weight, volume (of rinse) or area swabbed. Please indicate units as reported.
Samples per pool	Please indicate the number of samples pooled together prior to analysis.
Pool weight	The sum of the pooled sample weight. Please indicate units as reported.
Serovar(s) targeted or recovered	Please specify as reported by the author (e.g. S. Typhimurium, S. Enteritidis).
Raw/unadjusted data	
Total samples	The total number of samples analyzed. Please identify with relation to comparison groups (g0, g1, g2...gn).
Total samples *Salmonella* positive	The total number of samples analyzed that were positive. Please identify with relation to comparison groups (g0, g1, g2...gn).
Prevalence	Number of positive units divided by the total number of units. Please identify with relation to comparison groups (g0, g1, g2...gn).
Prevalence 95% CI low	95% confidence interval. Please identify with relation to comparison groups (g0, g1, g2...gn).
Prevalence 95% CI high	95% confidence interval. Please identify with relation to comparison groups (g0, g1, g2...gn).
Prevalence SE/SD	Standard error or standard deviation (please indicate which). Please identify with relation to comparison groups (g0, g1, g2...gn).
Concentration	The mean concentration (e.g. cfu) of *Salmonella* in a sample. Please identify with relation to comparison groups (g0, g1, g2...gn).
Concentration 95% CI low	95% confidence interval. Please identify with relation to comparison groups (g0, g1, g2...gn).
Concentration 95% CI high	95% confidence interval. Please identify with relation to comparison groups (g0, g1, g2...gn).
Concentration SE/SD	Standard error or standard deviation (please indicate which). Please identify with relation to comparison groups (g0, g1, g2...gn).
Mean diff concentration	The mean concentration (e.g. cfu) of *Salmonella* in a sample.
MD concentration 95% CI low	95% confidence interval.
MD concentration 95% CI high	95% confidence interval.
MD concentration SE/SD	Standard error or standard deviation (please indicate which).
Analysis	
Type of analysis	The model or statistical test conducted to give the measure of precision and p-value. For example, t-test, chi-square, ANOVA.
Model adjusted by, if applicable	List the random effects and fixed effects included in the model for these results.
Outcome type	Please specify the outcome. For example, binary (OR, RR), or continuous (mean difference, LS means). This may also include log OR, or a coefficient in the log scale.
Estimate of effect	Indicate the result of the test or model.
Measure of variability of effect estimate	This may be a SD, SE, or 95%CI. Please specify with the results.
P-value	The level of significance achieved by the risk factor.
IF	Infection factor as reported by author.
PF	Protection factor as reported by author.

Other	Open textbox for additional information.	
Additional comments	.....	If the reviewer feels there is something that was not captured in the tool but should be acknowledged.

Appendix 6. Data extraction form for prioritized prevalence and risk factor studies identified in a scoping review on *Salmonella* in broiler chickens for rigorous systematic review

GENERAL INFORMATION		
Variable	**Category**	**Explanation**
Ref ID from SRS	.....	
Journal name	.....	
Author(s) name	.....	
Publication year	.....	
Publication type	a. Peer reviewed b. Conference proceeding c. Thesis d. Government or research station report e. Other (please specifiy)	
Country/region/province/state where the study was carried out.	a. ..... b. Not reported	
In what year(s) was the data collected?	a. ..... b. Not reported	
Cumulative length of the study?	a. ..... b. Not reported	Time between first and last collection of samples, and/or survey or questionnaire data. Please specify as reported (days, months, or years).
Institution(s) that funded the study	a. ..... b. Not reported	
What is the study design? *** When more than one trial exists in a study: indicate in the text box an acronym per study design (e.g. T1, T2, T3) and follow this notation through the tool.*	a. Cohort study..... b. Case-control study..... c. Cross-sectional study..... d. Prevalence Survey..... e. Longitudinal prevalence..... f. Other (specify)..... g. Not an observational study, **stop reviewing and contact Ashley Farrar**	**Trial:** One of a number of repetitions of an experiment. a. **Cohort study:** A group of animals exposed to a hypothesized risk factor (exposure), and a group not exposed to the factor are selected and observed over the study period to record development of disease in each group. b. **Case-control study**: A group of diseased animals and a group of non-diseased animals are selected and compared with respect to the presence of the hypothesized risk factor (exposure). c. **Cross-sectional study**: A study done at a single point in time to investigate the prevalence and distribution of disease and hypothesized risk factors within the

		population. d. **Prevalence survey:** A study that measures outcome (prevalence and distribution of disease only) at a single point in time. e. **Longitudinal prevalence:** A study that measures outcome (prevalence and distribution of disease only) at multiple points in time on the same population. f. **Other:** Hybrid or other designs.
POPULATION		
What is the target population that the sample population is representing?	a. described by author ..... b. not described by author	
Age-group(s) of the study population	a. Broiler Eggs..... b. Day old chicken..... c. Broilers (indicate age/size)..... d. Broiler breeders (hens/cocks)..... e. Other (specify)..... f. Not reported	a. **Broiler eggs:** are eggs intended for hatcheries that produce broiler chickens. b. **Day old chickens:** are from hatching to 72 hours old. c. **Broilers:** are chickens, usually 6-8 weeks old and 3-5 pounds, raised primarily for meat. d. **Broiler breeders:** include broiler egg laying hens and cocks. e. **Other:** Please specify
What is the breed(s) of broilers sampled in this study?	a..... b. Not reported	
Point(s) in chain where sampling took place.	a. Farm b. Transport c. Processing d. Other (specify).....	a. **Farm** includes breeding, hatchery and grow-out farms b. **Transport** involves catching and transport to slaughter c. **Processing** includes: primary and secondary processing. Secondary processing includes cut-up, de-boning, partitioning and grinding of raw chicken carcasses up to the point of freezing.
Farm (if applicable)		

If farm level, what was the setting?	a. Commercial farm b. Research farm	a. **Commercial farms** include operations rearing broilers in a commercial setting. b. **Research farms** include operations affiliated with Universities and/or research organizations. Usually they are smaller farms with high biosecurity standards and do not resemble commercial farms.
If farm level, what is the type of farm sampled?	a. Broiler breeder farm (parent/ grandparent flock) b. Hatcheries c. Grow-out farms (broiler farm) d. Other (specify).....	a. **Broiler breeder farms** produce hatching eggs intended for hatcheries. b. **Hatcheries** produce day old broiler chickens. c. **Grow out farms** produce broilers to be marketed for meat production.
If commercial farm, indicate size of farm.	a. ..... b. Not reported c. Not applicable	Please specify as reported. May include the number of broilers produced per production cycle year, the average, median or range of flock size.
Transport (if applicable)		
If transport, what was the number of crates/cages per truck?	a. ..... b. Not reported	Crates/cages are the containers used for transporting broilers.
If transport, what was the number of broilers per crate?	a. ..... b. Not reported	Please indicate as reported by the author.
If transport, what was the number of vehicles used?	a. ..... b. Not reported	Vehicles include trucks, cars, and rail.
If transport, what was the duration of transport from farm to the slaughter plant?	a. ..... b. Not reported	Please specify minutes/hours/days.
Processing (if applicable)		
If commercial processing, indicate the slaughter capacity	a. ..... b. Not reported	Please indicate the number of broilers slaughtered per hour/ day/week/month/year or the average, median or range of broilers slaughtered.
Number sampled		
Please indicate the total number of each sampled.	a. Farms ..... b. Hatcheries..... c. Barns/House..... d. Flocks..... e. Transport vehicles..... f. Day old chickens..... g. Broiler chickens..... h. Breeding poultry..... i. Broiler eggs	Please provide data as reported. For example, if they give the number of farms/flocks and chickens, but not the number of barns, do not put an answer in barns. **Note that this is the study total, not how many flocks are in a barn.**

	j. Slaughter plants..... k. Batches/lots..... l. Carcasses/pieces..... m. Other..... n. Not reported	a. **Farms** are plots of land devoted to raising animals, for example, broilers. b. **Hatcheries** a place where broiler eggs are hatched. c. **Barns** are usually large buildings for the storage/housing of broiler chickens. d. **Flocks** are groups of broilers raised together. e. **Transport vehicles** include trucks, cars or trains used to transfer broiler chickens from the barn to processing facilities. f. **Day old chickens** are from hatching to 72 hours old. g. **Broilers** are chickens, usually 6-8 weeks old and 3-5 pounds, raised primarily for meat. h. **Breeding poultry** include broiler egg laying hens and cocks. i. **Broiler eggs** are eggs intended for hatcheries that produce broiler chickens. j. **Slaughter plants** are buildings where broiler chickens are slaughtered and processed. k. **Batches** are groups of chickens that come from the same farm or source. l. **Carcasses** include whole carcasses, part of the carcass or skin from carcasses. m. **Other:** if category is not specified above, indicate here and provide total sampled.
Inclusion/Exclusion criteria		
List the inclusion/exclusion criteria reported in the study for the farm level.	a. Farms ..... b. Hatcheries..... c. Barns/House..... d. Flocks..... e. Transport vehicles..... f. Day old chickens..... g. Broiler chickens..... h. Breeding poultry..... i. Broiler eggs ..... j. Slaughter plants..... k. Batches/lots..... l. Carcasses/pieces.....	a. **Farms** are plots of land devoted to raising animals, for example, broilers. b. **Hatcheries** a place where broiler eggs are hatched. c. **Barns** are usually large buildings for the storage/housing of broiler chickens. d. **Flocks** are groups of broilers raised together. e. **Transport vehicles**

	m. Other..... n. Not reported	include trucks, cars or trains used to transfer broiler chickens from the barn to processing facilities. f. **Day old chickens** are from hatching to 72 hours old. g. **Broilers** are chickens, usually 6-8 weeks old and 3-5 pounds, raised primarily for meat. h. **Breeding poultry** include broiler egg laying hens and cocks. i. **Broiler eggs** are eggs intended for hatcheries that produce broiler chickens. j. **Slaughter plants** are buildings where broiler chickens are slaughtered and processed. k. **Batches** are groups of chickens that come from the same farm or source. l. **Carcasses** include whole carcasses, part of the carcass or skin from carcasses. m. **Other:** if the category is not specified above, indicate here and provide total sampled.
OUTCOME		
What test(s) were used to monitor *Salmonella*?	a. Bacteriological culture b. PCR (Polymerase Chain Reaction) c. Other.....	Please specify as reported by the author.
If bacterial culture, specify the culture media used.	a. Enrichment media..... b. Enrichment time..... c. Enrichment temperature ..... d. Culture media..... e. Culture time..... f. Culture temperature..... g. Confirmation method..... h. Not applicable	a. **Enrichment media**: Please specify, for each different enrichment media, all culture media names and supplements used to culture (e.g. phosphate buffered peptone water, Rappaport-Bassiliadis broth, tetrathionate broth). b. **Enrichment time:** Please specify the enrichment time (e.g. 24 hours). c. **Enrichment temperature:** Please specify the enrichment temperature (e.g. 37°C). d. **Culture media:** Please specify, for each different plating media, all the plating media names and

		supplements used for plating (e.g. BG Sulfa plates containing 200 ppm nalidixic acid, modified lysine iron agar plates, Rambach agar). e. **Culture time:** Please specify the enrichment time (e.g. 24 hours). f. **Culture temperature:** Please specify the enrichment temperature (e.g. 37°C). g. **Confirmation method:** Please indicate how *Salmonella* was confirmed (e.g. Agglutination, PCR). If referenced in another paper, please answer ref in the text box.
If PCR, please specify the reported PCR used with basepairs.	a. PCR type..... b. Cycle conditions..... c. Primer Name..... d. Target sequence..... e. Not applicable	a. **PCR type:** For example real-time, multiplex, reverse transcriptase. b. **Cycle conditions:** What DNA polymerase enzyme was used, denaturation time/temperature, annealing time/temperature and extension time/temperature. c. **Primer name:** If applicable, indicate the name of the primers used, often the same name as the target sequence with different word formatting. If not applicable, provide author reference name and target sequence (e.g. Oresnik et al. 2006 – *Eco*R1). d. **Target sequence:** Please report the gene targeted by PCR to be amplified, as reported by the author. If referenced in another paper, please answer ref in the text box.
If reported, specify sensitivity (Sn) and specificity (Sp) of the test(s) used.	a. Sn..... b. Sp..... c. Not reported	If applicable please indicate the Sn and Sp of all tests used.
What cut-off level/unit of sampling was used to classify farm/flock/bird	a. Farms..... b. Hatcheries..... c. Barns/House.....	If applicable, indicate the cut off level used for *Salmonella* positivity of all

positive?	d. Flocks..... e. Trucks..... f. Broiler chickens..... g. Breeding poultry..... h. Broiler eggs..... i. Slaughter plants..... j. Batches/lots..... k. Carcasses/pieces..... l. Other..... m. Not reported	levels. a. **Farms** are plots of land devoted to raising animals, for example, broilers. b. **Hatcheries** a place where broiler eggs are hatched. c. **Barns** are usually large buildings for the storage/housing of broiler chickens. d. **Flocks** are groups of broilers raised together. e. **Transport vehicles** include trucks, cars or trains used to transfer broiler chickens from the barn to processing facilities. f. **Day old chickens** are from hatching to 72 hours old. g. **Broilers** are chickens, usually 6-8 weeks old and 3-5 pounds, raised primarily for meat. h. **Breeding poultry** include broiler egg laying hens and cocks. i. **Broiler eggs** are eggs intended for hatcheries that produce broiler chickens. j. **Slaughter plants** are buildings where broiler chickens are slaughtered and processed. k. **Batches** are groups of chickens that come from the same farm or source. l. **Carcasses** include whole carcasses, part of the carcass or skin from carcasses. m. **Other:** if the category is not specified above, indicate here and provide total sampled.
Serovar(s) targeted or recovered?	a. *S.* Typhimurium..... b. *S.* Enteritidis..... c. Other.....	Please specify as reported by the author.

RESULTS (use one column per *Salmonella* x risk factor combination)

General Sample Data

Trial # (if applicable)	Text	
Time	Only for longitudinal studies. Indicate time relative to other samples	
Place	Only for multi-stage prevalence. Indicate the point of sampling in the continuum.	

If this is a cohort study	What was the prevalence of *Salmonella* shedding at the beginning of the study?
Unit of observation?	Level at which data was gathered
Sample type	**Text** Feces Fecal swab Cecal Swab Intestinal contents Crop contents Lymph nodes Carcass rinse Carcass swab Skin sample Fluff Other (specify)
Sample weight	The measure of weight, volume (of rinse) or area swabbed. Please indicate units as reported.
Samples per pool	Please indicate the number of samples pooled together prior to laboratory analysis
Pool weight	The sum of the pooled sample weight. Please indicate units as reported (eg. grams)
Overall Prevalence/Concentration (Any observational design may provide overall prevalence, except CC, or concentration)	
Total samples	The total number of samples analyzed.
Total samples *Salmonella* positive	The total number of samples analyzed that were positive.
Prevalence	Number of positive units divided by the total number of units.
Prev 95% CI low	95% confidence interval.
Prev 95% CI high	95% confidence interval.
Prev_SE/SD	Standard error or standard deviation (please indicate which).
Concentration	The mean concentration (e.g. cfu) of *Salmonella* in a sample.
Conc 95% CI low	95% confidence interval.
Conc 95% CI high	95% confidence interval.
Conc_SE/SD	Standard error or standard deviation (please indicate which).
Mean diff Concentration	The mean concentration (e.g. cfu) of *Salmonella* in a sample.
MD Conc 95% CI low	95% confidence interval.
MD Conc 95% CI high	95% confidence interval.
MD Conc_SE/SD	Standard error or standard deviation (please indicate which).
Other	Open textbox for additional information.

Empty cells indicate that information was not reported.

Risk Factor Data (cohort, case-control, X-sectional designs)	
Describe the risk factor:	What is the risk factor as described by the author. If some sort of treatment for example, also fill in the other details provided by the author. (cohort, XS, CC designs)
a- RF route of application	If applicable
b- RF dose/ concentration	If applicable
c-RF length of application	If applicable
d-RF frequency of application (time between)	If applicable
Raw Risk Factor Data	
RF+ n (number sampled)	Total number of samples in the risk factor +ve group.

RF+/ Salmonella +	Binary result = number of salmonella positive samples in the risk factor +ve group
RF+ Salmonella concentration	Continuous outcome in the risk factor +ve group
RF+ measure of variability	Measure of precision e.g. SE, SD, 95% CI in the risk factor +ve group
RF- n (number sampled)	Total number of samples in the risk factor -ve group.
RF- / Salmonella +	Binary result = number of salmonella positive samples in the risk factor -ve group
RF- Salmonella concentration	Continuous outcome in the risk factor -ve group
RF- measure of variability	Measure of precision e.g. SE, SD, 95% CI in the risk factor -ve group.
Risk Factor Analysis – adjusted and/or unadjusted	
Type of Analysis (stat test/ model)	The model or statistical test conducted to give the measure of precision and p-value.
Risk Factor	This is the type of exposure variable(s) of interest that is potentially associated with Salmonella presence
Outcome type	Please specify the outcome. For example, binary (OR, RR), or continuous (mean difference, LS means). This may also include log OR, or a coefficient in the log scale.
Specify comparison groups (if applicable)	What groups are being compared?
estimate of effect	If this was analysed in a regression model the coefficient may be available or Odds ratio or relative risk or concentrations etc. (e.g. concentration difference) (please indicate units.)
95% Conc_low	95% confidence interval.
95% Conc_high	95% confidence interval.
RF_SE/SD	Standard error or standard deviation (please indicate which).
P-value	The level of significance achieved by the risk factor.
Model adjusted by	List the random effects and fixed effects included in the model for these results.
Other information	Open textbox for additional information.
Other Outcome	
IF – infection factor	Infection factor as reported by author.
PF – protection factor	Protection factor as reported by author.

Appendix 7. Guidelines for relevance tool 1 in a scoping review of *Salmonella* in broiler chickens

1. What to do with surveillance reports?

Please classify as primary research, because most of the time they report original routine (passive) diagnostic data, and sometimes active surveillance data. This will be documented as a specific feature of this review.

Those surveillance reports that only mention pullorum/gallinarum serovars will be excluded through question 2.

Those reports that report serovars of public health importance will be included in question 3 as prevalence studies (either inside or outside of North America).

2. What to do with studies that measure serovars prevalence?

Please include these studies (under prevalence studies in question 3) as long as they report serovars of public health importance.

3. What to do with studies that measure contamination not in broilers?

For studies that evaluate interventions for which the outcome (Salmonella) was not measured either in hatching eggs, alive chicken (including faecal samples) or raw poultry should be excluded.

4. What is the difference between an intervention study and a risk factor study?

In short, an intervention study is one of experimental design (randomized control trial or challenge trial) and a risk factor study is an observational study (cohort, cross-sectional, case-control). However, it is possible that some interventions may be studied with a cohort design. If a study uses a cohort design to evaluate an intervention, it should be classified as an intervention. In addition, some risk factors are interventions (things that we do to the poultry while raising them) and some are just observations (age/sex etc. that may modify the outcome for some reason).

In the case of observational studies that model a number of risk factors including management interventions, in question three, please just answer yes to risk factor study (and not intervention study also).

Appendix 8. Guidelines for relevance tool 2 in a scoping review of *Salmonella* in broiler chickens

Intervention study designs: Interventions are most likely going to be studied as controlled trials or challenge trials. In rare cases, cohort study designs may be used to evaluate an intervention. A controlled trial/challenge trial is any situation where the researcher manipulates the environment. Some will be conducted under field conditions and others will be conducted under lab or unnatural conditions (such a research farm). In a challenge trial, the researcher will artificially infect the broiler/raw chicken product with *Salmonella.* In a controlled trial, *Salmonella* measured is from natural infection.

Risk factor study designs: Risk factor studies will mostly refer to observational studies. Observational studies are all studies where a sampling frame is selected 1) without prior knowledge of E/D status (cross-sectional), 2) selected by E status and followed for D status (cohort), and 3) selected by D status and examined for E status (case-control). Some studies may model a number of "risk factors" (for example, management). In this case, the study should be included as a risk factor study, not an intervention study.

Prevalence study designs: Prevalence studies often follow cross-sectional designs. We will come across several types in this SR: 1) one measure at one stage in the processing chain, 2) multiple measures (longitudinal) at one stage in the processing chain, 3) single measure at more than one stage in the processing chain (multi-stage prevalence), and 4) multiple measures (longitudinal) at more than one stage in the processing chain (multi-stage prevalence).

In order to be classified as a prevalence study the paper must provide: a numerator, denominator, and the point in chain prevalence was measured. If a paper does not report estimates in one of the above formats, it is not a prevalence study.

Additional points of interest
- Please label a study with each category it applies to. For example, a study can be both a risk factor and prevalence study.

Sample abstracts

Intervention – challenge trial

The influence of a feed additive level of virginiamycin on the course of an experimentally induced Salmonella typhimurium infection in broilers.
Abou-Youssef, M. H., Di Cuollo, C. J., Free, S. M., and Scott, G. C.

The purpose of this study was to determine the effect of virginiamycin on the course of an experimentally induced infection of Salmonella typhimurium in broilers. Several parameters were evaluated, including effects on the persistence and duration of shedding of the infecting Salmonella organism and its antibiotic resistance patterns. Virginiamycin was administered to the experimentally infected group for 8 weeks in feed at concentrations of 25 g/ton. This was compared to an infected control group not receiving the antibiotic. No effects were exhibited by virginiamycin on Salmonella typhimurium shedding and antibiotic resistance patterns.

Intervention – controlled trial

Control of Salmonella by acid disinfection of chicks food
Hinton, M., Linton, A. H., and Perry, F. G.

Four groups, each of 125 chicks, were fed a ration containing 0, 0.25%, 0.5% or 0.75% of an 85% formic acid preparation. Weekly sampling of cloacal faeces and caecal contents for 7 weeks revealed Salmonella of three serotypes in 52.5% of controls, 69% of the group fed 0.25%, and none of those fed 0.5% or 0.75%.

Risk factor – observational study

A retrospective study on Salmonella infection in Danish broiler flocks.
Angen, O., Skov, M. N., Chriél, M., Agger, J. F., and Bisgaard, M.

A retrospective longitudinal study was conducted to identify risk factors associated with Salmonella enterica infection in Danish broiler production. The study was based on information in the ante-mortem database (AM database) where data were available for all broiler flocks slaughtered over the 2-year period from 1992-93 in Denmark. The AM database contains information collected by the ante-mortem veterinarians, from the slaughterhouses, and from the salmonella examinations carried out at the National Veterinary Laboratory. The epidemiological unit was the individual broiler flock. The salmonella status of the flock was determined by examining the caecal tonsils from 163-week-old chickens from each flock. This procedure would detect a salmonella-infected flock, with a probability >95%, if the prevalence is >20%. Furthermore, the structure and quality of the collected data have been evaluated. 14 variables were selected for analysis by multivariable logistic regression. An increased risk of salmonella infection in the broiler flocks was associated with the biggest hatcheries and feedmill, with an increasing number of houses on the farm, if the preceding flock was infected, and if the flock was reared in the autumn. Additionally, the main variables of the model were analysed by including a random effect at the house level. This resulted only in minor changes of the parameter estimates.

Prevalence study
Characterization of Salmonella isolates from beef cattle, broiler chickens and human sources on Prince Edward Island
Abouzeed, Y. M., Hariharan, H., Poppe, C., and Kibenge, F. S. B.

Non-typhoid Salmonella serovars remain a potential threat to human health, and beef cattle and broiler chickens are possible sources of these organisms on Prince Edward Island (PEI). In this study, the ceca of beef cattle belonging to fasted and non-fasted groups, and broiler chickens were examined for Salmonella at the time of slaughter. The characteristics of the isolates, including antimicrobial resistance patterns and virulence genes, were studied along with the isolates obtained from cases of human salmonellosis on PEI during the study period (1996-97). The prevalence of Salmonella in beef cattle was 4.6% (11/240). The rate was significantly higher in fasted cattle (7.46%), than in non-fasted cattle (0.94%). The prevalence rate in chickens was 32.5% (39/120). In beef cattle, Salmonella typhimurium phage type (PT) or definitive type (DT) 104 which was resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole and tetracycline, was the most predominant type (64%). In chickens, S. heidelberg, with resistance to gentamicin, streptomycin and sulfisoxazole, predominated. Of 26 isolates from humans, the most common serovar was S. typhimurium, including a multidrug-resistant strain of DT104. Examination by PCR revealed presence of the virulence gene invA in all serovars, and the spvC gene in all S. typhimurium isolates, of both beef cattle and human origin. Among the other serovars the latter gene was found in 7 human isolates, but in none of the chicken or beef isolates. All but 3 of the spvC-positive isolates possessed a 90 kilobasepair (kbp) plasmid suggesting that the 3 isolates had the spvC gene on their chromosome. These findings were confirmed by plasmid DNA isolation using 3 different protocols and by sequence analysis of the spvC-PCR product.

Multi-stage prevalence
Effects of water chillers on bacterial quality of poultry carcasses in industrial slaughterhouses of Tehran and Gilan provinces.
Akhondzadeh, A., Misaghi, A., Bokaei, S., Zahraei Salehi, T., and Eshpari, H.

Objective: To study the effects of water chiller on microbiological quality of poultry carcasses before and after chilling process in 11 slaughterhouses of Tehran and Gilan provinces, Iran. Samples: 75 poultry carcasses were collected from 11 industrial slaughterhouses of Tehran and Gilan provinces. Method: 51 poultry carcasses were from 9 industrial slaughterhouses of Tehran province and 24 poultry carcasses from 2 industrial slaughterhouses of Gilan province, before and after chilling process, were collected and analysed bacteriologically according to American Public Health Association method. The free chlorine content and temperature of water for every chiller was also measured. Results: Total coliform count of poultry carcasses which were collected after chilling showed higher microbial loads than before chilling in Tehran. Paired-samples T test indicated a significant difference ($P<0.05$). One of 51 carcasses which were collected after chilling in Tehran and all the carcasses collected in Gilan, before and after chilling, were positive for Escherichia coli. The isolated serotypes were

O119:B14, O128:K67, O78:K80, O2:K1 and H7. One of 51 poultry carcasses from Tehran province was Salmonella enteritidis positive after chilling. Free chlorine content of water in 8 slaughterhouses located in Tehran province was not measurable. Therefore, it was measured in water of one of the slaughterhouses in Tehran and 2 slaughterhouses of Gilan which were 0.5, 1.0 and 0.1 ppm, respectively. The mean temperatures+or-standard error of water in chillers of the slaughterhouses of Gilan were 6.1+or-1.4 and 6.5+or-0.7 degrees C respectively. Conclusion: According to the results, water chillers may be considered as a risk for bacterial contamination of poultry carcasses. Therefore, hygienic quality control is very important.

Appendix 9. Guidelines for methodological assessment of studies in prioritized systematic reviews

General Questions

Q. How do I know which questions to answer for which study design?

A. Please refer to the hard copy of the QA tool. Study designs appropriate for each question will also be listed after the question in SRS.

Q. What to do when a study has more than one trial and/or both intervention and prevalence data?

A: If answers are the same, answer QA. If answers are different please contact Ashley and she will duplicate the reference in SRA and the QA form will be filled out twice.

Q. I found a paper that is not relevant, what do I do with it?

A. Please email Ashley and do not QA until further notice.

Major criteria for intervention studies to keep an eye out for:

1) Measurement must be in broilers (papers that only use environmental samples or evaluate in vitro should be excluded at RT 2).
2) Data must be from chickens only. Data collapsed with other types of animals and poultry should be excluded at RT 2. This includes data collapsed between broilers and layers.

Major criteria for prevalence/risk factor studies to keep an eye out for:

1) Must include a numerator, denominator and location of sampling.
2) Any studies that measure prevalence in clinically ill/dead birds should be excluded at RT 2.
3) Measurement must be in broilers (papers that only use environmental samples or evaluate in vitro should be excluded at RT 2).

Q. I have been assigned some papers that haven't been uploaded into SRS. Why?

A. These are being uploaded as we receive them.

Q. I have been assigned some refids that are abstracts or conference proceedings, what do I do with these?

A. Ashley has been contacting authors to try and get full papers of those she could not link to a publication already in the database. If full papers cannot be found, we need to assess whether or not results are provided → If yes, QA; If no, please contact Ashley and do not QA until further notice.

Q. I have full papers with no or missing results (for example, tables missing from paper). What do I do with these?

A. Please QA these articles and make a note in the final QA question textbox indicating what is available (if anything) if you don't think any data is useful.

Question 1: "What is the study design specified by the author?"

Q. How specific does the study design have to be for the "other" category?

A. The definition in the tool specifies that the "other" category is for hybrid or other designs that are clearly stated in the article (title, keywords, abstracts, methods). This means they must be very specific. If an author identifies it as an observational study, trial, project etc., the answer is not reported.

Question 1/2: "What is the study designed specified by the author/identified by the reviewer?"

Q. If the investigators rent a corner of a large commercial operation and apply a treatment to 250 birds, is this a field based trial?

A. Yes – for the purposes of questions 1,2, answer either CT or ChT (depending on study design), but the fact that it is a field trial has implications on whether or not questions in the tool are applicable (questions 4, 5, 7, 8).

Question 3: "Was the sample size justified?"

Q: Is this question asking if the sample size is justified, or if the sample population is representative of the target population?

A: We want to know if they said why or how they decided to sample what they did (whether or not it was justified). Question 7 will assess the representativeness of the sample.

Questions 5,6,7: "How were operations (hatchery/farm/processors) or (batch/flock/pen) or (egg/broiler/carcass) selected to participate in this study?"

Q: How are we to answer if there are different answers for different levels (e.g. processor is convenient, and farm is random)?

A: There will be a textbox for each answer in SRS where you can indicate which level your response is for.

Question 8: "Were the birds housed, grouped or slaughtered in a way that is representative of field conditions?"

Q: How do I know if the conditions are representative?

A: The minimum number per house is 15, 000, but please also refer to the environment and density.

Question 11: "Were the exposure and/or risk factors sufficiently described and measured appropriately?"

Q: What's the difference between exposure and risk factors?

A: The exposure in cohort, case control and cross-sectional studies is whatever the researcher is measuring. For example, exposure could be a vaccine, antimicrobial in feed, single vs. triple tank, etc. In some studies "exposure" and "risk factors" will be very clearly separate, and in others, it will be quite muddled.

Q: Is this referring to risk factors the authors mention in the paper, or are we expected to know the range of risk factors they should be considering?

A: This is specifically a reported variable. If the author stated that big barns are at greater risk, did they define what a big barn is?

Question 13: "Were the intervention protocols described in sufficient detail to allow reproduction of the experiment?"

Q. If a study does not fully specify the composition of the competitive exclusion culture, how should this question be answered?

A. Please answer yes. This problem will be dealt with at DE.

Question 16: "Was the time from intervention administration to first measurement of outcome reasonable for the intervention to have had an effect?"

Q: How do I know if the time was reasonable?

A: Please refer to the guidelines for this question.

Question 17: "Was the sample population tested for *Salmonella* status/prevalence at the beginning of the study?"

Q. If they used a strain of *Salmonella* that is selected for resistance to NA or some other antimicrobial should the answer be not applicable?

A. Yes, however, if they used a special or rare strain but also tested the sample population for *Salmonella* prior to the intervention, the answer should be yes.

Q. A study tested paper liners prior to the intervention administration, how do I answer this question?

A. Please answer yes (this will be checked with Bob Wills and reviewers will be notified of any changes).

Q. A study used specific pathogen free (SPF) birds, how do I answer this question?

A. Please answer no unless they mention testing the population for *Salmonella* prior to intervention administration (this will be checked with Bob Wills and reviewers will be notified of any changes).

Question 19: "Did the author report that blinding was used?"

Q: Is this question specific to blinding of the outcome assessor?

A: No, the answer may be yes for this question if other types of blinding are used (for example, if a farmer is blinded to the status of his birds – treatment or no treatment, the answer would be yes).

Q: Does this have to be specified by the author in the paper to answer yes?

A: Yes, the author must clearly specify in the paper that blinding was used.

Question 20: "Were the reasons for, and the proportion of, prospective participants that declined participation reported?"

Q: Do both parts of this question have to be met to answer yes?

A: Yes, the reasons and the proportion must be reported to answer yes.

Question 21: "Were mortality, withdrawals and/or loss to follow-up <15%?"

Q: If the author doesn't report loss to follow-up, how should we answer?

A: For studies that don't indicate that there were any losses, the answer should be yes (unless we can see from the initial enrolment to the sample size in the results there

has been a loss greater than 15%). For studies that report losses but don't provide reasons and/or proportion, we would answer no (again, unless we can see this in initial enrolment and sample size in the results). Basically, any study that provides initial enrolment and sample size in the results, we need to check to make sure there hasn't been a loss greater than 15% in order to answer yes (however, not all studies will provide this, and so the above guidelines should be followed in those cases).

Question 22: "Was the statistical analysis described adequately so they can be reproduced?"

Q: What if no statistical analysis is performed?

A: As indicated in question 23, if no statistical analysis is performed the answer should be statistical analysis not done.

Q: What if I don't see a statistical analysis, but a p-value is provided in the paper?

A: Any study that provides a p-value we will consider having conducted a statistical analysis (including one-way ANOVA and t-tests). If the study provides percentage with no p-value, this is not a statistical analysis (including IF's and PF's). Please note that we are only interested in *Salmonella* as an outcome, not other outcomes (such as performance measurements).

Question 23: "Were identified confounders controlled for or tested?"

Q: Is this referring to confounders the authors mention in the paper, or are we expected to know the range of confounders they should be considering?

A: This is specifically a reported variable. Only confounders that the author identifies in the paper are of concern to us.

Question 24: "Based on the study design, was clustering accounted for appropriately in the analysis?"

Q. How do I know if clustering is present?

A. Is there group hierarchy, repeated measures or multiple replicates? Were the results collapsed/pooled over the hierarchy, repeated measures or multiple replicates?

If yes = Clustering → Did they use something to account for the clustering in the analysis → If yes, answer yes on QA form; If no, answer no on QA form.

If no = No clustering → Answer N/A on QA form

Question 27: "Were model diagnostics presented?"

Q: Does ANOVA count as a model?

A: Yes, ANOVA may be included as a model. However, one-way ANOVA's are not considered to be a model (answer 26 with this information, and answer N/A for question 27).

Question 28: "Additional comments"

Q: What information are you looking for here?

A: This is a place the reviewer can comment if they feel there is something that was not captured in the tool but should be acknowledged during QA.

Appendix 10. Reasons studies excluded from data extraction (n=43) in a systematic review on the use of competitive exclusion in *Salmonella* in broiler chickens

FIRST AUTHOR	TITLE	JOURNAL	YEAR	VOL(ISSUE), PAGES
NO RAW/UNADJUSTED OR ADJUSTED DATA WITH MEASURES OF VARIABILITY (n=40)				
Adler, H.E.	Effect of ingested Lactobacilli on *Salmonella* infantis and *Escherichia* coli and on intestinal flora, pasted vents, and chick growth	Avian Dis.	1980	24(4), 868-78
Andreatti Filho, R.L.	Use of anaerobic cecal microflora, lactose and acetic acid for the protection of broiler chicks against experimental infection with *Salmonella* typhimurium and *Salmonella* enteritidis	Braz. J. Microbiol.	2000	31(2), 107-12
Awaad, M.H.H.	Effect of *Pediococcus* acidilactici and *Saccharomyces* boulardii as probiotics on intestinal and caecal colonization of *Salmonella* typhimurium and *Clostridium* perfringens in broiler chickens	Egypt. J. Vet. Sci.	2003	37, 127-136
Baba, E.	The role of intestinal microflora on the prevention of *Salmonella* colonization in gnotobiotic chickens	Poult. Sci.	1991	70(9), 1902-7
Bailey, J.S.	Resistance to challenge of breeders and their progeny with and without competitive exclusion treatment to *Salmonella* vaccination programs in broiler breeders	Int. J. Poult. Sci.	2007	6(6), 386-92
Barnes, E.M.	Manipulation of the crop and intestinal flora of the newly hatched chick	Am. J. Clin. Nutr.	1980	33(11), 2426-33
Bolder, N.M.	Effect of antibiotic treatment on competitive exclusion against *Salmonella* enteritidis PT4 in broilers	Vet. Rec.	1995	137(14), 350-1
Corrier, D.E.	Control of *Salmonella* colonization of poultry: Dietary lactose enhances natural resistance mechanism	Proceedings of the 39th Western Poultry Disease Conference	1990	92-93

DeLoach, J.R.	Defined competitive exclusion culture for prevention of *Salmonella* in poultry	Proceedings of the 45th Western Poultry Disease Conference	1996	243-5
Dudikova, G.	*Salmonella* and E. coli associated infections at a poultry farm	Biotechnology in Agriculture and the Food Industry	2004	23-33
Gil de los Santos, J.R.	*Bacillus* cereus var. toyoii and *Saccharomyces* boulardii increased feed efficiency in broilers infected with *Salmonella* enteritidis	Br. Poult. Sci.	2005	46(4), 494-7
Goren, E.	Termination of *Salmonella* enteritidis shedding and carriage by treatment with enfrofloxacin followed by application of intestinal microflora	Proceedings of the 42nd Western Poultry Disease Conference	1993	72-3
Goren, E.	Termination of *Salmonella* enteritidis shedding and carriage by treatment with enfrofloxacin followed by application of intestinal microflora	Proceedings of the 42nd Western Poultry Disease Conference	1993	72-3
Goren, E.	Protection of chicks against *Salmonella* infantis infection induced by strict anaerobically cultured intestinal microflora	Vet. Q.	1984	6(1), 22-6
Goren, E.	Protection of chicks against *Salmonella* infection induced by spray application of intestinal microflora in the hatchery	Vet. Q.	1984	6(2), 73-9
Hudault, S.	Elimination of *Salmonella* typhimurium from the digestive tract by the flora of conventional chicken	8th International Symposium on Germfree Research	1984	163-7
Impey, C.S.	Attempt to control stunting in chickens by "competitive exclusion"	Vet. Rec.	1984	115(2), 36-7
Impey, C.S.	Evaluation of treatment with defined and undefined mixtures of gut microorganisms for preventing *Salmonella* colonization in chicks and turkey poults	Food Microbiol.	1984	1(2), 143-7
Jones, F.T.	Microbial compound holds promise as *Salmonella* control	Feedstuffs	1990	62(26), 13-14
Koscova, J.	Effect of two plant extracts and Lactobacillus fermentum on colonization of gastrointestinal tract by *Salmonella*	Biologia	2006	61(6), 775-8

	enterica var. Dusseldorf in chicks			
Lafont, J.P.	Practical limits to the use of competitive exclusion for the protection of poultry against *Salmonella*	Priority aspects of salmonellosis research. A workshop held in Brussels	1984	323-30
Lafont, J.P.	Experimental study of some factors limiting "competitive exclusion" of *Salmonella* in chickens	Res. Vet. Sci.	1983	34(1), 16-20
Martin, C.	Drinking water delivery of a defined competitive exclusion culture (Preempt) in 1-day-old broiler chicks	J. Appl. Poult. Res.	2000	9(1), 88-91
Nisbet, D.	Defined competitive exclusion cultures in the prevention of enteropathogen colonisation in poultry and swine	Antonie Van Leeuwenhoek	2002	81(1-4), 481-6
Nuotio, L.	Use of competitive exclusion to protect newly-hatched chicks against intestinal colonisation and invasion by *Salmonella* enteritidis PT4	Br. Poult. Sci.	1992	33(4), 775-9
Obsioma, V.P.	Comparison of competitive exclusion culture, inulin and flavophospholipol treatments in controlling *Salmonella* colonization in broilers	Philipp. J. Vet. Med.	2006	43(1), 18-25
Rambousek, M.J.	The effect of carbohydrate administration on experimental infection with *Salmonella* serotypes in chickens	Rev. Microbiol.	1995	26(1), 32-6
Reynolds, D.J.	Evaluation of combined antibiotic and competitive exclusion treatment in broiler breeder flocks infected with *Salmonella* enterica serovar enteritidis	Avian Pathol.	1997	26(1), 83-95
Salvat, G.	Use of competitive exclusion product (Broilact) to prevent *Salmonella* colonization of newly hatched chicks	Int. J. Food Microbiol.	1992	15(3-4), 307-11
Schneitz, C.	Effect of Broilact on the physicochemical conditions and nutrient digestibility in the gastrointestinal tract of broilers	Poult. Sci.	1998	77(3), 426-32
Schneitz, C.	Research note: Automated droplet application of a competitive exclusion preparation	Poult. Sci.	1992	71(12), 2125-8
Schneitz, C.	Automated droplet application of a competitive exclusion preparation	Poult. Sci.	1992	71(12), 2125-8
Schneitz, C.	Droplet application for protecting chicks against	Vet. Rec.	1990	126(20), 510

	Salmonella colonisation by competitive exclusion			
Schneitz, C.	Pilot-scale testing of the competitive exclusion method in chickens	Br. Poult. Sci.	1991	32(4), 881-4
Sterzo, E.V.	Time required to protect the intestinal tract of chicks against *Salmonella* enterica serovar enteritidis using competitive exclusion	Rev. Bras. Cienc. Avi.	2005	7(2), 119-22
Toro, H.	Use of bacteriophages in combination with competitive exclusion to reduce *Salmonella* form infected chickens	Avian Dis.	2005	49(1), 118-24
Weinack, O.M.	Therapeutic trials with native intestinal microflora for *Salmonella* typhimurium infections in chickens	Avian Dis.	1985	29(4), 1230-4
Yamamoto, S.	Inhibitory effect of normal flora on prevalence of *Salmonella* typhimurium in experimentally infected broiler chickens	Proceedings of the 5th AAAP Animal Science Congress	1990	202
Zacconi, C.	Effect of administration of Lactobacillus salivarius and lactic microflora in chick digestive tract	Ann. Microbiol. Enzimol.	1999	49(2), 117-123
NO CONTROL GROUP (n=2)				
Mead, G.C.	Control of *Salmonella* colonization in poultry flocks by defined gut-flora treatment	Proceedings of the International Symposium on *Salmonella*	1984	74-5
Hudault, S.	Efficiency of various bacterial suspensions derived from cecal floras of conventional chickens in reducing the population level of *Salmonella* typhimurium in gnotobiotic mice and chicken intestines	Can. J. Microbiol.	1985	31(9), 832-8
DUPLICATE (n=1)				

Appendix 11. Studies included and reasons excluded in the meta-analyses-meta-regression of primary research evaluating competitive exclusion in broiler chickens

FIRST AUTHOR	TITLE	JOURNAL	YEAR	VOL(ISSUE), PAGES
STUDIES INCLUDED IN BOTH THE META-ANALYSIS (n=59) AND META-REGRESSION (n=104)				
Ali, H.A.[1,4]	Recycling poultry waste as feed for chickens and its protective effect on *Salmonella* typhimurium infection	Vet. Med. J. Giza	1996	44(4), 709-17
Andreatti Filho, R.L. [1,4]	Use of cecal microflora cultured under aerobic or anaerobic conditions in the control of experimental infection of chicks with *Salmonella* Enteritidis	Vet. Microbiol.	2003	92(3), 237-44
Bailey, J.S.[1]	Effect of *Salmonella* in young chicks on competitive exclusion treatment	Poult. Sci.	1998	77(3), 394-9
Barbour, E.K. [1]	Emergence of *Salmonella* enteritidis outbreaks in broiler chickens in the Lebanon: Epidemiological markers and competitive exclusion control	Rev. Sci. Tech.	1999	18(3), 710-8
Barnes, E.M. [1]	Competitive exclusion of *Salmonellas* from the newly hatched chick	Vet. Rec.	1980	106(3), 61
Blanchfield, B. [1,4]	Minimum intestinal inoculum for Nurmi cultures and a new method for determining competitive exclusion of *Salmonella* from chicks	J. Food Prot.	1984	47(7), 542-545
Blanchfield, B. [1]	Nurmi concept for preventing infection of chicks by *Salmonella:* Comparison of fecal suspensions and fecal cultures administered into the crop and in drinking water	J. Food Prot.	1982	45(4), 345-7
Blankenship, L.C. [1]	Two-step mucosal competitive exclusion flora treatment to diminish *Salmonellae* in commercial broiler chickens	Poult. Sci.	1993	72(9), 1667-72
Cameron, D.M. [1]	Evaluation of Aviguard against a *Salmonella* enteritidis infective model in broiler chickens	Western Poultry Disease Conference	1996	256-9
Cameron, D.M. [1,4]	Evaluation of the efficacy of Broilact in preventing infection of broiler chicks with *Salmonella* enteritidis PT4	Int. J. Food Microbiol.	1992	15(3-4), 319-26
Chen, M. [1,4]	Administering mucosal competitive exclusion flora for	J. Appl. Poult. Res.	1998	7(4), 384-91

	control of *Salmonellae*			
Corrier, D.E.[2]	Dosage titration of a characterized competitive exclusion culture to inhibit *Salmonella* colonization in broiler chickens during growout	J. Food Prot.	1998	61(7), 796-801
Corrier, D.E.[2]	Effect of simultaneous or delayed competitive exclusion treatment on the spread of *Salmonella* in chicks	J. Appl. Poult. Res.	1998	7(2), 132-7
Corrier, D.E.[2]	Spray application and dosage titration of characterized CF3 competitive exclusion culture for *Salmonella* control in broiler chickens	Western Poultry Disease Conference	1996	272-5
Corrier, D.E.[2,4]	Control of *Salmonella* typhimurium colonization in broiler chicks with a continuous-flow characterized mixed culture of cecal bacteria	Poult. Sci.	1995	74(6), 916-24
Corrier, D.E. [1]	Treatment of commercial broiler chickens with a characterized culture of cecal bacteria to reduce *Salmonellae* colonization	Poult. Sci.	1995	74(7), 1093-1101
Corrier, D.E.[2]	Resistance against *Salmonella* enteritidis cecal colonization in Leghorn chicks by vent lip application of cecal bacteria culture	Poult. Sci.	1994	73(5), 648-52
Corrier, D.E.[2,4]	Competitive exclusion of *Salmonella* enteritidis in Leghorn chicks: Comparison of treatment by crop gavage, drinking water, spray, or lyophilized alginate beads	Avian Dis.	1994	38(2), 297-303
Ferreira, A.J. [1,4]	Comparison of three commercial competitive-exclusion products for controlling *Salmonella* colonization of broilers in Brazil	J. Food Prot.	2003	66(3), 490-2
Goren, E. [1,4]	Protection of chicks against *Salmonella* infection induced by spray application of intestinal microflora in the hatchery	Vet. Q.	1984	6(2), 73-9
Higgins, J.P. [1]	Temporal effects of lactic acid bacteria probiotic culture on *Salmonella* in neonatal broilers	Poult. Sci.	2007	86(8), 1662-6
Higgins, S.E. [1]	Effect of probiotic treatment in broiler chicks on intestinal macrophage numbers and phagocytosis of *Salmonella* enteritidis by abdominal exudate cells	Poult. Sci.	2007	86(11), 2315-21

Hollister, A.G.[3,4]	Effect of lyophilization in sucrose plus dextran and rehydration in thioglycollate broth on performance of competitive exclusion cultures in broiler chicks	Poult. Sci.	1995	74(3), 586-90
Hollister, A.G.[3]	Effect of cecal cultures lyophilized in skim milk of Reagent 20 on *Salmonella* colonization in broiler chicks	Poult. Sci.	1994	73(9), 1409-16
Hume, M.E.[3,4]	Early *Salmonella* challenge time and reduction in chick cecal colonization following treatment with a characterized competitive exclusion culture	J. Food Prot.	1998	61(6), 673-6
Hume, M.E.[2]	Reduction of *Salmonella* crop and cecal colonization by a characterized competitive exclusion culture in broilers during grow-out	J. Food Prot.	1996	59(7), 688-93
Impey, C.S. [1]	Fate of *Salmonellas* in the alimentary tract of chicks pre-treated with a mature caecal microflora to increase colonization resistance	J. Appl. Bacteriol.	1989	66(6), 469-75
Impey, C.S. [1]	Influence of continuous challenge via the feed on competitive exclusion of *Salmonellas* from broiler chicks	J. Appl. Bacteriol.	1987	63(2), 139-46
La Ragione, R.M.[2,4]	In vivo characterization of Lactobacillus johnsonii FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry	Lett. Appl. Microbiol.	2004	38(3), 197-205
La Ragione, R.M.[2,4]	Competitive exclusion by Bacillus subtilis spores of *Salmonella* enterica subtype Enteritidis and *Clostridium* perfringens in young chickens	Vet. Microbiol.	2003	94(3), 245-56
Maruta, K.[2]	Exclusion of intestinal pathogens by continuous feeding with Bacillus subtilis C-3102 and its influence on intestinal microflora in broilers	Anim. Sci. Tech.	1996	67(3), 273-80
Nisbet, D.J.[2]	Maintenance of the biological efficacy in chicks of a cecal competitive exclusion culture against *Salmonella* by continuous-flow fermentation	J. Food Prot.	1996	59(12), 1279-83
Nisbet, D.J.[3]	Cecal propionic acid as a biological indicator of the early establishment of a microbial ecosystem inhibitory to *Salmonella* in chicks	Anaerobe	1996	2(6), 345-50

Nurmi, E. [1,4]	New aspects of *Salmonella* infection in broiler production	Nature	1973	241(5386), 210-11
Orencia, M.B. [1]	Efficacy of two preparations for controlling cecal colonization of *Salmonella* by competitive exclusion in broiler chicks	Phillipp. J. Vet. Med.	2001	38(2), 75-8
Oyarzabal, O.A.[2]	Application of direct-fed microbial bacteria and fructooligosaccharides for *Salmonella* control in broilers during feed withdrawal	Poult. Sci.	1996	75(2), 186-90
Pivnick, H. [1]	Comparison of fresh faeces with lyophilized and frozen cultures of faeces as inocula to prevent *Salmonella* infection in chicks	J. Food Prot.	1982	45(13), 1188-94, 1196
Pivnick, H. [1]	Prevention of *Salmonella* infection in chicks by treatment with fecal cultures from mature chickens (Nurmi cultures)	J. Food Prot.	1981	44(12), 909-16
Prukner-Radovcic, E.[2]	Competitive exclusion against *Salmonella* enterica subspecies enterica serovar Enteritidis infection in chickens	Vet. Arhiv	2003	73(3), 141-52
Rantala, M. [1]	Cultivation of a bacterial flora able to prevent the colonization of *Salmonella* infantis in the intestines of broiler chickens, and its use	Acta Pathol. Microbiol. Scandin.	1974	82(1), 75-80
Rantala, M. [1 4]	Prevention of the growth of *Salmonella* infantis in chicks by the flora of the alimentary tract of chickens	Br. Poult. Sci.	1973	14(6), 627-30
Revolledo, L. [1]	Comparison of experimental competitive-exclusion cultures for controlling *Salmonella* colonization in broiler chicks	Braz. J. Microbiol.	2003	34(4), 354-8
Schneitz, C. [1,4]	Comparison of two different types of competitive exclusion products	Lett. Appl. Microbiol.	1998	26(5), 338-41
Schneitz, C. [1]	Efficacy of different microbial preparations for controlling *Salmonella* colonisation in chicks and turkey poults by competitive exclusion	Br. Poult. Sci.	1992	33(1), 207-11
Schneitz, C. [1]	The anaerobically cultured cecal flora of adult fowls that protects chickens from *Salmonella* infections	Acta Pathol. Microbiol. Scandin.	1981	89(2), 109-16
Seuna, E. [1]	An epizootic of *Salmonella* typhimurium var. copenhagen in broilers and the use of cultured chicken intestinal flora for its	Br. Poult. Sci.	1978	19(3), 309-14

	control			
Snoeyenbos, G.H. [1,4]	Protecting chicks and poults from *Salmonellae* by oral administration of "normal" gut microflora	Avian Dis.	1978	22(2), 273-87
Soerjadi, A.S. [1,4]	The influence of lactobacilli on the competitive exclusion of paratyphoid *Salmonellae* in chickens	Avian Dis.	1981	25(4), 1027-33
Soerjadi, A.S. [1]	Some measurements of protection against paratyphoid *Salmonella* and *Escherichia* coli by competitive exclusion chickens	Avian Dis.	1981	25(3), 706-12
Stavric, S. [1,4]	Experience of the use of probiotics for *Salmonellae* control in poultry	Lett. Appl. Microbiol.	1992	14(3), 69-71
Stavric, S. [1]	Competitive exclusion of *Salmonella* from newly hatched chicks by mixtures of pure bacterial cultures isolated from fecal and cecal contents of adult birds	J. Food Prot.	1985	48(9), 778-82, 785
Stern, N.J.[2,4]	Comparison of mucosal competitive exclusion and competitive exclusion treatment to reduce *Salmonella* and *Campylobacter* spp. colonization in broiler chickens	Poult. Sci.	2001	80(2), 156-60
Stersky, A. [1]	Reduction of *Salmonella* excretion into drinking water following treatment of chicks with Nurmi culture	J. Food Prot.	1981	44(12), 917-920
Tellez, G.[2]	Evaluation of avian-specific probiotic and *Salmonella* enteritidis-, *Salmonella* typhimurium-, and *Salmonella* heidelberg-specific antibodies on cecal colonization and organ invasion of *Salmonella* enteritidis in broilers	J. Food Prot.	2001	64(3), 287-91
Watkins, B.A. [1,4]	Competitive gut exclusion of avian pathogens by Lactobacillus acidophilus in gnotobiotic chicks	Poult. Sci.	1983	62(9), 1772-9
Weinack, O.M. [1,4]	Reciprocal competitive exclusion of *Salmonella* and *Escherichia* coli by native intestinal microflora of the chicken and turkey	Avian Dis.	1982	26(3), 585-95
Wolfenden, A.D. [1]	Evaluation of spray application of a Lactobacillus-based probiotic on *Salmonella* enteritidis colonization in broiler chickens	Int. J. Poult. Sci.	2007	6(7), 493-6
Wolfenden,	Effect of a defined competitive exclusion culture for	Int. J. Poult. Sci.	2007	6(7), 489-92

A.D.[1]	prophylaxis and reduction of horizontal transmission of *Salmonella* enteritidis in broiler chickens			
Zhang, G.[3]	*Salmonellae* reduction in poultry by competitive exclusion bacteria Lactobacillus salivarius and Streptococcus cristatus	J. Food Prot.	2007	70(4), 874-8
STUDIES INCLUDED IN THE META-REGRESSION, EXCLUDED FROM THE META-ANALYSIS (n=59)				
COMBINATION INTERVENTION (n=37)				
Andreatti Filho, R.L.[4]	Ability of bacteriophages isolated from different sources to reduce *Salmonella* enterica serovar Enteritidis in vitro and in vivo	Poult. Sci.	2007	86(9), 1904-9
Bailey, J.S.	Effect of fructooligosaccharide on *Salmonella* colonization of the chicken intestine	Poult. Sci.	1991	70(12), 2433-8
Bailey, J.S.	Effect of anticoccidial and antimicrobial feed additives on prevention of *Salmonella* colonization of chicks treated with anaerobic cultures of chicken feces	Avian Dis.	1988	32(2), 324-9
Bilgili, S.F.	Influence of whey and probiotic-supplemented withdrawal feed on the retention of *Salmonella* intubated into market age broilers	Poult. Sci.	1990	69(10), 1670-4
Corrier, D.E.[4]	Inhibition of *Salmonella* enteritidis cecal and organ colonization in Leghorn chicks by a defined culture of cecal bacteria and dietary lactose	J. Food Prot.	1994	57(5), 377-81
Corrier, D.E.[4]	Protective effect of used poultry litter and lactose in the feed ration on *Salmonella* enteritidis colonization of leghorn chicks and hens	Avian Dis.	1993	37(1), 47-52
Corrier, D.E.	Development of defined cultures of indigenous cecal bacteria to control salmonellosis in broiler chicks	Poult. Sci.	1993	72(6), 1164-8
Corrier, D.E.	Effect of dietary lactose on *Salmonella* colonization of market-age broiler chickens	Avian Dis.	1990	34(3), 668-676
Fernandez, F.[4]	Evaluation of the effect of mannan-oligosaccharides on the competitive exclusion of *Salmonella* enteritidis colonization in broiler chicks	Avian Pathol.	2000	29(6), 575-81
Fukata, T.[4]	Inhibitory effects of competitive exclusion and	J. Food Prot.	1999	62(3), 229-33

	fructooligosaccharide, singly and in combination, on *Salmonella* colonization of chicks			
Hinton, A. Jr.	Comparison of the efficacy of cultures of cecal anaerobes as inocula to reduce *Salmonella* typhimurium colonization in chicks with or without dietary lactose	Poult. Sci.	1991	70(1), 67-73
Hinton, M.	Protection of chicks against environmental challenge with *Salmonella* enteritidis by "competitive exclusion" and acid-treated feed	Lett. Appl. Microbiol.	1991	12(3), 69-71
Hinton, A. Jr.	Biological control of *Salmonella* typhimurium in young chickens	Avian Dis.	1990	34(3), 626-33
Hollister, A.G.[4]	Effect of cecal cultures encapsulated in alginate beads or lyophilized in skim milk and dietary lactose on *Salmonella* colonization in broiler chicks	Poult. Sci.	1994	73(1), 99-105
Hollister, A.G.	Comparisons of effects of chicken cecal microorganisms maintained in continuous culture and provision of dietary lactose on cecal colonization by *Salmonella* typhimurium in turkey poults and broiler chicks	Poult. Sci.	1994	73(5), 640-7
Humbert, F.	Effect of four antibiotic additives on the *Salmonella* contamination of chicks protected by an adult caecal flora	Avian Pathol.	1991	20(4), 577-84
Impey, C.S.[4]	Competitive exclusion of *Salmonellas* from the chick caecum using a defined mixture of bacterial isolates from the caecal microflora of an adult bird	J. Hyg. (Lond.)	1982	89(3), 479-90
Jarquin, R.L.	The evaluation of organic acids and probiotic cultures to reduce *Salmonella* enteritidis horizontal transmission and crop infection in broiler chickens	Int. J. Poult. Sci.	2007	6(3), 182-6
Kogut, M.H.	Effect of *Eimeria* tenella infection on resistance to *Salmonella* typhimurium colonization in broiler chicks inoculated with anaerobic cecal flora and fed dietary lactose	Avian Dis.	1994	38(1), 59-64
Kubena, L.F.	Cecal volatile fatty acids and broiler chick susceptibility to *Salmonella* typhimurium colonization as affected by aflatoxins and T-2 toxin	Poult. Sci.	2001	80(4), 411-7

McReynolds, J.L.	Evaluation of a competitive exclusion culture and megan vac 1 on *Salmonella* typhimurium colonization in neonatal broiler chickens	J. Appl. Poult. Res.	2007	16(3), 456-63
Nisbet, D.J.[4]	Inoculation of broiler chicks with a continuous-flow derived bacterial culture facilitates early cecal bacterial colonization and increases resistance to *Salmonella* typhimurium	J. Food Prot.	1994	57(1), 12-5
Nisbet, D.J.	Effect of dietary lactose and cell concentration on the ability of a continuous-flow-derived bacterial culture to control *Salmonella* cecal colonization in broiler chickens	Poult. Sci.	1994	73(1), 56-62
Nisbet, D.J.	Effect of a defined continuous-flow derived bacterial culture and dietary lactose on *Salmonella* typhimurium colonization in broiler chickens	Avian Dis.	1993	37(4), 1017-25
Nisbet, D.J.	Effect of mixed cecal microflora maintained in continuous culture and of dietary lactose on *Salmonella* typhimurium colonization in broiler chicks	Avian Dis.	1993	37(2), 528-35
Nurmi, E.	The influence of zinc bacitracin on the colonization of *Salmonella* infantis in the intestine of broiler chickens	Res. Vet. Sci.	1974	17(1), 24-7
Opitz, H.M.[4]	Effectiveness of five feed additives in chicks infected with *Salmonella* enteritidis phage type 13a	J. Appl. Poult. Res.	1993	2(2), 147-53
Qin, Z.R.	Effect of lactose and Lactobacillus acidophilus on the colonization of *Salmonella* enteritidis in chicks concurrently infected with *Eimeria* tenella	Avian Dis.	1995	39(3), 548-53
Rahimi, S.	Prevention of *Salmonella* infection in poultry by specific egg-derived antibody	Int. J. Poult. Sci.	2007	6(4), 230-5
Seo, K.H.[4]	Elimination of early *Salmonella* enteritidis infection after treatment with competitive-exclusion culture and enrofloxacin in experimentally infected chicks	Poult. Sci.	2000	79(10), 1408-13
Seuna, E.	Combined therapy of *Salmonella* infection in chickens by antimicrobial agents followed by cultured cecal bacteria	Poult. Sci.	1980	59(6), 1187-92
Snoeyenbos, G.H.[4]	Large-scale trials to study competitive exclusion of *Salmonella* in chickens	Avian Dis.	1985	29(4), 1004-11

Snoeyenbos, G.H.	Further studies on competitive exclusion for controlling *Salmonellae* in chickens	Avian Dis.	1979	23(4), 904-14
Weinack, O.M.[4]	Influence of Mycoplasma gallisepticum, infectious bronchitis, and cyclophosphamide on chickens protected by native intestinal microflora against *Salmonella* typhimurium or *Escherichia* coli	Avian Dis.	1984	28(2), 416-25
Wolfenden, A.D.	Effect of organic acids and probiotics on *Salmonella* enteritidis infection in broiler chickens	Int. J. Poult. Sci.	2007	6(6), 403-5
Zacconi, C.	Competitive exclusion of *Salmonella* kedougou in kefir fed chicks	Microbiol. Ali. Nutr.	1994	12(4), 387-90
Ziprin, R.L.	Intracloacal *Salmonella* typhimurium infection of broiler chickens: Reduction of colonization with anaerobic organisms and dietary lactose	Avian Dis.	1990	34(3), 749-53
NOT CECAL CONTENTS, CECA, CECAL TONSILS (n=14)				
Fritts, C.A.[4]	Bacillus subtilis C-3102 (calsporin) improves live performance and microbiological status of broiler chickens	J. Appl. Poult. Res.	2000	9(2), 149-55
Hume, M.E.[4]	Effect of a characterized continuous-flow culture of cecal bacteria on *Salmonella* typhimurium crop colonization in broiler chicks	Avian Dis.	1996	40(2), 391-7
Khaksefidi, A.	Effect of probiotic inclusion in the diet of broiler chickens on performance, feed efficiency and carcass quality	Asian-Aust. J. Anim. Sci.	2005	18(8), 1153-6
Lloyd, A.B.[4]	Prevention of *Salmonella* typhimurium infection in poultry by pretreatment of chickens and poults with intestinal extracts	Aust. Vet. J.	1977	53(2), 82-7
Netherwood, T.[4]	Probiotics shown to change bacterial community structure in the avian gastrointestinal tract	Appl. Environ. Microbiol.	1999	65(11), 5134-8
Palmu, L.	The use of competitive exclusion in broilers to reduce the level of *Salmonella* contamination on the farm and at the processing plant	Poult. Sci.	1997	76(11), 1501-5
Rigby, C.E.	Observations on competitive exclusion for preventing *Salmonella* typhimurium infection of broiler chickens	Avian Dis.	1980	24(3), 604-15

Seuna, E.	Sensitivity of young chickens to *Salmonella* typhimurium var. Copenhagen and S. infantis infection and the preventive effect of cultured intestinal microflora	Avian Dis.	1979	23(2), 392-400
Soerjadi, A.S.	Streptococcus faecalis, a bacterial isolate which protects young chickens from enteric invasion by *Salmonellae*	Aust. Vet. J.	1978	54(11), 549-50
Takahashi, S.E.	Efficiency of prebiotics and probiotics on the performance, yield, meat quality and presence of *Salmonella* spp. in carcasses of free-range broiler chickens	Rev. Bras. Cienc. Avi.	2005	7(3), 151-7
Weinack, O.M.	A supplemental test system to measure competitive exclusion if *Salmonellae* by native microflora in the chicken gut	Avian Dis.	1979	23(4), 1019-30
Weinack, O.M.[4]	Further studies on competitive exclusion of *Salmonella* typhimurium by lactobacilli in chickens	Avian Dis.	1985	29(4), 1273-6
Wierup, M.	Experience of a 10-year use of competitive exclusion treatment as part of the *Salmonella* control programme in Sweden	Int. J. Food Microbiol.	1992	15(3-4), 287-91
Wierup, M.	Epidemiological evaluation of the *Salmonella*-controlling effect of a nationwide use of a competitive exclusion culture in poultry	Poult. Sci.	1988	67(7), 1026-33
NOT DAY OLD CHICKS (n=8)				
Avila, L.A.	Effects of probiotics and maternal vaccination on *Salmonella* enteritidis infection in broiler chicks	Avian Dis.	2006	50(4), 608-12
Bailey, J.S.	Commercial field trial evaluation of mucosal starter culture to reduce *Salmonella* incidence in processed broiler carcasses	J. Food Prot.	2000	63(7), 867-70
Bailey, J.S.	Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers	Poult. Sci.	1998	77(3), 405-10
Brzoska, F.[4]	The effects of lactic acid bacteria and mannan oligosaccharide, with or without fumaric acid, on chicken performance, slaughter yield and digestive tract microflora	J. Anim. Feed Sci.	2007	16(2), 241-51
Cox, N.A.	Research note: In ovo administration of a competitive exclusion culture treatment to broiler embryos	Poult. Sci.	1992	71(10), 1781-4
Goren, E.	Reduction of *Salmonella* infection of broilers by spray	Vet. Q.	1988	10(4), 249-55

	application of intestinal microflora: A longitudinal study			
Jin, L.Z.	Influence of dried Bacillus subtilis and lactobacilli cultures on intestinal microflora and performance in broilers	Asian-Aust. J. Anim. Sci.	1996	9(4), 397-403
Rantala, M.	Hazards involved in the use of furazolidone for the prevention of salmonellosis in broiler chickens	J. Hyg.	1974	72(3), 349-54
STUDIES EXCLUDED FROM BOTH THE META-ANALYSIS AND META-REGRESSION NO AVAILABLE PREVALENCE OR CONCENTRATION DATA (n=31)				
Abu-Ruwaida, A.S.	*Salmonella* exclusion in broiler chicks by the competitive action of adult gut microflora	Microbios	1995	83(334), 59-69
Behling, R.G.	Competitive exclusion of *Salmonella* enteritidis in chicks by treatment with a single culture plus dietary lactose	Int. J. Food Microbiol.	1994	22(1), 1-9
Bolder, N.M.	Prevention of colonization by *Salmonella* enteritidis PT4 in broiler chickens	Int. J. Food Microbiol.	1992	15(3-4), 313-7
Bowman, P.J.	Further studies on competitive exclusion in controlling *Salmonella* infections in young chickens	Proceedings of the First Australasian Poultry and Stock Feed Convention	1976	318-319
Chambers, J.R.	Probiotics and maternal vaccination for *Salmonella* control in broiler chickens	J. Appl. Poult. Res.	2002	11(3), 320-7
Corrier, D.E.	Control of established *Salmonella* typhimurium intestinal colonization with in vivo-passaged anaerobes	Avian Dis.	1993	37(1), 183-8
Corrier, D.E.	Control of *Salmonella* in broiler chicks inoculated with continuous-flow culture probiotic	Proceedings of the 19th World's Poultry Congress	1992	1, 440-4
De Oliveira, G.H.	Prevention of *Salmonella* infection by contact using intestinal flora of adult birds and/or a mixture of organic acids	Braz. J. Microbiol.	2000	31(2), 116-20
Droleskey, R.E.	Colonization of cecal mucosal epithelium in chicks treated with a continuous flow culture of 29 characterized bacteria: Confirmation by scanning electron microscopy	J. Food Prot.	1995	58(8), 837-42
Gleeson, T.M.	Protection of chicks against *Salmonella* infection with a mixture of pure cultures of intestinal bacteria	Avian Dis.	1989	33(4), 636-42
Hoszowski, A.	Prevention of *Salmonella* typhimurium caecal colonisation by	Comp. Immunol.	1997	20(2), 111-7

	different preparations for competitive exclusion	Microbiol. Infect. Dis.		
Javed, T.	Competitive exclusion of *Salmonella* by lactobacilli: A strategy to control salmonellosis in chicken	Proceedings of Pakistan Congress of Zoology	1993	13, 493-500
Lapinskaite, R.	The curative effect of preparation STF on chickens infected with *Salmonella* typhimurium	Biologija	1999	2, 40-3
Mahajan, P.	Effect of probiotic (Lacto-Sacc) feeding and season on poultry meat quality	Indian J. Poult. Sci.	2000	35(3), 297-301
Massa, S.	Protection of young chicks against *Salmonella* kedougou by administration of intestinal microflora	Int. J. Food Microbiol.	1998	40(1-2), 123-6
Mead, G.C.[4]	Control of *Salmonella* colonization in poultry flocks by defined gut-flora treatment	Proceedings of the International Symposium on *Salmonella*	1984	72-9
Meijerhof, R.	In ovo injection of competitive exclusion culture in broiler hatching eggs	J. Appl. Poult. Res.	1997	6(3), 260-6
Methner, U.[4]	Combination of competitive exclusion and immunization with an attenuated live *Salmonella* vaccine strain in chickens	Avian Dis.	2001	45(3), 631-8
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[1] Included in the prevalence meta-analysis
[2] Included in both the prevalence and concentration meta-analysis
[3] Included in the concentration meta-analysis
[4] This study was conducted in chicks of a laying breed