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Application of evidence-based medicine to veterinary science and food safety

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Application of evidence-based medicine to veterinary science and food safety

by

Thomas Nishantha Denagamage

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Veterinary Preventive Medicine

Program of Study Committee:
Annette O'Connor, Major Professor
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Ames, Iowa

2008

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TABLE OF CONTENTS

GENERAL INTRODUCTION	1
CHAPTER 1. AN INTRODUCTION TO EVIDENCE-BASED APPROACHES TO VETERINARY SCIENCE AND FOOD SAFETY: APPLICATION TO <i>SALMONELLA</i> IN SWINE	2
CHAPTER 2. AN INTRODUCTION TO THE TEST BASE AREA: <i>SALMONELLA</i> IN SWINE AS A FOODBORNE PATHOGEN	19
CHAPTER 3. EFFICACY OF VACCINATION TO REDUCE <i>SALMONELLA</i> PREVALENCE IN LIVE AND SLAUGHTERED SWINE: A SYSTEMATIC REVIEW OF LITERATURE FROM 1979 TO 2007	42
CHAPTER 4. A REVIEW OF ANTIBIOTICS TO REDUCE <i>SALMONELLA</i> IN SWINE: A QUANTITATIVE ANALYSIS OF BINOMIAL AND CONTINUOUS DATA	72
CHAPTER 5. EVALUATING THE SENSITIVITY AND SPECIFICITY OF CULTURE AND PCR METHODS FOR DETECTION OF <i>SALMONELLA</i> IN FIELD SAMPLES USING THE STARD APPROACH	108

GENERAL CONCLUSIONS.....130

ACKNOWLEDGMENTS.....133

GENERAL INTRODUCTION

Thesis organization

This thesis is organized as follows. The 1st chapter is a brief discussion of the concepts of evidence-based medicine (EBM) and their potential for application to agri-food arena. The 2nd chapter is a brief background discussion of *Salmonella* in swine focusing on pre-harvest interventions available for controlling *Salmonella*. The subsequent chapters 3, 4, and 5 are examples of approaches used over the past 18 months to apply EBM principles to *Salmonella* in swine. Chapter 3 “Efficacy of vaccination to reduce *Salmonella* prevalence in live and slaughtered swine: a systematic review of literature from 1979 to 2007” is a systematic review of vaccine interventions for *Salmonella* and illustrates the application of the evidence pyramid and systemic review approach to *Salmonella* in swine. Chapter 4 “A review of antibiotics to reduce *Salmonella* in swine: a quantitative analysis of binomial and continuous data” illustrates the application of the evidence pyramid and systemic review approach including meta-analysis for continuous and categorical data to *Salmonella* in swine. Chapter 5 “Evaluating the sensitivity and specificity of PCR and culture method for detection of *Salmonella* in field samples using the STARD approach”, a diagnostic test evaluation study for *Salmonella* PCR and culture, illustrates the use of application of EBM statements study design, in particular, the STARD statement. The final discussion concludes the significance and impact of research findings of the present study.

CHAPTER 1

**AN INTRODUCTION TO EVIDENCE-BASED APPROACHES TO
VETERINARY SCIENCE AND FOOD SAFETY: APPLICATION TO
SALMONELLA IN SWINE**

Evidence-based medicine (EBM) is an area of clinical medicine that uses aims to combine the best scientific evidence with practical experience to make the decision about a patient's outcome. The concept has been used in human medicine for the past 15-20 years and has expanded to include areas of evidence-based public health and evidence based nursing.

In veterinary science and food safety, recent publications have discussed the need for evidence-based approaches to study design and literature review in agri-food public health to make informed decisions for food safety policy makers and other decision makers in food production continuum (Sargeant et al., 2006a; Sargeant et al., 2006b). The idea of formalizing the evidence-based approach to decision making is therefore relatively new to veterinary science and food safety. Veterinary science and food safety differ greatly from clinical medicine in many important areas including the unit of concern, i.e. individual versus groups, prevention of outcomes rather than treatment of disease, the influence of publication bias and the availability of challenge models to assess outcomes. Therefore, translation of EBM concepts to veterinary science and food safety will require careful consideration of what is applicable and when are changes needed for these unique fields.

Central to the concept of EBM is the idea that some information sources have greater evidentiary value and greater weight should be given to information obtained from higher quality information sources. A common information source is results from primary research studies, and central to EBM is the concept that individual studies should be reported in such a way that the quality of the study can be assessed. Further, when evaluating a body of work to reach a decision about treatment or intervention, the study design and the quality with which it was executed should be a consideration, and greater weight given to results obtained from well executed high quality designs. To promote these concepts, there are three primary tools in clinical EBM;

- 1) an evidence pyramid that described the hierarchical value of information sources
- 2) a series of statements that describe the elements within a study design that should be reported so that quality can be assessed and
- 3) a formal structure for incorporating 1 and 2 when evaluating a body of work i.e. systemic reviews which may include meta-analysis.

These tools have been designed for assessment of human therapy purposes. The purpose of this thesis has been to explore how these tools can be applied and issues associated with that application, to the food safety decision arena. The test base for the application is pre-harvest prevention of food borne disease associated with *Salmonella* in swine.

1) The evidence pyramid in human medicine

An evidence pyramid provides the framework for ranking information sources that evaluate health care interventions (Figure 1). The most clinically relevant study

designs are located at the top of the pyramid and the least clinically relevant sources such as in-vitro test tube studies are at the bottom of the pyramid. The value of evidence provided by an information source is associated with statistical power and potential for bias, with the quality of the individual studies critically evaluated. The statistical power of a study is dependent on sample size, magnitude of the effect of the intervention and the natural variation (Cockcroft and Holmes, 2003). The potential for bias such as confounding, selection and misclassification bias in an information source is higher in studies in lower positions on the pyramid. For example, when addressing therapy questions, randomized controlled trials (RCT) provide the best opportunity for examining effectiveness of interventions, as random allocation to treatment groups under natural conditions minimizes the potential for confounding bias compared to cohort studies and case-control studies. Further, based on EBM concepts, a large well-executed RCT would provide better evidence than a small well executed RCT. The highest form of evidence for a therapy is a systemic review with meta-analysis, which is a combination of several RCT with the same therapy and disease classification. A combination of well-executed RCT is considered better evidence than a single well-executed RCT because the potential impact of bias will be less and the number of unit studies is higher.

Challenges for veterinary science and food safety

There are numerous examples of RCTs available in human medicine for a specific question to be addressed in a systematic review. But, in veterinary medical context, specifically in on-farm intervention strategies RCTs are rare and research evidence primarily comes from observational studies such as cohort studies, case control studies and cross-sectional studies. Observational studies are easy to conduct in commercial

livestock production systems than the experimental RCTs. These study designs are useful for addressing issues about causation. Cohort studies and case control studies are useful for testing hypothesis, and cross-sectional studies are used to generate hypothesis. The hierarchy of evidence in the human evidence pyramid is limited to the available medical literature. In the absence of randomized controlled trials in veterinary science and agri-food research, there is a need to understand the evidentiary value of available research in these fields, and rank in the evidence pyramid.

Another unique characteristic of veterinary science and food safety is the challenge study design, an experimental study design that uses artificial inoculation of the animal with the disease agent (Sargeant et al., 2006b). Challenge studies provide the evidence of effectiveness of intervention under controlled conditions in the species of interest, but not under naturally occurring commercial setting with natural disease infection. In human therapy, this design is not available, and experimental challenge models are rated very poorly because the natural environment is missing and the species is not human. In veterinary science and food safety what value should this design have? It is unclear how this design should be valued as although the ability to experimentally infecting the species of interest is important, the absence of the natural environment is a serious limitation. Veterinary science has many examples of intervention efficacy under laboratory conditions in the species of interest that have not translated into field efficacy.

Challenge study outcomes may not reflect field outcomes for many reasons including the multi-factorial nature of disease and the clustering of animals in agriculture. Agricultural animals can be grouped into litters, pens, barns or lots within a commercial operation. Therefore, when applying interventions, the effect of animal clustering should

be considered. Most often challenge studies and even RCTs studies are conducted using small number of animals or groups, and limiting the findings of these studies to the commercial settings.

Publication bias refers to the higher probability of studies with positive results being published, and it is well known that only a proportion of research projects ultimately reach publication in peer reviewed journals (Egger et al., 2001). Publication bias occurs when sponsors do not allow studies unfavorable to their products to be published or allow only selective reporting of favorable studies. A condition of this funding is that data are owned by the firm and this may lead to publication bias more frequently in challenge trials than in observational research where the presence of industry is much limited. Other potential forms of publication bias include publishing funded research in supplements as proceedings of symposia. Researchers have raised concerns that studies with negative results may remain unpublished and their failure to appear in the literature can distort the conclusions that we obtain from clinical experiments regarding the best available evidence. Perhaps, this is a major concern in the field of veterinary science and food safety intervention studies since research in these areas get less government funding than the relevant human medicine research. Also, in human medicine, it is necessary to register all government funding research trials in clinical trial register in the United States (Anonymous, 2008a). But, there is no obligation to register privately funded trials in veterinary science and food safety research studies. Therefore, if evaluating only peer-reviewed literature might have the potential to distort evidence, and hence food-safety policy.

2) A series of statements that describe the elements within a study design - CONSORT statement and STARD statement

To improve the quality of reporting for studies, several standardized statements for reporting studies in human medicine have been developed; the consolidated standards of reporting trials (CONSORT) statement, the standards for the reporting of diagnostic accuracy studies (STARD) statement, and the reporting of meta-analysis of observational studies in epidemiology (MOOSE).

The first and most widely known statement is designed for RCTs, the consolidated standards of reporting trials (CONSORT) statement. which comprises a checklist and flow diagram for reporting a RCT (Begg et al., 1996; Moher et al., 2001). The CONSORT is primarily intended for use in writing, reviewing or evaluating reports of RCT, although it is frequently used as a tool for designing RCT. The aim of the CONSORT is to allow readers to assess the execution of the study design, and therefore, the evidentiary value of the results. The CONSORT statement checklist helps researchers write a clear and comprehensive report of the RCT while the flowchart helps the scientist and readers follow participants or subjects through the RCT. Using the checklist provided by the CONSORT, readers can determine if adequate information is given to assess the study execution, and based on that assessment the applicability and validity of the trial results. In theory, at least, if a trial is in adequately reported, the results are not given further consideration. The CONSORT statement has been adopted by more than 150 medical, clinical and psychological journals, and has been translated into several different languages (Anonymous, 2008b).

The CONSORT statement and checklist is primarily focusing on RCTs.

Application of CONSORT statement and checklist to veterinary science and food safety intervention studies is limited since the lack of such trials. It is possible to modify and adopt such statement for veterinary science and food safety intervention studies by modifying the checklist to fit for the challenge studies and observational studies. So far, CONSORT statement has been endorsed by only two journals that publish research articles related to veterinary science; The Veterinary Journal and The Equine Veterinary Journal (Anonymous, 2008b).

Another example of a standardized statement is the STARD which related to studies of diagnostic tests. Studies of diagnostic tests are required to validate new diagnostic tests before they are introduced into practice. These studies typically report sensitivity and specificity, likelihood ratios, or area under a receiver operating characteristics (ROC) curve as measures of diagnostic performance. Using this information enables judgments to be made relating to the potential suitability of new tests for clinical practice. However, improperly conducted and incompletely reported studies are likely to include bias that in turn, may lead to overly optimistic estimations of the diagnostic value of the test. Exaggerated results may lead to premature adoption of diagnostic tests and to incorrect clinical decisions. To improve the quality of reporting of diagnostic accuracy studies, the STARD statement consist of a checklist of 25 items and it is recommended to use of a flow diagram which describes the design of the study and the flow of patients (Bossuyt et al., 2003). This allows improving the accuracy and completeness of reporting of studies of diagnostic accuracy, to allow readers to assess the potential for bias in the study and to evaluate its generalisability to the external

populations. One of the ultimate benefits for using the STARD initiative is to develop a consistent reporting format across all types of diagnostic tests and clinical disciplines.

STARD statement and check list has been adopted by many leading medical journals (Anonymous, 2008c). So far, veterinary journals that publish diagnostic test accuracy studies have not adopted the STARD statement. Reporting diagnostic test accuracy studies related to veterinary science and food safety interventions with deficient information will lead to adopt inaccurate diagnosis tests by practitioners and producers. This may result in unhygienic food available in the market for human consumption.

3) A formal structure for incorporating the hierarchy of information sources and the quality of design execution when evaluating a body of work

EBM uses a very formal approach to incorporate the key EBM concepts into decision making, and this approach is referred to as systemic review.

Systematic reviews

Systematic reviews are focused on specific review questions and for clinical human medicine; this is usually about the efficacy of a treatment for a particular condition in a subgroup of the population. The systematic review process describes procedures for obtaining primary research papers that will be considered for answering the review question, assessing the quality of conduct of primary research, and when appropriate, synthesizing data quantitatively. The purpose of these procedural details is to increase the transparency of the review and allow readers to assess the potential for bias to influence the review conclusions, as they would on primary research using the standardized reporting guidelines. In fact, there is a standardized reporting guideline for systemic review (Begg et al., 1996; Moher et al., 2001; Stroup et al., 2000)

Assessment of methodological quality such as design, conduct and analysis of studies included in systematic review to evaluate the overall strength of evidence is a major departure from the traditional narrative review. Consistent with EBM the aim is to reproduce review, if the reader followed the procedure details in the systematic review. For example, if the systemic review describes a search of PubMed with particular terms and date restrictions, a reader should be able to reproduce the search results. The systemic review process does not prevent bias, but aims to enable readers to identify bias. For example, if a systemic review identified a PubMed search, and the reader notices a critical term is missing, then the reader can identify a bias. In a narrative review, with no search terms published, the reader cannot identify the bias.

The characteristics of narrative versus systematic reviews are listed in Table 1. Narrative reviews often focus on a wide range of objectives, without a clear question and therefore may not provide clarification about the review process. Therefore, traditional narrative reviews are often biased. In contrast to the narrative reviews, systematic reviews critically appraise a specific clinical question using explicit methods in a transparent manner allowing reproducibility. Therefore, systematic reviews are less likely to have biases in the selection of studies. Assessment of methodological quality of studies in systematic reviews provides overall strength of the evidence. Systematic review allows scientists to establish whether findings are consistent and generalizable across populations, or whether findings are varied significantly by particular sub group (Akobeng, 2005; Sargeant et al., 2006a).

Two primary factors have been the motivators for the development of systematic approaches to translating and summarizing a body of scientific literature. First, more than

two million biomedical articles are published annually in over 20,000 journals. This massive amount of information necessitates a compression of information. This role can be filled by narrative or systematic reviews. However, in the early 80's, critical analysis of medical reviews indicated that these reviews did not routinely use scientific methods to identify, assess and synthesize information, i.e. the reviews were not subjected to the scientific process of repeatability. It is obvious that review authors were weighing the value of studies differently or using different methods to identify literature, therefore, the methods differed between reviews, but these methods were not reported. The first attempt to address this issue was a review which reported standard factors that should be included in a review i.e. standard reporting guidelines (Mulrow, 1987). This movement also later included the "MOOSE" statement for how to report reviews (Stroup et al., 2000).

Current to this process of "how " to report a review, was the EBM movement which address the 1st major difference between reviews i.e. authors giving different weight to studies. The evidence based movement, recognized that different studies and study design features increase the value of information obtained. In evidence based medicine, the greatest statistical certainty comes from meta-analyses that incorporate a number of RCTs.

The combination of trying to make "reviews" repeatable and weighing the values of studies consistently within a review represents the key features of a systemic review and several organizations became in outlining the methodologies required for a systematic review. The most important of these are Cochrane Collaboration, BMJ evidence-based medicine group, Campbell Collaboration, NHS Centre for Reviews and Dissemination, and US centers for Evidence-Based Medicine which are all involved in

coordinating systematic review methodology in medical field. Since the beginning of 90's, systematic reviews have been well established for health care providers, researchers and policy makers to efficiently integrate existing information and provide data for rational decision making (Cook et al., 1997; Mulrow, 1994).

Another aspect of EBM with the concept of systematic reviews is meta-analysis. In many cases, individual studies often failed to detect significant difference between two treatments due to inadequate sample size. On the other hand, it is not economical or practicable to conduct large studies. An alternative to such large studies is to conduct meta-analysis using similar studies to address a specific question (Egger et al., 2001). Meta-analysis is the process synthesizing research results by using various statistical methods to retrieve, select and combine results from separate but related studies, and to investigate reasons for variations among studies. In meta-analysis, weighted averages of effects are combined to make summery estimate, so that larger studies have more influence than smaller studies taking into account both individual estimates and their standard errors (Dohoo I et al., 2003; Egger et al., 2001). If the treatment effect is consistent among various strata of the population, generalisability of scientific findings can be established. The other advantage of meta-analysis is to increase the statistical power specifically in studies with relatively low incident rates, and small effect sizes are being assessed (Mulrow, 1994). Cumulative meta-analysis is the process of repeating meta-analysis whenever new studies become available to include for the previous meta-analysis. Thereby, increasing the sample size results in narrowing of confidence interval so that precision of the estimates become increased if the new treatment appears to be significantly effective. It is possible to conduct meta-analysis on a group of studies

without considering their evidentiary value, therefore, meta-analyses are not unique to EBM or systematic reviews, and however they are a common feature.

Very few systematic reviews related to veterinary science have been published in peer-reviewed journals. Because of scarcity of RCT in the field of veterinary science and food safety intervention studies, researchers need to identify other study design that are available to address issues related to food safety. The required information may not be available in published journals. Therefore, searching for unpublished studies, expert opinion, expert reports and conference proceedings may help to identify available information.

Conclusion

The general aim of this thesis has been to apply some of the concepts of EBM to veterinary science and food safety using interventions designed for pre-harvest interventions in *Salmonella* in swine. Research is conducted so that science may reduce our uncertainty about the outcome and decisions. Individual research papers contribute a small amount to understanding. However, scientists should be able to evaluate a body of work on a topic and report what the body of work is “telling us”. This formal approach to the evaluation of a body of work is a relatively new field in veterinary science and food safety. The aim is to translate the body of work into a format that makes it consumable by the end user. There are many methods of translating a body of scientific literature for decision makers such as risk assessment, expert opinion, narrative review, meta-analysis and the approach evaluated in this thesis i.e. systematic review which may include meta analysis.

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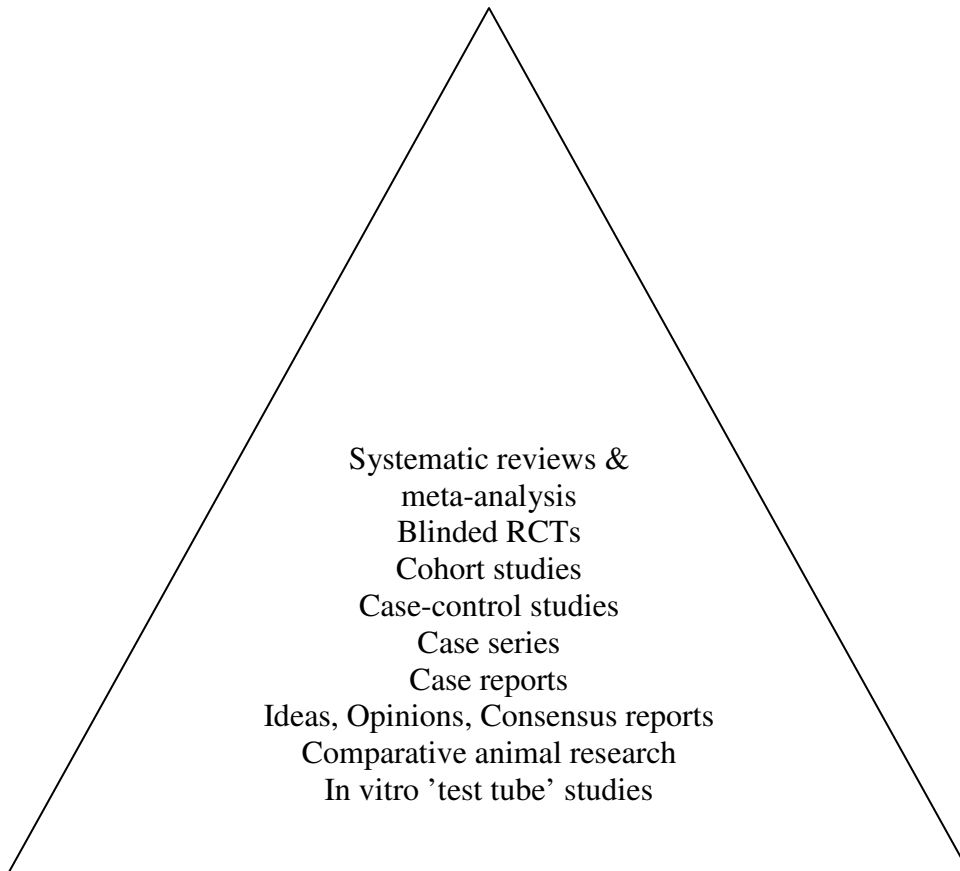
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Table 1. Differences between traditional narrative reviews and systematic reviews describing essential features of these reviews.

Essential feature	Traditional narrative review	Systematic review
1. Review question	Often broad in scope	Often a focused clinical question
2. Literature sources & search strategy	Usually not provided	Comprehensive search of multiple databases and explicit search strategy provided
3. Selection of studies	Usually not provided, potentially biased	Criterion-based selection process uniformly applied
4. Review and appraisal	Varies, depend on the reviewer	Rigorous critical appraisal
5. Study quality	No formal quality assessment	Always quality assessment included
6. Data synthesis	Often a qualitative summary	Qualitative and quantitative if the data can be pooled
7. Inference	Sometimes evidence-based	Typically evidence-based

Figure 1. The hierarchy of evidence illustrated as a pyramid of evidence in EBM. The most clinically relevant, strongest evidence are at the top of the pyramid and gradually become weaker towards the bottom of the pyramid.



CHAPTER 2

AN INTRODUCTION TO THE TEST BASE AREA: *SALMONELLA* IN SWINE AS A FOODBORNE PATHOGEN

Salmonella is a zoonotic pathogen capable of colonizing many vertebrates.

Infection due to *Salmonella* in human and domestic animals remains a serious worldwide problem, and the outcome of the infection varies from asymptomatic carrier stage to serious systemic infection. *Salmonella* infection in animals are of importance due to the direct economic consequences of mortality and morbidity associated with clinical disease, and due to human health consequences of salmonellosis acquired by direct or indirect contact with animals or animal products. These consequences have resulted in increased efforts in many countries to reduce the prevalence of *Salmonella* in food-producing animals.

Nomenclature of *Salmonella*

The genus *Salmonella* consists of a taxonomic group that contains over 2500 recognized serotypes (serovars) (Popoff et al., 2004; Popoff and Le Minor, 1997). There are two species of *Salmonella*: *S. enterica* and *S. bongori*. The species *S. enterica* is divided into six subspecies: *enterica*, *salame*, *arizonae*, *diarizonae*, *houtenae*, and *indica*. About sixty percent of serovars of *Salmonella* belong to *S. enterica* sub species *enterica*, and these are present in avian and mammalian hosts. *S. enterica* sub species *enterica* contains all serovars that cause disease in domestic animals and human, and only less than fifty serovars account for most of the cases of disease in animals and humans.

Characteristics of *S. enterica*

Salmonella, a member of the family *Enterobacteriaceae* are facultative anaerobic non spore forming, gram-negative rods. Most isolates grow optimally at 37° C, however, salmonellae can grow between 7° C and 48° C. Growth can also occur between p^H 4 to 8. Salmonellae are readily killed by heat and acid, but can survive freezing and drying. The ubiquitous presence in animals and environment make salmonellae one of the most important foodborne pathogens in the United States (Baird-Parker, 1990).

With the exception *S. enterica* sub species *arizonae* and *diarizonae*, all the other *Salmonella* are non-lactose fermenting. A common distinguishing family characteristic is the ability to produce acid through fermentation of glucose (Cowan, 1974). *Salmonella* are negative in the indole, Voges-Proskauer and urease tests, and positive in methyle red, citrate utilization, H₂S production, lysine decarboxylation, and ornithine decarboxylase tests. Recovery of *Salmonella* from fecal or environmental samples is often required to diagnose disease or to identify sources of infection in animals. These samples usually contain a large number of non-*Salmonella* bacteria and thus isolation of *Salmonella* requires inhibition of non-*Salmonella* bacteria while ensuring the growth of *Salmonella* (Libby et al., 2004). Therefore, combination of many pre-enrichment, selective enrichment and indicative plating are required to identify *Salmonella* in these samples.

***Salmonella* in swine and food safety**

In swine, several *Salmonella* serotypes are frequently isolated from clinical and non-clinical animals. Infection with serovar Typhimurium of *S. enterica* is associated with enterocolitis and often develops a carrier state in swine (Fedorka-Cray et al., 1995).

Many serotypes of *S. enterica* are responsible for illness in human (Centers for Disease Control, 2005) (Table 1). The four serotypes mostly found in human cases in United States from 1970 to 2005 are *S. Typhimurium*, *S. Enteritidis*, *S. Heidelberg* and *S. Newport* (Centers for Disease Control, 2005). Of the top twenty serovars most commonly associated with human infections, four serovars (*S. Typhimurium*, *S. Heidelberg*, *S. Agona*, and *S. I 4,[5],12:i:-*) are also found among the top serovars of swine (Table 2) during the year 2005 (Centers for Disease Control, 2005).

Foodborne salmonellosis: clinical disease in humans

In 1999, it was estimated that 1.4 million cases of human salmonellosis occurred in the United States annually, resulting more than 15,000 hospitalizations and over 500 deaths. *S. enterica* is considered as the second most important cause of bacterial foodborne disease in the United States (Mead et al., 1999). The number of reported cases of *Salmonella* in humans in Europe in 2005 was greater than 175,000, and the estimated number of unreported cases was several folds higher than the reported cases (European Food Safety Authority (EFSA), 2005). In the United States, 36184 human *Salmonella* cases has been reported to Centers for Disease Control (CDC) during the year 2005 (Centers for Disease Control, 2005). The under-reporting of *Salmonella* has been estimated 38 times higher than the reported cases in the United States (Chalker and Blaser, 1988). The main reservoir of non-typhoidal *Salmonella* is food animals, and the main sources of infections in developed countries are food of animal origin, mainly, poultry, pork, beef and eggs (Mayrhofer et al., 2004; Rose et al., 2002; Sanchez et al., 2002). It is estimated in Europe that 9 to 15% of human *Salmonella* cases are estimated to

be due to pork (Hald et al., 2004). Accordingly, in order to reduce the potential risk to consumers by *Salmonella* contaminated food, it is crucial to reduce the pathogen load intake into the food chain.

On farm *S. enterica* fecal prevalence is reported to be around 3.4% compared to 71.8 % at the abattoir-collected samples from market swine slaughtered in Iowa (Hurd et al., 2001). In another study, there was sevenfold-higher *S. enterica* isolation rate from pigs slaughtered at the abattoir (39.9%) compared to those slaughtered on the farm (5.3%) (Hurd et al., 2002). Therefore, it appears to be 40-70% of market swine are positive for *S. enterica* at the abattoir compared to 3-5% on-farm prevalence. However, the fecal isolation of *S. enterica* serotypes from naturally contaminated swine differs among culture methods (Rostagno et al., 2005). Prevalence estimates of individual studies are an important tool to evaluate the presence of *Salmonella* at a point in time, and provide information for control strategies.

Culture methods for *Salmonella*

A three-stage procedure involving pre-enrichment in non-selective broth, enrichment in selective broth, and subsequent detection on selective and indicative agar media is required to detect *Salmonella* in samples with low initial cell numbers or with bacteria those are stressed due to physical or chemical injury. Pre-enrichment broths available are buffered peptone water, lactose broth and brilliant green broth lauryl tryptose broth, manitol purpal suger broth, and nutrient broth can also be used (Hoorfar and Baggesen, 1998). This procedure is used on environmental and food samples and fecal and tissue samples from animals without clinical disease. Enrichment broths

recommended for *Salmonella* isolation are tetrathionate and Rappaport-Vassiliadis (RV) (Hammack et al., 1999; U.S.Food and Drug Administration, 1998). Selective enrichment with RV is generally carried out at 42° C for 24 hours. Selective and indicative media used for salmonellae are *Salmonella-Shigella* (SS) agar, bismuth sulfite (BS) agar, and xylose lysine desoxycholate (XLD) agar, brilliant green (BG) agar and desoxycholate citrate agar. MacConkey agar, Hektoen Enteric (HE) agar desoxycholate agar, and eosin-methylene blue (EMB) agar are less selective but primarily differential. Typical growth characteristic of *Salmonella* on commonly used selective and differential media are listed in Table 3.

Once potential salmonellae are identified, biochemical profiling and serological testing are performed for confirmation. A battery of biochemical tests recommended for further confirmation of *Salmonella*. (U.S.Food and Drug Administration, 1998). The majority of biochemical tests are incorporated by inoculation into triple sugar iron (TSI) and lysine iron agar (LIA) followed by incubation for 18 to 24 hours at 37° C (Davies et al., 2000; Waltman et al., 1991). Once an isolate is confirmed as *Salmonella*, serological testing is carried out with commercially available *Salmonella* ‘O’, ‘H’ and ‘Vi’ antibodies. Serotyping based on Kauffman-White scheme requires two consecutive agglutination tests. First, ‘O’ antigen is identified in a slide agglutination test by mixing a saline suspension of bacterial cells mixed with different anti ‘O’ sera. Subsequently, ‘H’ antigen is determined by use of formalin killed broth culture mixed with different anti ‘H’ sera in tube agglutination test. To confirm the specific antigens in ‘O’ and ‘H’ test, absorbed single-factor anti-sera are used (Brenner et al., 2000). For epidemiological

purposes, *Salmonella* serotypes are further differentiated by phage typing which is based on reactivity with a defined set of bacteriophages.

Rapid detection techniques

The relative insensitivity and time consuming nature of routine culture and biochemical methods available for *Salmonella* isolation and identification prompted researchers to develop rapid methods such as serological and genetic techniques. Most of these rapid methods that detect *Salmonella* antigen/s still necessitate culturing *Salmonella* until the cell numbers reach a range of 10^4 to 10^5 cells per ml. Therefore, most rapid antigen detection methods require 8 to 24 hours for completion although they are more convenient and less labor-intensive than the conventional methods (Molbak et al., 2006).

Enzyme-linked Immunosorbent Assay (ELISA) to detect antibodies to *Salmonella*

This test is used for mass screening of past/present *Salmonella*-infected animals on herd basis. The ELISA test is sensitive, cost effective, available for automation, and no prior incubation is required to increase the number of bacterial cells. The constraint with the test is that it is less useful for testing individual animals. Anti-*Salmonella* antibodies often appear only 1 to 2 weeks post-infection, and is always not indicative of a current infection (Hanes, 2003). This method is being widely used in Denmark, The Netherlands and in Germany in *Salmonella* pre-harvest control programs for poultry and pigs (Nielsen et al., 1998; Wiuff et al., 2000). There are different types of ELISAs that utilize different *Salmonella* antigens. One assay utilizes LPS of *S. Typhimurium* and *S. Choleraesuis* to detect antibodies to *Salmonella* O antigen (Nielsen et al., 1994). This

assay can be used at the herd level but not suitable to test individual animals. Another ELISA test uses heat-extracted antigen to detect antibodies in *Salmonella* carrier swine (Kramer et al., 1994). This ELISA was later developed to utilize a different type of heat-extract antigen in mixed ELISA format to offer with a better sensitivity and specificity (Gray and Fedorka-Cray, 1999). Countries such as the USA, Germany, France, UK and the Netherlands have used different ELISA techniques that have been developed to detect *Salmonella* serovars prevalent in the country. In Denmark, the mixed ELISA has been used for routing screening of breeding, multiplying and slaughter swine herds (Nielsen et al., 2001).

Enzyme-linked Immunosorbent assay (ELISA) to detect *Salmonella* antigens

There are a number of commercial kits available to detect *Salmonella* antigens. However, it requires prior pre-enrichment and selective enrichment to achieve enough *Salmonella* required for detection limit of the test (Swaminathan and Feng, 1994). Some ELISAs detect *Salmonella* antigens in swine feces, whereas, some other ELISAs are performed on swine lymph nodes and cecal contents (Araj and Chugh, 1987; Van Poucke, 1990). Sensitivity and specificity of these antigen ELISAs heavily depend on the cleanliness of the sample (Harvey et al., 1999).

DNA-based detection methods

A number of PCR techniques have been developed for the rapid detection of *Salmonella* in clinical samples (Nowak et al., 2007; Sibley et al., 2003; Stone et al., 1994). These techniques are based on amplification of targets such as replication genes,

fim genes and *inv* genes of *Salmonella* (Sibley et al., 2003; Stone et al., 1994)). One method that uses PCR amplification of 457-bp sequence covering *invE* and *invA* genes coupled with Southern hybridization was sensitive enough to detect about 9 CFU of *Salmonella* organisms in pure culture and 300 fg of purified chromosomal DNA. When this PCR-hybridization assay was coupled with a brain heart infusion enrichment step for 2 hours, it could detect as few as 80 CFU of *Salmonella* organisms in seeded feces (Stone et al., 1994). Most of the PCR assays had a low detection limit and detected *Salmonella* serovars in clinical samples with the same sensitivity and specificity as did the culture methods. However, these tests detected *Salmonella* more quickly than conventional culture techniques. Spiking of samples with appropriate serovars of *Salmonella* has been proved to increase the sensitivity of the PCR assays (Eriksson and Aspan, 2007). Recent attempts have been also made to develop and validate a LightCycler real-time PCR assay (LC-PCR) and a PCR-enzyme-linked immunosorbent assay (PCR-ELISA) for detecting *Salmonella* in meat samples (Perelle et al., 2004).

Intervention strategies to reduce *Salmonella* in swine

Salmonella in swine is a high priority research area due to its impact on swine health and production as well as its ability to cause foodborne illnesses in human. Therefore, control of *Salmonella* in swine is important in both swine and public health standpoints. Numerous intervention strategies have been attempted to reduce foodborne illness and economic loss to the swine caused by *Salmonella*. Various intervention strategies which are being practiced to reduce *Salmonella* levels throughout the pork production system can be categorized under three broad areas: on-farm or pre-harvest

interventions, interventions during the processing or harvesting at the abattoirs, and post-harvest interventions. On-farm intervention strategies include vaccination, management practices, type of feed, use of antibiotics, acids, prebiotics, probiotics, and phage therapy in feed, sanitation and bio-security measures. Of these aforementioned intervention strategies, this dissertation will be focused only on the use of vaccines and antimicrobials to reduce *S. Typhimurium* shedding in finisher swine.

Vaccines to reduce *Salmonella* in swine

Both innate and adaptive branches of the immune system play a role in host defenses against the bacterium *Salmonella* in swine. Gastric pH, intestinal motility, host-derived proteins such as defensins and proteolytic enzymes, normal intestinal microflora, non-specific host immune cells such as natural killer cells and $\gamma\delta$ T cells are important innate host defenses against the infection. As *Salmonella* has the ability to inhabit extracellular and intracellular niches of the host, antibody-mediated and cell-mediated responses of adaptive immune response are vital to control the infection (McSorley and Jenkins, 2000; Mittrucker et al., 2002; Tizard, 2004; Yrlid et al., 2001). The ideal vaccine against *Salmonella* should prevent colonization, shedding of the organism, the development of carrier stages and development of clinical disease, and promote elimination of the organism from the host (Haesebrouck et al., 2004). Live vaccines are considered to offer a better protection against *Salmonella* infection than the killed or inactivated vaccine. This superior protection could be related to better cell-mediated immune system elicited and induction of mucosal IgA antibody production by live vaccine strains (Hackett, 1990). Also, live vaccines are thought to provide protection

against both homologous and heterologous *Salmonella* infections (Chu et al., 2007; Matsui and Arai, 1992).

Three types of live-attenuated vaccines have been tested experimentally. One vaccine type contains strains attenuated without targeting a specific gene or marker, for example temperature-sensitive mutants of *Salmonella* (Hooke et al., 1993). Another vaccine type contains strains attenuated by mutating genes required for bacterial metabolism. This category of vaccines includes auxotrophic strains generated by mutating genes such as *galE* (mutation in the gene required for GalE enzyme synthesis), *purABEH* (mutation in genes required for purine synthesis) and *aroACD* (mutation in genes required for aromatic amino acid synthesis), and strains carrying mutations in the regulatory genes such as *cya* (a gene required for the synthesis of adenylate cyclase), *crp* (a gene required for the synthesis of cAMP receptor protein) and *pho* (a global regulator gene) (Coe and Wood, 1992; Curtiss, III et al., 1989; Hoiseth and Stocker, 1981; McFarland and Stocker, 1987; Nnalue and Stocker, 1987). The third type of vaccine includes strains that carry mutations in the virulence genes such as *inv* genes located on a virulence plasmid of *Salmonella* (Kramer et al., 1992). Vaccine strains containing mutations in virulence genes are generally considered to be safer than the auxotrophic or temperature-sensitive mutants because they are unlikely to revert to the wild-type strain. In some auxotrophic vaccine strains, additional safety has been ensured by introducing a second mutation or curing virulence plasmids from the already attenuated strain (Chu et al., 2007).

Because of the ubiquitous nature of *Salmonella* infection, and the use of *Salmonella* vaccines in control programs and *Salmonella* serology in control programs

recent attempts have been made to construct negative-marker vaccine strains so that antibodies produced against *Salmonella* can be distinguishable from those produced subsequent to infection (Selke et al., 2007). The ability to distinguish vaccinated from naturally infected animals would be advantageous because vaccination would not hinder current control programs dependent on serological tests.

Antibacterial agents to reduce *Salmonella* in swine

Antibacterial agents act on targets that are essential for bacterial survival and growth. These targets vary with the class of antibacterial agent and include bacterial cell wall biosynthesis (peptidoglycan), bacterial protein synthesis (ribosomes or enzymes involved in protein synthesis), bacterial DNA replication and repair, and bacterial cell membrane (Walsh, 2000). Antibiotics belongs to various groups of antibiotics are being used in swine for production enhancement as well as prophylactic for *Salmonella*. There has been various experimental challenge studies conducted to evaluate effectiveness of antibiotics to control *Salmonella* shedding in swine. Most of the studies had undergone microbial culture as to determine the presence or absence of *Salmonella* in feces while culture methods differ among studies.

Although vast amount of literature describing detection and intervention strategies to control *Salmonella* in swine are available, no evidence-based approach has been carried out to analyze these data. Therefore, the objective of this dissertation is to summarize the available evidence for detection and control foodborne *Salmonella* in swine. In particular, the first objective of this thesis was to apply EBM approach to analyze studies of vaccine to control *Salmonella* in swine. Second objective was to

quantitative analysis of binary and continuous data of antibiotic studies to control *Salmonella* in swine using EBM approach. The third objective was to compare the sensitivity and specificity of in-house culture method to two PCR assays using Bayesian analysis in the absence of a gold standard test.

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Table 1. The 20 most frequently reported *Salmonella* serotypes from human sources reported to CDC during the year 2005

Rank	Serotype
1	Typhimurium
2	Enteritidis
3	Newport
4	Heidelberg
5	Javiana
6	14,[5],12:i:-
7	Montevideo
8	Muenchen
9	Saintpaul
10	Braederup
11	Ornienburg
12	Mississippi
13	Infantis
14	Paratyphi B var. L(+) tartrate +
15	Thompson
16	Agona
17	Typhi
18	Hartfold
19	Stanley
20	Berta

Source: <http://www.cdc.gov/ncidod/dbmd/phlisdata/salmtab/2005/>

Table 2. Non-clinical *Salmonella* isolates from porcine reported to CDC and National Veterinary Services Laboratories by serotype during the year 2005

Serotype	Non-clinical non-human source
Derby	192
Typhimurium	139
Heidelberg	43
Worthington	33
Mbandka	25
Agona	16
14,[5], 12i:-	9
Anatum	6
Braenderup	4
Johansburg	4
Newport	4
Lille	2
Meleagridis	2
Brandenburg	1
Norwich	1
Ohio	1
Saintpaul	1
Senftenberg	1

Source: <http://www.cdc.gov/ncidod/dbmd/phlisdata/salmtab/2005/>

Table 3: Typical growth characteristics of *Salmonella* on some commonly used selective and differential media

Media	Colony appearance
<i>Salmonella-Shigella</i> agar	Colorless colonies on a pink background
Bismuth sulfite (BS) agar	Black colonies surrounded by a brown to black zone that casts a metallic sheen
Brilliant green (BG) agar	Pink colonies surrounded by red zone
Xylose lysine desoxycholate (XLD)	Black-centered red colonies with H ₂ S producers, red colonies with non-producers
MacConkey (MA) agar	Uncolored, transparent colonies
Eosin-methylene Blue (EMB) agar	Translucent amber to colorless colonies

Source: (Guthrie, 1992)

CHAPTER 3

EFFICACY OF VACCINATION TO REDUCE *SALMONELLA* PREVALENCE IN LIVE AND SLAUGHTERED SWINE: A SYSTEMATIC REVIEW OF LITERATURE FROM 1979 TO 2007

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ABSTRACT

A systematic review was conducted to evaluate the efficacy of vaccination to reduce *Salmonella* prevalence in market weight finisher swine. A search of online databases, and selected conference proceedings was conducted to identify relevant studies. The review process followed relevance screening, methodological quality assessment and data extraction. Although multiple outcomes were frequently reported, only outcomes describing culture of *Salmonella* were extracted. Five clinical trials and 23

challenge studies were considered likely relevant to the review as they described vaccination to reduce *Salmonella* in swine. Five clinical trials reported vaccination was associated with reduced isolation of *Salmonella* in market weight pigs, however, information required to assess the internal validity of the study was often not described in the manuscripts. All challenge studies assessed vaccine efficacy in pigs aged less than 15 weeks reducing the relevance of results to the review which focused on market weight pigs. Only five of the 23 challenge studies reported the majority of information necessary to evaluate the quality of vaccine studies. Given large variability in population type, sample size, type of vaccine, dose and dosing regimens, and type of outcomes observed, pooled data analysis was not possible, and therefore, a qualitative synthesis of the studies was conducted. Available evidence suggests that vaccination is associated with reduced *Salmonella* prevalence in swine at or near harvest; however, this conclusion is based on studies with design and reporting deficiencies that could potentially indicate biases with the outcome.

INTRODUCTION

SALMONELLA is considered one of the major foodborne pathogens transmitted by pork and pork products. Estimates of the proportion of human cases of *Salmonella* attributable to pork vary from 5% to 15% depending on the attribution approach used (Batz et al., 2005; Frenzen et al., 1999; Hald et al., 2004; Miller et al., 2005).

Vaccination of pigs against *Salmonella* may be one means of reducing pre-harvest *Salmonella* prevalence and therefore, the number of swine carrying *Salmonella* into the slaughter plant. Numerous studies reported vaccination to reduce *Salmonella* prevalence,

however, diffuse sources of data, multiple outcomes and the volume of work mean it is not readily apparent in what circumstances vaccination against *Salmonella* may be an effective means of pre-harvest control of *Salmonella*. The aim of this review was to appraise and synthesize studies describing vaccination as a method of reducing *Salmonella* prevalence in market weight finisher swine. To synthesize these studies, a systematic review approach rather than a traditional narrative review was used (Cook et al., 1997; Mulrow, 1987; Sargeant et al., 2006b).

Systematic reviews evaluate a topic using transparent, repeatable methods, and assess the internal and external validity of the primary research to the review question. The objective is to convey to the consumer of the review, not only the conclusion of the review, but sufficient information for the consumer to determine their agreement with the conclusion. Inclusion of descriptive information about scope of the literature search, criteria used for inclusion of studies, and assessment of study quality informs the review consumer of potential biases and represents a major departure from traditional narrative reviews.

MATERIALS AND METHODS

To review the efficacy of vaccination against *Salmonella* and the association with *Salmonella* prevalence in swine, a stepwise procedure was followed.

Question definition

The study question was “In market weight finisher swine or sows in production systems of the European Union and developed nations of the Pacific Rim, what is the

association between *Salmonella* prevalence and vaccination against *Salmonella*”?

Vaccination was defined as any intervention designed to stimulate a systemic immune response to protect against *Salmonella*. Although many manuscripts reported multiple outcomes, only outcomes describing culture of *Salmonella* post-vaccination were considered for the review. The association of vaccination with antibody production or clinical disease occurrence was not considered for this review.

Literature search and relevance screening

A literature search was conducted of thirteen electronic online bibliographic databases, from their inception to April 2007. No language or publication restrictions were imposed for the searches. The databases selected as relevant to the review topic were AGRICOLA (1970-2007), AGIRS (1975-2007), Biological and Agricultural Index (1983-2007), Biological Abstracts (1980-1989), Biosis Previews (1980-2007), CAB Abstracts (1973-2007), Current Contents (1998-2007), Dissertation Abstracts (ProQuest Digital Dissertations, 1961-2007), Food Science and Technology Abstracts (1969-2007), Ingenta Gateway (1997-2007), MEDLINE (1950-2007), PubMed (1965-2007), and Web of Science (1945-2007). Three major conference proceedings were searched by hand, starting at their inception: American Association of Swine Veterinarians (1973-2007), International Pig Veterinary Society Congress (1969-2007), and International Symposium on the Epidemiology and Control of *Salmonella* in Pork (1996-2007). Reference lists of studies identified as relevant were checked for additional references. The search combined sets of terms related to swine; (1) population, (2) interventions, and (3) outcome. Search terms within each component were combined using “OR” and each

component was combined using “AND” (Population terms AND intervention terms AND outcome terms). The population terms used in the original search were hogs, hog, swine, pig, pigs, gilts, sows, market-weight, finishers, boars and porcine. The original search was designed to capture studies describing various pre-harvest interventions associated with *Salmonella* reduction in swine, but the specific terms used to identify vaccine studies were vaccines, vaccination, immunization, intervention and management practice. The outcome terms included were carcass swabs, culture, serology, prevalence, incidence, risk factors, cecal lymph nodes, pharyngeal swabs, feces, fecal contents, rectal swab, rectal contents, mesenteric lymph nodes and salmonell*.

Reference management (Reference Manager 11, Thomson ResearchSoft, USA) and electronic systematic review software (Systematic Reviews SRS 3.0, TrialStat Corporation, Canada) were used to coordinate the review process. For each citation identified, abstracts were uploaded into the reference management program. After removing duplicates, citations were uploaded into the electronic systematic review software, and screening for relevance to the review question was conducted. All four reviewers initially read the same fifty abstracts, and the results were discussed to reach agreement about which papers should be included. Inclusion criteria for relevance screening were 1) primary research in English, 2) Ph.D. theses in English, 3) citations from conference proceedings 4) described evaluation of vaccination against *Salmonella* in swine in a challenge trial or clinical trial, and 5) reported ante-mortem or post mortem presence of *Salmonella* in swine. Exclusion criteria included 1) primary research in languages other than English, 2) Ph.D. theses in languages other than English, 3) literature reviews, and 4) other articles, such as letters, comments, and editorials. The

abstracts were assigned to reviewers in blocks, because random assignment was not possible using the commercial software. Two reviewers read each abstract independently, and excluded references not meeting the inclusion criteria. During relevance screening, the reviewers were blinded to the source of each reference, but the author was known. In cases where the two reviewers disagreed, discussions were held to resolve the disagreement.

Assessment of the methodological soundness of the relevant studies

Using full articles, the methodological soundness and completeness of all relevant studies was independently assessed by two reviewers using checklists to assess challenge trials and clinical trials. The checklists had been created by agreement of the authors. The assessment specifically evaluated the materials and methods section of the manuscripts for complete descriptions of the vaccination, challenge, and outcome measurement protocols. A complete description of the vaccination protocol included vaccine organism (*S. choleraesuis* or *S. typhimurium*, or subunit), route of administration, frequency of administration and dose. A complete description of the challenge included identification of the organism used (*S. choleraesuis* or *S. typhimurium*), dose, route and timing of administration relative to vaccination. Age of pigs at vaccination or challenge with *Salmonella* was not considered as part of the quality assessment. A complete description of the outcome included the timing of sample collection after vaccination and challenge, the type of sample (fecal or tissue), size of sample, and the culture method. A “low” grade was assigned to a manuscript if both reviewers agreed that either randomization was not explicitly stated or that vaccination protocol, challenge protocol, outcome

assessment or statistical analysis was not described fully. A “high” grade was assigned when both reviewers agreed that these criteria were satisfactorily met. As few studies reported blinding, this factor was excluded from the methodology assessment and reported separately. Prior to starting the assessment, 5 articles were reviewed by all reviewers and agreement was assessed. Thereafter, two reviewers independently assessed each article. Disagreements between reviewers were resolved by consensus. The checklists for the methodology assessment are available from the first author.

Data extraction and summarization

A standardized form was used to extract information from each manuscript for the most externally valid studies only (Sargeant et al., 2006a). The assessment of external validity was based on the measurement of *Salmonella* in market weight swine. One reviewer (TD) was responsible for extraction of primary data. Data extraction included the characteristics of population, intervention and level of allocation to treatment groups, outcome, and results. Only outcomes describing ante-mortem or post-mortem culture of *Salmonella* post-vaccination were extracted.

For data extraction we aimed to extract and report the most informative data available that conveyed the study result, therefore we tried to extract raw data with prevalence of *Salmonella* in the vaccinated and unvaccinated group and to report the p value associated with these data. If raw data information were not reported, we extracted summary data such as proportions, percentages, relative risk or vaccine efficacy with confidence intervals or p values, if available. If these data were not available we tried to report the results of hypothesis testing with preference given to reporting the exact p

value. If the exact p value was not reported ,we would extract information that indicated the result of significance testing either as $p < 0.05$ and finally, only if no other information was available we reported that the association was significant or not significant if that was all that was provided.

RESULTS

Studies identified, relevance screening, quality assessment, and data extraction

20,814 references were identified, 20,655 references identified in the 13 bibliographic electronic data bases and 159 references in the three major conference proceedings. After duplicate removal, 8,087 unique references were identified for relevance screening. Based on the title and the abstract, 7,807 references did not meet the inclusion criteria. Of the 280 abstracts that passed initial relevance screening based only on the abstract, 254 were deemed not relevant based on evaluation of the full article, leaving 27 articles that reported vaccination against *Salmonella* in swine. One article described two trials, a challenge trial and a clinical trial, therefore, 28 unique studies were considered for the review. Table 1 describes the methodology grade received, randomization and blinding for the 28 studies that discussed vaccination against *Salmonella*. Only 5 of the 28 articles received a high methodological quality score.

Characteristics of the studies

From the manuscripts identified, 5 clinical trials and 23 challenge studies were reported. Twenty two studies were conducted in North America; 20 and two in the USA

and Canada, respectively. Five studies were conducted in Europe; three studies in Germany, and one each in the United Kingdom and Ireland. The other study was carried out in Brazil. Studies were published between 1979 and 2006, and the sample size ranged from 8 to 7,000 pigs. Not all the studies tested for *Salmonella* status prior to the intervention. Table 2 and Table 3 summarize the characteristics of challenge studies and clinical trials, respectively, and describe the type of pig studied, the setting, age at vaccination and age at outcome assessment. None of the challenge studies were conducted using market weight finisher swine. The outcome of the five clinical trials was assessed in market weight finisher swine, and therefore, data were extracted from only these trials.

Evidence for an association between vaccination and Salmonella status in pigs

Vaccination protocols and outcomes from the five clinical trials reporting recovery of *Salmonella* in market weight finisher pigs are reported in Table 3. The outcomes measured differed for each study, however, each trial reported vaccination was associated with reduced *Salmonella* prevalence. We were unable to extract and report raw data from any of the five trials. Summary data were reported for one trial, which suggested that vaccination with a live modified *S. choleraesuis* vaccine twice at 3 and 16 weeks of age was effective in reducing *Salmonella* prevalence in ileocecal lymph nodes at slaughter (Maes et al., 2001). The prevalence of *Salmonella* in the unvaccinated barns was 7.2% compared to 0.6% in vaccinated barns (Relative risk: 0.083, 95% confidence interval 0.019 - 0.35) (Maes et al., 2001). The remaining four clinical trials also reported *Salmonella* prevalence was reduced in vaccinated animals, however, it is not possible to

report the magnitude of *Salmonella* reduction as these four trials reported only p-values or the number of *Salmonella* positive animals without indicating how many animals were in the study group. (Maes et al., 2001) found that following use of a commercially available *S. choleraesuis* live vaccine, only 0.6% and 7.2% of the ileocecal lymph nodes were positive for *Salmonella* in the vaccinated and control group, respectively, at slaughter ($p < 0.001$). Whereas, (Baum et al., 1997) observed a significant difference in the culture prevalence of *S. typhimurium* serogroups B and C1, but not serogroups C2 and E in response to vaccination. Further, the authors reported that the total significance difference of *Salmonella* recovery from vaccinated and nonvaccinated groups was $p < 0.05$. In a trial carried out by (Kolb et al., 2002), vaccinated pigs showed significant reduction in percentage of carcasses culture positive for *Salmonella* culture at slaughter ($p = 0.02$). The authors also reported a reduction in *Salmonella* prevalence in spiral colon fecal samples ($p = 0.1$) as a result of vaccination. In a study conducted by (Roesler et al., 2006) using a homologous inactivated herd-specific *Salmonella* vaccine in sows, *S. typhimurium* could not be detected in fecal samples from the piglets of vaccinated sows, and subsequently, in gilts. Also, a large trial carried out by (Schwarz et al., 2007) reported that following use of a commercially available *S. choleraesuis* vaccine, significant reduction in the rate of *Salmonella* isolation from mesenteric lymph nodes ($p < 0.05$).

All five clinical trials received a low methodology quality score (Table 1). Common features of the studies were failure to report a statistical method that would account for clustering and failure to report blinding. One study reported randomization at the group level ($n = 12$, 6 vaccinated groups and 6 control groups), but did not report

blinding at outcome assessment or withdrawals from the study (Maes et al., 2001). The prevalence of *Salmonella* in the unvaccinated barns was 7.2% compared to 0.6% in vaccinated barns (Relative risk: 0.083, 95% confidence interval 0.019 - 0.35). Another study reported random allocation of 8 buildings (4 vaccinated, 4 control) to treatment groups however it was not possible to fully assess if clustering had been accounted for as the descriptions of statistical methods were short (Baum et al., 1997). Although the number of *Salmonella* positive animals was described, the number of animals tested was not clear, therefore, primary data could not reliably be extracted. The other three clinical trials (Kolb et al., 2002; Roesler et al., 2006; Schwarz et al., 2007) failed to provide enough data for re-analysis or assessment of the controlling for biases (Table 3). Therefore, consistent with systematic review methodologies, these studies provided little strong evidence to support the efficacy of vaccine against *Salmonella* in market weight finisher swine.

Five challenge studies received a high methodology score; however, *Salmonella* status was not assessed in market age swine. The remaining studies were not used as evidence of an association, because they had a low methodology score and *Salmonella* status was not assessed in market age swine. The majority of these studies reported a positive association of vaccination with *Salmonella* culture measured either in feces or tissue samples.

DISCUSSION

The association between vaccination and *Salmonella* reduction in market weight finisher swine is promising but not definitive. Unfortunately, none of these studies

contributed high quality evidence supporting the use of vaccination to reduce *Salmonella* in market weight finisher swine because of failures to report key components of the study necessary to assess the evidentiary value of the research. If the evidentiary value and disparity in outcome measures are ignored, all five clinical trials suggest that vaccination is associated with reduction in *Salmonella* (Table 3). Our conclusion is that current evidence suggests some vaccines are effective, however, because of the methodological quality of the studies identified for this review; it is possible that this conclusion is incorrect. Another limitation to this conclusion is analysis based on binomial outcome. Enumeration data of the effect of vaccination on *Salmonella* microbial load reflect the risk better than the prevalence. Therefore, if enumeration data were available in manuscripts, may capture different and potentially more relevant effects as microbial load, as well as prevalence, are likely associated with the human health risk. Unfortunately, none of the manuscripts identified reported enumeration data.

Systematic reviews assess two features of studies, external validity and internal validity. External validity refers to the applicability of the study to the review question and depends upon the population studied and the outcome reported. The internal validity refers to accurate measurement of the outcome in the study population apart from random error. Biases that prevent internal validity include selection bias, misclassification bias and confounding bias. In studies of interventions, randomization and blinding limit the impact of confounding and misclassification bias, respectively. The best evidentiary value for an intervention such as vaccination comes from randomized clinical trials with high external and internal validity i.e. provide a high level of evidentiary value (Persaud and Mamdani, 2006). In this review, we identified five clinical trials with high external

validity, based on the population and outcomes reported. However, our rationale for tempering our review conclusion is based on internal validity. Four of the five clinical trials reported randomization at the group/building level in the study population of interest. However, these studies failed to report analyses that would account for clustering, and therefore, introduced the possibility of bias. Failure to account for clustering increases chance of rejecting the null hypothesis when it is true. This problem is addressed by (Cornfield, 1978); “randomization by cluster accompanied by an analysis appropriate to randomization by individual is an exercise in self-deception and should be discouraged”.

Similarly, blinding is an essential feature associated with internal validity to limit the introduction of differential misclassification bias (Montori et al., 2002). Without a formal description of a blinding protocol, it is not possible to assess the extent to which this bias has influenced the study outcome. It could be argued that *Salmonella* culture is a blinded outcome, however, for barn level studies it is possible that awareness of the vaccination status of the barn being cultured could lead to increased scrutiny of cultures or re-run doubtful results, and therefore, different sensitivity and specificity for culture results for vaccinated and unvaccinated barns. We acknowledge it is also possible that studies used but failed to report formal randomization and blinding, and if this is the case, our assessment of the literature may be overly conservative (Callaham et al., 1998).

Veterinary vaccine challenge studies should provide evidence for vaccine efficacy, because researchers are able to control internal biases and have fewer barriers to studying the animal population of interest. All challenge studies, however, lack some external validity due to the inability to replicate field conditions such as concurrent

disease/organism exposure, which may be important in disease ecology (Table 2). The challenge for the reviewer is to balance the information from a challenge study with the relevance of the field environment. In selecting which studies to extract data and report, we chose only the most relevant based on our assessment that differences in immune system of young and mature pigs and the absence of concurrent disease challenge were sufficient, for us, to conclude these studies were not applicable to the review question (Baum et al., 1997; Charles et al., 1999; Charles et al., 2000a; Charles et al., 2000b; Coe et al., 1992; Draayer, 1986; Foster et al., 2003; Gibson et al., 1999; Groninga et al., 2000; Hanna et al., 1979; Husa et al., 2006; Kennedy et al., 1999; Kern, 1994; Kolb et al., 2001; Kramer et al., 1987; Kramer et al., 1992; Ku et al., 2005; Letellier et al., 2000; Lumsden et al., 1991; Neubauer and Roof, 2005; Roesler et al., 2004; Roof and Doitchinoff, 1995; Springer et al., 2001). This assessment of relevance is subjective and review consumers may disagree, however, communication of the value of information was considered for the review as part of the systematic review process. Likely, similar judgments are made by traditional reviews although the process may not be as apparent. Further, it is important to note that a lack of external validity was a consequence of our review question. Many studies had the primary function to assess clinical disease associated with *S. choleraesuis* and fecal recovery of *Salmonella* was a secondary outcome, a different review question would reach different conclusion about the external validity of the study. Unfortunately, many of the challenge studies also failed to control for internal biases with blinding and randomization.

An ancillary finding in the review was the large amount of detail often excluded from study reports. Only 5 of 28 studies provided a complete description of the

vaccination, challenge and outcome protocol, suggesting a common problem with reporting and manuscript preparation that likely limits the usefulness and reproducibility of these studies. “Low” methodology scores were often, but not always, associated with conference proceedings, for example, three of the clinical trials reported were identified in conference proceedings and contained little detail of the study protocols (Baum et al., 1997; Kolb et al., 2002; Schwarz et al., 2007). Obviously, the limitations on space in conference proceedings prevent authors from including critical information. Another consideration should be that published reports suggest conference proceedings tend to present more favorable results than subsequent peer-reviewer publications of the same material (Callahan et al., 1998; Hopewell et al., 2006).

We did not report a meta-analysis as part of the systematic review. Meta analysis is the quantitative method use to make pooled estimate of effect measure of individual studies. Systematic reviews may or may not include a meta-analysis. It is important to recognize the distinction between systematic review and meta-analysis, because it is always appropriate and desirable to systematically review a body of data, but it may be inappropriate or even misleading, to statistically combine results from independent studies (Egger et al., 2001). As reported, most studies provided little detail about the studies; therefore, we could not assess if the population type, sample size, type of vaccine, dose and dosing regimens, and type of outcomes observed were sufficiently similar among all five clinical trials, to warrant calculation of a combining effect measures (Moher et al., 2001; Naylor, 1995). Further, even if we were assured it was sensible to estimate a single effect or distribution of effect, only one of five studies reported the data needed for a meta-analysis. As meta-analysis works best when studies

have homogeneous settings, designs and quality, and outcome measures with small sample sizes, therefore, data from one study is insufficient for a meta-analysis (Moher et al., 2001; Naylor, 1995).

CONCLUSION

The evidence available suggests that *Salmonella* vaccines are associated with reduced *Salmonella* prevalence in swine at or near harvest. However, this conclusion is based on studies with design and manuscript deficiencies that could potentially indicate biases such as confounding, allocation and assessment bias associated with the outcome but not the result of vaccination.

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TABLE 1. Randomization, Blinding and Methodological Quality Grade of 28 Studies Reporting Vaccination of Swine Against *Salmonella* Associated with Foodborne

<i>Author</i>	<i>Outcomes</i>		
	<i>Randomization</i>	<i>Blinding at outcome assessment</i>	<i>Overall methodology assessment</i>
<i>Clinicaltrials</i>			
Baum <i>et al.</i> , 1997	Barn level allocation with 8 barns of 1100 head: 4 vaccinated barns, 4 nonvaccinated barns	Not reported	Low
Kolb <i>et al.</i> , 2002	Barn level allocation with 12 vaccinated barns and 12 nonvaccinated barns. The group size was not described.	Not reported	Low
Maes <i>et al.</i> , 2001	Barn level allocation with 12 groups of 380 pigs: 6 vaccinated barns and 6 nonvaccinated barns	Not reported	Low
Roesler <i>et al.</i> , 2006	Not reported	Not reported	Low
Schwarz <i>et al.</i> , 2006	Barn level allocation of 3,500 pigs to both treatment and control groups: number of barns not described	Not reported	Low

Challenge studies

Charles <i>et al.</i> , 2000 ^a	Individual random allocation	Not reported	High
Husa <i>et al.</i> , 2006	Individual random allocation to groups, and blocked by weight and sex	Adequate	High
Letellier <i>et al.</i> , 2000	Individual random allocation to groups	Not reported	High
Lumsden <i>et al.</i> , 1991	Blocked by litter and randomized with block	Adequate	High
Neubauer <i>et al.</i> , 2005	Reported without description	Adequate	High
Baum <i>et al.</i> , 1997	Reported without description	Not reported	Low
Charles <i>et al.</i> , 1999	Reported without description	Not reported	Low
Charles <i>et al.</i> , 2000 ^b	Reported without description	Not reported	Low
Coe <i>et al.</i> , 1992	Not reported	Adequate	Low
Draayer 1986	Not reported	Not reported	Low
Foster <i>et al.</i> , 2003	Not reported	Not reported	Low
Gibson <i>et al.</i> , 1999	Blocked by sex and weight	Not reported	Low
Groninga <i>et al.</i> , 2000	Reported without description	Not reported	Low
Hanna <i>et al.</i> , 1979	Not reported	Not reported	Low
Kennedy <i>et al.</i> , 1999	Blocked by weight and randomized with blocks	Adequate	Low
Kern <i>et al.</i> , 1994	Reported without description	Not reported	Low

Kolb <i>et al.</i> , 2003	Blocked by sex and weight	Not reported	Low
Kramer <i>et al.</i> , 1987	Blocked by sex and weight	Not reported	Low
Kramer <i>et al.</i> , 1992	Individual random allocation to groups	Double blinded	Low
Roesler <i>et al.</i> , 2004	Not reported	Adequate	Low
Roof <i>et al.</i> , 1995	Individual random allocation to groups	Not reported	Low
Springer <i>et al.</i> , 2001	Not reported	Not reported	Low
Ku <i>et al.</i> , 2005	Not reported	Not reported	Low

TABLE 2. THE COUNTRY, ANIMAL TYPE AND AGE OF ANIMALS IN THE CHALLENGE STUDIES REPORTING VACCINATION OF SWINE AGAINST *SALMONELLA* ASSOCIATED WITH FOODBORNE OUTCOMES

<i>Author</i>	<i>Country</i>	<i>Type of pig</i>	<i>Age at enrollment</i>
Baum <i>et al.</i> , 1997	USA	Commercial	3 weeks
Charles <i>et al.</i> , 1999	USA	Commercial	3-4 weeks
Groninga <i>et al.</i> , 2000	USA	Commercial	3 weeks
Kolb <i>et al.</i> , 2003	USA	Commercial	2 weeks
Kramer <i>et al.</i> , 1992	USA	Commercial	Nursery pigs
Kramer <i>et al.</i> , 1987	USA	Commercial	Weaned
Neubauer <i>et al.</i> , 2005	USA	Commercial	3 weeks
Roof <i>et al.</i> , 1995	USA	Commercial	3-4 week
Charles <i>et al.</i> , 2000 ^a	USA	Experimental	3-4 weeks
Charles <i>et al.</i> , 2000 ^b	USA	Experimental	3-4 weeks
Kennedy <i>et al.</i> , 1999	USA	Experimental	7 weeks
Ku <i>et al.</i> , 2005	USA	Experimental	5-8 weeks
Springer <i>et al.</i> , 2001	Germany	Experimental	3-4 week
Coe <i>et al.</i> , 1992	USA	SPF	8 weeks
Hanna <i>et al.</i> , 1979	Ireland	SPF	7 weeks
Lumsden <i>et al.</i> , 1991	Canada	SPF	5-6 weeks
Roesler <i>et al.</i> , 2004	Germany	SPF	4 weeks

Foster <i>et al.</i> , 2003	UK	Gnotobiotic	5 days
Draayer 1986	USA	Not defined	5 weeks
Gibson <i>et al.</i> , 1999	USA	Not defined	3-4 weeks
Husa <i>et al.</i> , 2006	USA	Not defined	3 weeks
Kern <i>et al.</i> , 1994	USA	Not defined	3-4 weeks
Letellier <i>et al.</i> , 2000	Canada	Not defined	12 days

TABLE 3. COUNTRY, TYPE OF VACCINE AND REGIMEN, TYPE OF SAMPLE AND OUTCOME REPORTED IN CLINICAL TRIALS
 REPORTING VACCINATION OF SWINE AGAINST *SALMONELLA* ASSOCIATED WITH FOODBORNE OUTCOMES

<i>Author*</i>	<i>Country</i>	<i>Type of vaccine</i>	<i>Vaccine regimen</i>	<i>Type of sample used</i>	<i>Outcome reported</i>
Baum <i>et al.</i> , 1997	USA	Avirulent <i>S. choleraesuis</i> live culture vaccine (SC540 - NOBL Laboratories Inc.)	Orally, two weeks after entering finishing site	swine to assess <i>Salmonella</i> in market weight	Serogroup B (p=0.0209) Serogroup C1 (p=0.0001) Serogroup C2 (p=0.0510) Serogroup E (p=0.0007) All serogroups (p<0.05)
Kolb <i>et al.</i> , 2002	USA	Avirulent live <i>S. choleraesuis</i> (Enterisol® SC-54)	Orally	Ileocecal lymph nodes and spiral colon fecal content	Carcass (p=0.02) Fecal (p=0.1)

Maes <i>et al.</i> , 2001	USA	<i>S. choleraesuis</i> live vaccine (Argus SC™) Intervet Inc.	Twice orally, at week 3 and 16	Ileocecal lymph nodes	(p<0.001)
Roesler <i>et al.</i> , 2006	Germany	Inactivated herd specific <i>S. typhimurium</i>	6 weeks ante-partum	Fecal	Significant **
Schwarz <i>et al.</i> , 2006	Brazil	Attenuated <i>S. choleraesuis</i> (Enterisol SC®, Boehringer Ingelheim Vetmedica, Inc.)	Once, day old pigs	Mesenteric lymph nodes	(p<0.05)

* All the clinical trials were conducted in commercial available finisher pigs.

** Only percentage of pigs positive for *Salmonella* were reported

CHAPTER 4

A REVIEW OF ANTIBIOTICS TO REDUCE *SALMONELLA* IN SWINE: QUANTITATIVE ANALYSIS OF BINOMIAL AND CONTINUOUS DATA

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Summary

The administration of sub-therapeutic levels of antibiotic as a prophylactic in swine feed is commonly practiced in swine production systems. To summarize the efficacy of antibiotic use in feed to control *Salmonella* Typhimurium shedding in swine, a systematic review of the literature and a meta-analysis were conducted. Multiple online data bases were searched through May 2007 with combination of search terms describing; population, intervention and outcome of interest for the review. Two reviewers independently applied selection criteria, carried out quality assessment, and one reviewer extracted the data. Fourteen challenge trials described the efficacy of different group of antibiotics in swine after challenged with *S. Typhimurium*. The most widely used group of antibiotic was tetracycline group either alone or combined with neomycin next to bambarmycin group. Both binary and continuous outcome were assessed for variation between multiple time points within studies, and between studies. Continuous data were analyzed using standardized mean differences and 95% confidence intervals while relative risks and 95% confidence intervals were calculated for binary data. The analysis of statistical heterogeneity between multiple time points in studies

suggest no statistical heterogeneity between multiple time points of individual studies for binary and continuous outcome. However, the assessment of heterogeneity between studies evaluating the same group of antibiotic showed statistically significant evidence of heterogeneity in tetracycline group of studies and studies of tetracycline with neomycin. There was no statistical heterogeneity between two studies of bambamycin, and summary Mantel-Haenszel risk ratio and 95% confidence interval (0.80, 0.65 - 0.98) was calculated. Limitations of these findings were that these studies were challenge studies, and outcome was measured before the age of market weight. Therefore, length of follow-up has been too short to assess the efficacy of antibiotics in market weight swine.

Introduction

Salmonella Typhimurium is a foodborne pathogen commonly associated with consumption of pork however infection with *S. typhimurium* in swine generally leads to asymptomatic carrier state, although the animals may shed the organism in feces (Schwartz, 1991; Wilcock and Olander, 1978). As part of a larger project to evaluate the effectiveness of numerous interventions to reduce *Salmonella* in swine (O'Connor et al., 2006), we reviewed the literature describing the association between sub-therapeutic level of antibiotics use in swine feed and *S. Typhimurium* shedding. Shedding was evaluated using binary and continuous measures of *Salmonella*.

Antimicrobial agents have been used in swine feed supplements for many years to control *Salmonella* Choleraesuis infections. *S. Choleraesuis* is a host adapted pathogen that is associated with paratyphoid in swine (Schwartz, 1991). Growing interest in food safety has promoted several researchers to evaluate the efficacy of sub-therapeutic levels

of antibiotic in feed or water to reduce the presence of *S. Typhimurium* shedding or duration of *Salmonella* shedding in swine. However, no attempts have been made to summarize the efficacy of sub therapeutic level of antibiotics used to reduce *Salmonella* in swine across numerous studies. The magnitude of the expected impact of sub therapeutic levels of antibiotic on *S. Typhimurium* summarizing the efficacy across several studies should provide a better estimate of the overall expected effect. Therefore, this systematic review and meta-analysis is an attempt to summarize the efficacy of antibiotics from published studies.

Materials and methods

The objective of the systematic review and meta-analysis was to summarize the efficacy of the intervention of interest, sub-therapeutic antibiotics in feed or water , to reduce *S. Typhimurium* shedding in the population of interest i.e. finisher swine in modern swine production systems and compare the inference obtained from studies reporting continuous and categorical outcomes. Only field trials and challenge trials conducted to evaluate the efficacy of sub-therapeutic antibiotics were considered relevant for the review. The outcome of interest was the presence of *S. Typhimurium* in swine feces measured by culture and reported as either a binary variable (presence or absence of *S. Typhimurium*) or a continuous variable (number of colony forming units). Evidence of prior exposure to *Salmonella* measured by serological methods was not considered relevant to the review and these studies were excluded.

The 1st step in the review was to indentify studies relevant to the review topic; therefore, an extensive literature search was performed using online electronic data bases

from inception to May 15th, 2007. The databases were AGRICOLA (1970-2007), AGIRS (1975-2007), Biological and Agricultural Index (1983-2007), Biological Abstracts (1980-1989), Biosis Previews (1980-2007), CAB Abstracts (1973-2007), Current Contents (1998-2007), Dissertation Abstracts (ProQuest Digital Dissertations, 1961-2007), Food Science and Technology Abstracts (1969-2007), Ingenta Gateway (1997-2007), MEDLINE (1950-2007), PubMed (1965-2007), and Web of Science (1945-2007). To capture the relevant studies, search strings were designed to capture the population of interest, the intervention of interest and outcome of interest. Search terms within a group were combined with “OR”, and group of terms were combined with “AND”. The terms used to capture the population were: boar or porcine or gilt or sow or market-weight or finisher or pig or swine or hog. The terms used to capture the intervention used were additive or forage and feed science or antibiotic or ionophore or natural additive or feed concentrate or medicated feed or food microbiology or lactic acid bacteria or organic acid and salts or dietary mineral supplements or ergogenic acids or feed supplements or protein supplement or vitamin supplement or vitamin-mineral supplement or water acidifiers or water treatment or probiotic* or prebiotic*. Finally the terms used to capture the outcome were carcass swab or cecal lymph node or pharyngeal swab or feces or fecal content or rectal swab or rectal content or mesenteric lymph node or cecal content or fecal shedding or fecal shedding. The term salmonell* was included at the end of each string with “AND”. Language restrictions were not imposed for the literature search. To augment to electronic search, hand searching for relevant studies was also conducted of important conference proceedings: American Association of Swine Veterinarians (1973-

2007), International Pig Veterinary Society Congress (1969-2007), and International Symposium on the Epidemiology and Control of *Salmonella* in Pork (1996-2007).

After identification the potentially relevant citations were retrieved, and each citation was assessed by two reviewers. The screening questions were;

- (1) Does the abstract describe primary research in English?
- (2) Does the abstract describe the use of orally administered product in swine?
- (3) Does the abstract report ante-mortem or post-mortem presence of *Salmonella* in pigs?

The above screening questionnaire selected studies describing antibiotics use in swine, and acidification of feed or water in swine. This review will focus only on antibiotic use to reduce *Salmonella* in swine. Electronic systematic review software (Systematic Reviews SRS 3.0, TrialStat Corporation, Canada) was used to coordinate the review process. Disagreements between reviewers about the review questions were resolved by discussion during the relevant screening.

After identifying citations likely to be relevant to the review topic, full texts of potentially relevant citations were retrieved. The methodological qualities of the full manuscripts were again independently assessed by two reviewers. The checklist of quality assessment ensured the minimum quality required to evaluate the design and conduct of challenge trials and field trials. Studies that did not report the specific quality items within the checklist were excluded from the review.

Criteria applied for the quality assessment checklist of challenge studies were;

- (1) Were sampling units randomly assigned to the treatment groups?
- (2) Were the intervention protocols adequately described?
- (3) Was an appropriate control group used?

(4) Were the methods used to measure the outcome standard adequately described?

(5) Was the type of statistical analysis appropriate for the study design?

Criteria applied for the quality assessment checklist of field trials were;

(1) Were the animals housed or grouped in a way that is representative of field conditions?

(2) Were sampling units randomly assigned to the treatment groups?

(3) Was an appropriate control group used?

(3) Were the intervention protocols adequately described?

(4) Was the type of statistical analysis appropriate for the study design?

From each study that met the quality assessment criteria, binominal and continuous data describing *Salmonella* outcome were extracted from treatment and control group from multiple time points where the outcome were measured. When more than one antibiotic regimen was evaluated within a study, data from sub-therapeutic antibiotic treatment group compared with the control group was considered relevant for the review. For studies that reported *Salmonella* recovery on multiple days after challenge with *Salmonella*, data from each day was extracted. Binominal data extracted were number of animals per group, number of animals' positive and negative for *Salmonella*. For continuous data, either *Salmonella* counts in number of colony forming units per gram (cfu/g) of feces or mean number of cfu/g of feces or log mean number of cfu/g of feces or geometric mean number of cfu/g of feces isolated in each treated antibiotic and control groups were extracted. When only continuous data were reported these were also transformed into binary data. When data were only presented graphically,

the values were estimated by measurement from the graph. Data were extracted by one reviewer (TD) onto a data extraction form (Sargeant et al., 2006).

To assess the overall association between sub-therapeutic antibiotic use and *Salmonella* recovery in swine, a meta-analysis approach was used using separate analysis for binary and continuous data for each antibiotic family. For binary data, if the number of animals positive for *Salmonella* in both treatment and control group was zero for any given day within a study, that particular day was excluded from the analysis. If the number of animals positive was zero in either treatment or control group (i.e. only one cell of the 2 by 2 table), then 0.5 was added to each cell of the 2 x 2 table for that particular day (Egger et al., 2001).

If the continuous data were presented in the form of number of cfu for each individual animal, the mean log cfu for the group was calculated using the formula;

$$Y = \frac{\log_{10} X}{n}$$

Where Y is the mean of the log cfu in either antibiotic treated or control group for a given time point. X is the number of cfu in either antibiotic treated or control group for a given day and n is the number of animals in either antibiotic treated or control group.

When the group level variable “mean number of cfu” was reported this outcome was transformed into the log mean cfu using the formula:

$$Y = \log_{10} X$$

Where Y is the log mean number of cfu for either antibiotic treated or control group, and X is the mean number of cfu either antibiotic treated or control group.

When the geometric mean number of cfu per gram of feces per group was presented this was transformed into mean of the log cfu for the analysis using the formula:

$$Y = \log_{10} Z$$

Where Y is mean of the log cfu per gram of feces, and Z is the geometric mean number of cfu per gram of feces in either antibiotic treated or control group for a given day (Geometric means is the average of the logarithmic values of a data set, converted back to a base 10 number).

The statistical analyses were performed by Stata 8.2 (Statacorp, TX), SAS 9.1(SAS Institute Inc., Cary, NC), and Comprehensive Meta-Analysis 2.0 (Biostat, Englewood, NJ).

The statistical analysis required a step wise approach. The 1st step assessed the heterogeneity of the outcome within a study i.e. heterogeneity between multiple time points in individual studies. Statistical heterogeneity of effects measured relative risk (RR) across multiple time points in studies was assessed by the Cochran's Q statistic. Cochran's *Q* test was used to test the null hypothesis that the effect at multiple time points is equal for a binary outcome (RR). The null hypothesis was that the RR for multiple time points was not homogeneous. The p value for Cochran's Q statistic less than 0.1 was considered evidence against the null hypothesis. If there was no reason to reject the null hypothesis investigating the homogeneity for multiple time points in studies, the next analysis step was to combine days across a study to calculate a summary effect measure of the difference between the treatment and control groups for each study. The summary effects measure for binary data was expressed as study-specific Mantel-Haenszel risk

ratio ($\theta_{MH-study}$) with 95% confidence interval. If the summary $\theta_{MH-study}$ was less than one and 95% confidence interval did not include one, the interpretation was that the particular antibiotic was associated with reduced shedding of *S. Typhimurium*. When the confidence interval for $\theta_{MH-study}$ included the null value of one, the interpretation was no evidence in efficacy between treatment and control group. If the summary $\theta_{MH-study}$ was greater than one and 95% confidence interval did not include one, the interpretation was that the particular antibiotic was associated with increased shedding of *S. Typhimurium*.

For continuous data, statistical heterogeneity of effects measured standardized mean difference (SMD) across multiple time points in studies and assessed by the Cochran's Q statistic. Cochran's Q test was used to test the null hypothesis that the SMD for multiple time points was homogeneous. The p value for Cochran's Q statistic less than 0.05 was considered evidence against the null hypothesis. If not evidence was found to reject the null hypothesis when investigating the homogeneity for multiple time points in studies, the next analysis step was to combine the multiple time points with a study to calculate a summary effect measure (SMD) of the difference between the treatment and control groups for each study. The summary effect measure for continuous data was expressed as study-specific standardized mean difference (SMD_{study}) with 95% confidence interval. If the summary SMD_{study} was greater than zero and 95% confidence interval did not include zero, the interpretation was that the particular antibiotic was associated with reduced shedding of *S. Typhimurium* in the antibiotic treated group. If the summary SMD_{study} was less than zero and 95% confidence interval did not include zero, the interpretation was that the particular antibiotic was associated with increased shedding of *S. Typhimurium* in the antibiotic treated group.

After analysis of the individual study outcomes, the aim of the next analyses was to evaluate if studies reporting the use of the same antibiotic reported similar outcomes. Cochrane Q test statistic was calculated as above for multiple studies of same group of antibiotics using binary and continuous data. Finally if the analysis suggested the outcomes across the studies with the antibiotic group were homogeneous, a summary effect measure was calculated for the antibiotic group ($\theta_{MH \text{ antibiotic}}$, $SMD_{\text{antibiotic}}$). The formulae for Q statistics and summary effect measures are below.

Step 1: Heterogeneity between multiple time points in individual studies

To assess the heterogeneity between outcomes measured in multiple time points within each study for binary data, Cochrane Q statistic was calculated in STATA using metan command.

Q statistic is given by

$$Q = \sum w_i(\theta_i - \theta_{MH})^2$$

Where θ is the log relative risk

When the $P > 0.05$ for the Q statistic, we assumed that evidence against the null hypothesis, and therefore fixed effect model was used to calculate the summary relative risk. This approach treated days within a study as independent based on the Q statistics.

Heterogeneity between multiple time points in each study for the continuous outcome was assessed using Cochrane Q statistic given by

$$Q = \sum w_i(\theta_i - \theta_{IV})^2$$

where θ is the standardized mean difference

$$\theta_{IV} = \frac{\sum w_i \theta_i}{\sum w_i}$$

where θ is the standardized mean difference (SMD) for time point i

$$\theta = \frac{\text{MeanDifference}}{\text{PooledSD}}$$

The weight applied was inverse variance in fixed effect model.

weight is given by

$$w_i = \frac{1}{SE(\theta_i)^2}$$

Standard error of θ_{IV} is given by

$$SE(\theta_{IV}) = \frac{1}{\sqrt{\sum w_i}}$$

Values for Q statistic with a P value < 0.05 were considered as evidence of heterogeneity. When there was no evidence of heterogeneity between multiple time points in individual studies, it was decided that it is appropriate to combine the data within studies.

Step 2: The summary effect measure for binary data was given by the Mantel-Haenszel risk ratio (θ_{MHs}) given by

$$\theta_{MH} = \frac{\sum w_i \theta_i}{\sum w_i}$$

where θ is the risk ratio for time point i

weight is given by

$$w_i = \frac{c_i n_i}{N_i}$$

and c_i is the number of events occurred in the control group for time point i

and n_i is the number of individuals in the vaccinated group for time point i

and N_i is the number of individuals in the study for time point i

The summary effect measure for continuous data was given by summary standardized mean difference calculated for each study using mean difference and standard deviation in multiple days using metan command in STATA.

$$\theta_{IV} = \frac{\sum w_i \theta_i}{\sum w_i}$$

$$w_i = \frac{1}{SE(\theta_i)^2}$$

where θ is the standardized mean difference (SMD) for time point i

Step 3) Heterogeneity between same groups of antibiotic studies

Heterogeneity between studies of same antibiotic family was assessed using binary and continuous data where available. Each day an outcome reported was represented an independent observation in the analysis.

Values for Q statistic with P values < 0.05 were considered as evidence of to reject the null hypothesis and conclude that the studies were likely heterogeneous. When the P > 0.05 and the null hypothesis was not rejected i.e. studies were homogeneous, the summary θ_{MH} and 95% confidence interval of same group of antibiotics with binary data was calculated and for continuous data the SMD and 95% confidence interval were calculated. Under the fixed effect model it is assumed that the true effect measure is shared by each study, and the difference between studies results are due to within study variance.

Finally, comparisons of antibiotic efficacy were made comparing two data types in individual studies, binary data and continuous data of same group of antibiotic studies that provided usable information. The summary effects estimates for binary data were expressed as relative risk with 95% confidence interval. If Mantel-Haenszel risk ratio was less than one and confidence interval does not include one, interpretation was significant reduction in *Salmonella* shedding. Continuous data were expressed as standardized mean difference with 95% confidence interval. A positive SMD and confidence interval does not include zero, interpretation was significant reduction of *Salmonella* shedding.

Results

In the full review of interventions, 282 publications identified describing multiple interventions to reduce *Salmonella* prevalence in swine. 33 studies described antibiotic use to control *Salmonella* Typhimurium in swine. Four field trials (Funk et al., 2001; Nielsen et al., 1997; Roesler U. et al., 2004; Roesler et al., 2005) and 10 challenge trials (Abou et al., 1979; Baggesen et al., 1999; Culbreth et al., 1972; Edrington et al., 2001; Fenwick and Olander, 1987; Olson et al., 1972; Olson et al., 1977; Olson and Rodabaugh, 1977; Singh, 1968; Troutt et al., 1974) were excluded from the review based on the quality assessment criteria leaving 19 studies to be included in the further consideration. Five observational studies describing antibiotic use to control *Salmonella* are described in a separate systematic review and will be published elsewhere. Only fourteen studies, all challenge studies, met the quality assessment criteria and data was extracted from these studies. Four studies (Ebner and Mathew, 2000; Gutzmann et al., 1976; Jones et al., 1983; Williams et al., 1978) included multiple trials, therefore, the 14 studies described 19

antibiotic treated and control comparisons. Three studies (Delsol et al., 2003; Ebner and Mathew, 2000; Wilcock and Olander, 1978) did not provide enough usable data for the meta-analysis i.e. these studies only reported the outcome of significance testing rather than group level data with measures of variation (Figure 2).

The characteristics of 14 challenge studies are summarized in Table 1. Most of the studies used commercial pigs as study subjects. The number of animals in treatment group varied from 12 to 48. The age of pigs at the start of the antibiotic treatment was varied from 3 to 9 weeks, and duration of the studies lasted from 4 weeks to 8 weeks, except in one study which lasted 17 weeks in which specific pathogen free (SPF) pigs were used. Twelve out of 14 studies were conducted in USA.

All the studies allocated pigs into treatment and control groups either by simple randomization or blocked randomization (Table 2). The most commonly used group of antibiotic was the tetracycline group including chlortetracycline, oxytetracycline, and tetracycline (Claussen et al., 1998; Delsol et al., 2003; Evangelisti et al., 1975; Gutzmann et al., 1976; Jones et al., 1983; Williams et al., 1978) Four challenge studies described the use of tetracycline in combination with another antibiotic. (Ebner and Mathew, 2000; Girard et al., 1976; Gutzmann et al., 1976; Wilcock and Olander, 1978) Two studies described the use of bambamycin to reduce *S. Typhimurium* in swine. (Dealy and Moeller, 1976; Letellier et al., 2000) Aminoglycoside, lincosamide, macrolide and streptogramin were each only evaluated by a single study (DeGeeter et al., 1976; Jacks et al., 1988; Jones et al., 1983; Shryock et al., 1998). Within the same group of antibiotic, the sub-therapeutic dose differed from one study to another as did the level of *S. Typhimurium* used per pig for artificial challenge (Table 2).

Bacterial culture protocols for isolation and identification of *Salmonella* were differs among studies. Except one, all the other studies were based on 1g of feces. Only two studies described the use of pre-enrichment. Selective enrichment and selective media with various incubation period and incubation temperature has been used. Studies with provided level of detection of *Salmonella*, varied from 10^1 cfu/g to 10^2 cfu/g of feces (Table 3).

Nine trials from six studies provided continuous data (cfu/g of feces) (Table 4) in individual animals for multiple time points, and these data were dichotomized on the basis of whether presence or absence of the organism in microbiological culture (Evangelisti et al., 1975; Girard et al., 1976; Gutzmann et al., 1976; Jacks et al., 1988; Jones et al., 1983; Williams et al., 1978). One study reported group data (mean cfu/g of feces) in antibiotic treated and control groups separately for multiple time points (DeGeeter et al., 1976). The other study also reported group data (log mean cfu/g of feces) in antibiotic treated and control group for multiple time points (Shryock et al., 1998). Two studies reported only binary data for multiple time points (Claussen et al., 1998; Letellier et al., 2000). For all studies with suitable data, the analyses suggested no significant statistical heterogeneity between multiple time points in studies for binary and continuous outcome (Table 4). Therefore, summary Mantel-Haenszel risk ratio and SMD were calculated.

Three trials reported significant reduction of *Salmonella* shedding when binary data were analyzed (Claussen et al., 1998; Evangelisti et al., 1975; Girard et al., 1976). Using continuous data, six trials reported a significant reduction of *Salmonella* shedding (DeGeeter et al., 1976; Evangelisti et al., 1975; Girard et al., 1976; Gutzmann et al.,

1976; Shryock et al., 1998; Williams et al., 1978), while two trials showed significant increases in *Salmonella* shedding (Jacks et al., 1988; Williams et al., 1978) (Table 4). In only two studies was the administration of sub-therapeutic antibiotics associated with reduced *Salmonella* shedding as determined by binary and continuous measures of *Salmonella* (Evangelisti et al., 1975; Girard et al., 1976).

The assessment of homogeneity between studies using both binary and continuous data for the 6 tetracycline group studies showed statically significant evidence of heterogeneity (Cochrane Q statistic p value for binary data was 0.000 and for continuous data was 0.003). Also, using binary data, there was statistically significant heterogeneity in 2 studies of tetracycline with neomycin (Cochrane Q statistic p value for binary data= 0.000). There was no evidence to reject the null hypothesis of homogeneity between two studies of bambarmycin (Cochrane Q statistic p value for binary data was 0.6) (Table 5), and therefore summary Mantel-Haenszel risk ratio and 95% confidence interval (0.80, 0.65 - 0.98) was calculated.

Discussion

This systematic review and meta-analysis of the literature aimed to summarize the efficacy of antibiotic to reduce *S. Typhimurium* shedding in market weight finisher swine. Based on the quality criteria, only challenge studies were selected for the final review as all identified field trials failed to meet the quality assessment criteria, and were therefore, excluded from the review. Specifically, all the field trials did not report random allocation or blocking of pigs into treatment groups. The principle of randomization is to limit the confounding bias (Dohoo I et al., 2003). Our preference would be to report

outcomes from field trials as they are thought to better represent the “real world” however, the failure to describe randomization in the study suggests a potential bias and we have excluded studies based on this potential bias.

The review suggests that antibiotics can be associated with a reduction in *Salmonella* shedding although the effect does not occur for all antibiotics. On one occasion, antibiotics were associated with increased shedding. Further, *Salmonella* reduction is more likely to be detected if a continuous outcome is used rather than binary. This finding has implications for assessment of outcomes as it appears that antibiotic treatment decreases the amount of *Salmonella* but not necessarily the number positive animals. For food safety interventions traditionally the assessment has focused on the “number of positive pigs”, with the assumption that a reduction in positive pigs would translate to reduce *Salmonella* in pork. The validity of this assumption is unclear. However, clearly it is important to consider the outcome that should measure and which has the stronger association with food borne disease risk. If binary outcomes are related to risk, then only 3 of 12 studies suggest an association, however if continuous data are related to risk of 8 of 12 studies suggest an association (positive or negative).

Unfortunately, one outcome of the review is that we could not identify any studies that reported randomization conducted in the population of interest, finisher pigs. Although the pigs enrolled for the studies at the age of weaning, the outcome was measured before the age of market weight, despite the fact that recommended antibiotic withdrawal period before slaughter. Though, these trials were designed to assess the efficacy of antibiotic prophylaxis, the length of follow-up has been too short to assess the efficacy of antibiotics in market weight swine. Thus, extrapolating beneficial effect of

antibiotic use in these studies to market weight finisher swine, if any, is misleading. Multidrug resistant *S. typhimurium* is responsible for 10% - 30% of human salmonellosis in United States (Centers for Disease Control and Prevention (CDC), 2006). Prophylactic use of antibiotics in feed suppresses the normal intestinal flora of the pig, permitting *Salmonella* to increase in number or to establish a carrier state in the pig, and therefore increasing the number of resistant bacteria entering the abattoirs and thereby increasing the public health risk through food chain.

Studies selected for this systematic review has been conducted during the years starting from 1975 to 2003, and various culture protocols including pre-enrichment, enrichment, selective media, and duration of incubation for *Salmonella* isolation and identification has been practiced. The only similarity observed in most of these studies was the weight of feces used for the culture (Table 3)

Significant heterogeneity was found between studies of tetracycline, and studies of tetracycline with neomycin (Cochrane Q p value <0.05) (Table 5). It is possible that lack of consistency among study protocol, and different levels of detection contributed to the heterogeneity between outcomes of studies of same group of antibiotics. It was not possible to investigate sources of variation, although, sub-group analysis and meta-regression methodology exist to investigate the sources of variation (Egger et al., 2001). The summary M-H RR estimate of two studies describing bambermicin use in 4 -9 weeks old swine was 0.8 (confidence interval 0.65 to 0.98) in antibiotic treated group compared to control group (Figure 1). Although, the summary estimate suggests a 20% reduction in *Salmonella* shedding, the power of this estimate is limited as only two have contributed

information and the number animals included in antibiotic intervention and control groups were very low.

This review has several strengths. Comprehensive search for literature by exploring thirteen electronic databases and by hand searching three conferences proceedings relevant to the review topic captured all possible published literature. This comprehensive literature search minimized the possibility of publication bias. Screening for studies was conducted independently by two reviewers, as was quality assessment of included studies, and disagreements were resolved with discussion to minimize the reviewer bias, and conducted this systematic review and meta-analyses in accordance with published guidelines (Sargeant et al., 2006). In interpreting these results, some of the limitations of these studies must be taken into consideration. Assumption of independence of outcome measured in multiple time points is another concern. Finally, it is important to bear in mind that given the lack of relevance of these challenge studies to the natural infection under field conditions in finisher swine, no absolute conclusion can be drawn from this systematic review on the efficacy of antibiotic to reduce *Salmonella* shedding in market weight finisher swine (Griffith et al., 2006).

Other potential biases also have to be considered. We have restricted our review to articles published in English, because of the logistic difficulties of translation from other languages. We excluded trials that were only reported in abstract form, because of the impossibility of extracting the data for meta-analysis. To avoid potential biases, an adequate methodology must be used in designing field trials, including the definition of primary objective, a priori statistical evaluation of the number of animals to be included,

description of the randomization method and allocation concealment, and description of the blinding at outcome measure

In conclusion, from the available literature, although some antibiotics reduced the *Salmonella* shedding in swine in these challenge studies, extrapolation of these findings to finisher swine is not recommended.

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Table 1: Country, type of animal and number of animals used for the trial, age or weight at the enrollment, and study period for the challenge studies describing *S. typhimurium* measured by culture

Study	Country	Type of pig production	Total N*	Age or weight at enrollment	Study period in days
Claussen et al., 1998	USA	Commercial	20	at weaning	35
Dealy et al., 1976	USA	Commercial	20	4 to 5 weeks	49
DeGeeter et al., 1976	USA	Commercial	19	4 to 5 weeks	53
Delsol et al., 2003	UK	Commercial	12	3 weeks	41
Ebner et al., 2000	USA	Experimental	48	3 weeks	84
Evangelisti et al., 1975	USA	Commercial	20	9.6 kg	28
Girard et al., 1976	USA	Commercial	20	9.1 kg	28
Gutzmann et al., 1976	USA	Experimental	30	5 to 6 weeks	26
Jacks et al., 1988	USA	Commercial	20	6 to 7 weeks	56
Jones et al., 1983	USA	Commercial	12	6 weeks	31
Letellier et al., 2000	Canada	Commercial	20	12 days	28
Shryock et al., 1998	USA	Commercial	20	7 weeks	57
Wilcock et al., 1978	USA	SPF**	20	Weaned	120
Williams et al., 1978	USA	Commercial	14	8-18kg	57

* Total N represent only the number of animals in treatment group (challenged with *Salmonella* and antibiotic treated) and control group (challenged with *Salmonella* only)

** Specific pathogen free

Table 2. Method of allocation, antibiotic regimen and number of colony forming units used to induce artificial infection in studies selected for the review

Author	Method of allocation	Antibiotic regimen	Challenge Exposure
Claussen et al., 1998	Blocked by gender, and body weight	Chlortetracycline 200 g/ton of feed ad libitum	<i>S. Typhimurium</i> 2 x 10 ⁹ cfu twice orally
Dealy et al., 1976	within gender Blocked weight, sex and litter number	Oxytetracycline 5 mg/lb body weight, intramuscular on days 3, 4, 5, 17, 18, 19 post exposure Bambermycines 4.4 mg/kg of feed 5 days prior to challenge	on day 0 and day 14 <i>S. Typhimurium</i> 2.5 x 10 ¹¹ cfu orally
DeGeeter et al., 1976	Randomly assigned	Lincomycin 110 mg/kg of feed 7 days before exposure and throughout the experimental period	<i>S. Typhimurium</i> 1 x 10 ¹¹ cfu orally
Delsol et al., 2003	Randomly assigned	Aureomycin 1.5 mg/kg of feed for 7 days	<i>S. Typhimurium</i> 2x 10 ⁹ cfu orally
Ebner et al., 2000	Blocked by litter	T ₁ . Ceftiofur intramuscular for 3 days Oxytetracycline 100 g/ton of feed there after T ₂ . Apramycin 150 g/ton of feed for 14 days Oxytetracycline 100 g/ton of feed there after T ₃ . Carbadox 50 g/ton until pigs reached 35 kg	<i>S. Typhimurium</i> 3.6 x 10 ⁹ cfu intranasal <i>S. Typhimurium</i> 3.6 x 10 ⁹ cfu intranasal <i>S. Typhimurium</i> 3.6 x 10 ⁹ cfu intranasal

Evangelisti et al., 1975	Blocked by weight and sex	Oxytetracycline 150 g/ton of feed 5 days prior to exposure and throughout the experimental period	<i>S. Typhimurium</i> 1.4 x 10 ¹¹ cfu orally
Girard et al., 1976	Randomly assigned	Oxytetracycline 150 g and Neomycin 150 g/ton of feed 5 days prior to exposure and throughout the experimental period	<i>S. Typhimurium</i> 1.4 x 10 ¹¹ cfu orally
Gutzmann et al., 1976	Blocked by litter, sex and weight	T ₁ . Chlortetracycline 220.5 g/metric ton of feed starting from 5 days before exposure and there after continuous T ₂ . Chlortetracycline 110.2 g, Sulfamethazine 110.2 g and Penicillin G 55.1 g/metric ton of feed 5 days before exposure and there after continuous	<i>S. Typhimurium</i> 1 x 10 ¹¹ cfu orally <i>S. Typhimurium</i> 1 x 10 ¹¹ cfu orally
Jacks et al., 1988	Blocked by litter, sex and weight	Efretomycin 16mg/kg of feed ad libitum	<i>S. Typhimurium</i> 1.7 x 10 ¹⁰ cfu orally
Jones et al., 1983	Blocked by weight within sex	T ₁ . Chlortetracycline 55 mg/kg of feed ad libitum T ₂ . Virginiamycin 55 mg/kg of feed ad libitum	<i>S. Typhimurium</i> 5.1 x 10 ⁹ cfu orally <i>S. Typhimurium</i> 5.1 x 10 ⁹ cfu orally
Letellier et al., 1999	Randomly assigned	Flavomycin 0.5 g/ton of feed	<i>S. Typhimurium</i> 1 x 10 ⁷ cfu orally 14 days

Shryock et al., 1998	Blocked by weight and sex	Tylosin 100 g/ton of feed ad libitum	1st dose <i>S. Typhimurium</i> 5.2×10^{10} cfu 2nd dose <i>S. Typhimurium</i> 5.6×10^{10} cfu
Wilcock et al., 1978	Blocked by equal weight and sex	Neomycin 110 g and Oxytetracycline 110 g/ton of feed	<i>S. Typhimurium</i> 1×10^{10} cfu orally
Williams et al., 1978	Randomly assigned	T ₁ . Chlortetracycline 110 g/ton of feed 5 days prior to exposure and throughout the experimental period	<i>S. Typhimurium</i> 1.35×10^9 cfu orally
		T ₂ . Chlortetracycline 110 g/ton of feed 5 days prior to exposure and throughout the experimental period	<i>S. Typhimurium</i> 6.5×10^{10} cfu orally

Table 3. Variability of bacterial culture procedures describing *Salmonella* isolation and enumeration in studies conducted during 1975 to 2003

Study	Fecal	Pre-enrichment	Enrichment	Selective media	Level of detection
Claussen et al., 1998	1 g	TT at 42 ^o C for 48 h	RV R10 42 ^o C for 24 h	XLT4 at 42 ^o C for 24 h	Not reported
Dealy et al., 1976	1 g	Not described	TTBG	BGA at 37 ^o C for 36 h	10 ² cfu/g
DeGeeter et al., 1976	1 g	Not described	Not described	MA & BGA at 37 ^o C for 24 h	Not reported
Delsol et al., 2003	1 g	Not described	SCB at 37 ^o C for 18 h	MA	10 ² cfu/g
Ebner et al., 2000	*	Not described	i.TT at 42 ^o C for 24 h	XLT4 at 37 ^o C for 24 h	No enumeration
	*	Not described	ii.SCB at 37 ^o C for 24 h	XLT4 at 37 ^o C for 24 h	No enumeration
Evangelisti et al., 1975	1 g	Not described	BGA	BGSA at 37 ^o C for 48 h	10 ² cfu/g
Girard et al., 1976	1 g	Not described	BGA	BGSA at 37 ^o C for 48 h	10 ² cfu/g
Gutzmann et al., 1976	1 g	Not described	Not described	MA at 37 ^o C for 24 h	Not reported
Jacks et al., 1988	1 g	Not described	TT at 37 ^o C for 48 h	BGA at 37 ^o C for 24 h	10 ¹ cfu/g
					(1cfu/enriched)
Jones et al., 1983	1 g	Not described	TTBG at 37 ^o C for 18 h	BGA at 37 ^o C for 24 h	10 ² cfu/g
Letellier et al., 2000	1 g	NB at 37 ^o C for 18 h	TTBG at 37 ^o C for 48 h	BGSA at 37 ^o C for 48 h	Not reported

Shryock et al., 1998	3 g	Not described	BGB at 37 ⁰ C for 18 h	MA at 37 ⁰ C for 24 h	10 ¹ cfu/g
Wilcock et al., 1978	‡10 g	Not described	TT at 42 ⁰ C for 24 h	BGA at 42 ⁰ C for 15 h	Not reported
Williams et al., 1978	1 g	Not described	TT at 37 ⁰ C for 24 h	BGA at 37 ⁰ C for 24 h	10 ² cfu/g

BGA = Brilliant Green Agar

BGB = Brilliant Green Broth

BGSA = Brilliant Green Sulfur Agar

NB = Nutrient Broth

MA = MacConkey Agar

RV R10 = Rapaport-Vassiliadis R 10

SCB = Selenite Cysteine Broth

TT = Tetrathionate

TTBG = Tetrathionate Brilliant Green Broth

XLT4 = Xylose-Lysine-Tergitol-4

* Not provided

‡ Composite of five fecal samples from each pen

Table 4. Binary and continuous outcomes where available in studies which measured *Salmonella* outcome in multiple time points in fixed effect model

Author	Time points	Binary data		Continuous data		Effect
		MH RR [‡] (95% CI)	Effect	IV SMD [‡] (95% CI)	Effect	
Claussen et al., 1998	14	0.30 (0.16 to 0.54)	Significantly reduced	No continuous data	-	-
Dealy et al., 1976	9	No binary data	-	0.15 (-0.12 to 0.42)	-	Not significant
DeGeeter et al., 1976	10	1.04 (0.83 to 1.30)	Not significant	0.38 (0.04 to 0.72)	Significantly reduced	Significantly reduced
Evangelisti et al., 1975	6	0.70 (0.53 to 0.92)	Significantly reduced	0.50 (0.16 to 0.84)	Significantly reduced	Significantly reduced
Girard et al., 1976	7	0.26 (0.16 to 0.41)	Significantly reduced	1.44 (0.99 to 1.89)	Significantly reduced	Significantly reduced
Guttzmann et al., 1976 [†]	7	0.88 (0.64 to 1.21)	Not significant	0.65 (0.30 to 0.99)	Significantly reduced	Significantly reduced
Guttzmann et al., 1976 ^{††}	7	1.00 (0.75 to 1.34)	Not significant	0.24 (-0.09 to 0.57)	Not significant	Not significant
Jacks et al., 1988	13	1.12 (0.95 to 1.32)	Not significant	-0.31 (-0.57 to -0.05)	Significantly increased	Significantly increased
Jones et al., 1983 [§]	7	1.08 (0.67 to 1.73)	Not significant	0.39 (-0.21 to 0.99)	Not significant	Not significant

Jones et al., 1983 ^{§§}	7	1.21 (0.78 to 1.88)	Not significant	-0.06 (-0.63 to 0.50)	Not significant
Letellier et al., 2000	4	0.92 (0.45 to 1.86)	Not significant	No continuous data	-
Shryock et al., 1998	10	0.86 (0.66 to 1.12)	Not significant	0.71 (0.44 to 0.97)	Significantly reduced
Williams et al., 1978*	17	1.30 (1.06 to 1.61)	Not significant	-0.46 (-0.82 to -0.10)	Significantly increased
Williams et al., 1978**	21	0.87 (0.73 to 1.05)	Not significant	0.88 (0.54 to 1.22)	Significantly reduced

‡ A RR less than 1 indicates a reduction in *Salmonella* shedding in antibiotic treated group compared to the control group.

¥ A positive SMD indicates a reduction in *Salmonella* shedding in antibiotic treated group compared to the control group.

† chlortetracycline

†† chlortetracycline, sulfamethazine and penicillin G

§ chlortetracycline

§§ virginiamycin

* *S. typhimurium* resistant to chlortetracycline

** *S. typhimurium* susceptible to chlortetracycline

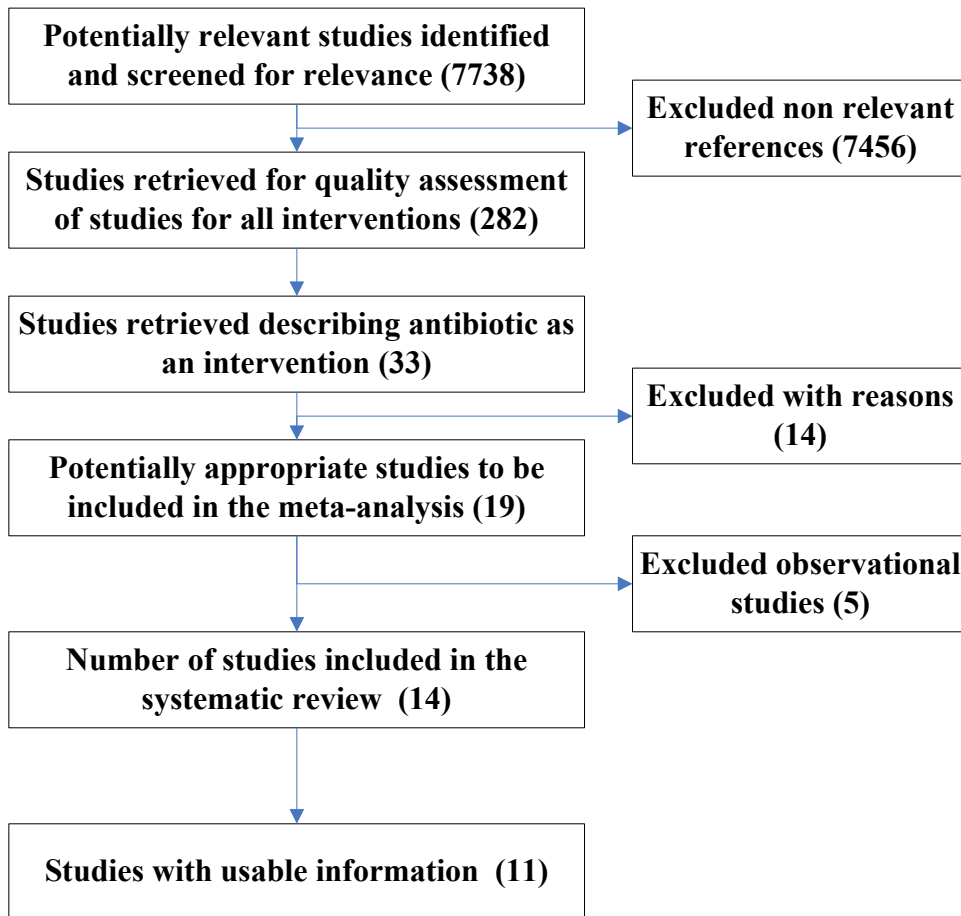
Table 5. Heterogeneity investigation between same family of antibiotic studies using binary and continuous data under fixed effect model

Antibiotic group	Binary data			Continuous data	
	Number of Studies	Number of time points	Cochrane Q p value	Number of time points	Cochrane Q p value
Tetracycline	6	73	0.000	38 [†]	0.003
Tetracycline & Neomycine	2	11	0.000	*	*
Bambermycine	2	15	0.6	*	*

[†] Number of time points for continuous data represents only from 5 studies

* No continuous data available

Figure 1. Flow chart of study selection process



CHAPTER 5

EVALUATING THE SENSITIVITY AND SPECIFICITY OF CULTURE AND PCR METHODS FOR DETECTION OF *SALMONELLA* IN FIELD SAMPLES USING THE STARD APPROACH

T. N. Denagamage

INTRODUCTION

In response to the ongoing United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) national initiative to reduce human salmonellosis by 50% there is a need to develop rapid, economical, and simple techniques for detection and ultimately enumeration of *Salmonella* in order to assess the effectiveness of Hazard Analysis and Critical Control Point (HACCP) interventions as well as to support on-going surveillance. Surveillance, therefore, requires a rapid, user-friendly method to accurately monitor a large number of samples with potentially low *Salmonella* prevalence.

Detection of *Salmonella* in pork products and porcine lymph nodes employs bacteriological isolation, which entails multiple sample transfers to multiple culture media. This process is time-consuming, prone to laboratory cross-contamination, and requires an experienced microbiologist to perform the test. PCR-based technologies may represent a technology that could be rapid and inexpensive and applied to *Salmonella* surveillance within the plant. The aim of this study was to evaluate a PCR for detection of *Salmonella* in field samples. As field samples were used, no gold standard test was available; therefore, Bayesian approaches to data analysis were used. Further, the design

of the study followed the principles of the standards for reporting of diagnostic accuracy (STARD) statement which aims to minimize the bias in reporting of sensitivity and specificity of diagnostic assays.

MATERIALS AND METHODS

In this study, the STARD checklist was used to report the assessment of sensitivity and specificity of PCR and culture methods for *Salmonella* detection in field samples (7,8).

Samples were collected from pigs slaughtered at a large scale abattoir in mid-west United States. The capacity of the abattoir was 2600 pigs per hour. All the pigs included in the study were finished on farms in Iowa. Transport time from farm to abattoir varied up to 3 hours, and lairage time varied to 2 hours from overnight. For the study, finisher pigs from 15 farms were selected, and collected on 15 sampling days during the period from July 2007 to December 2007. At each farm, 30 pigs were selected based on convenience and tattooed. At the slaughter plant, sub-iliac and mesenteric lymph nodes were collected. Mesenteric lymph nodes were collected from the gastro-intestinal viscera after evisceration and separation from the carcass. The sub-iliac lymph nodes were collected before chilling. The sub-iliac lymph nodes were identified at the individual animal level by a code comprising tattoo number of the pig. Mesenteric lymph node samples were separated from the carcass and therefore could only be identified at the farm level. Therefore, it was not possible to match sub-iliac and mesenteric lymph nodes at the individual animal level.

Mesenteric lymph nodes and sub-iliac lymph nodes were collected into labeled sterile plastic bags (Whirl-Pak bag, Fisher Scientific Inc) using sterile forceps and scissors. Samples were transported at ambient temperature to the laboratory within 2 hours of collection and stored at 4° C.

Using sterile forceps, the lymph node was removed from the whirl top bag and dipped in 70% ethanol, and briefly flamed. The flamed lymph node was placed in an appropriately labeled filter bag and macerated with a rubber mallet. 25 ml sterile phosphate-buffered saline was added to the macerated lymph node and the sample was stomached for 60 seconds at 230 rpm to create a lymph node homogenate for the bacterial culture and for the PCR assay. All mesenteric and sub iliac samples were processed immediately using culture, and homogenates were stored for later PCR analysis. Homogenates of mesenteric lymph node samples and sub-iliac lymph node samples were randomly selected (Microsoft Office Excel 2003) from the stored homogenates for real-time PCR assay.

Culture procedure was conducted by a research associate with a Master of Science with a microbiology emphasis.

Two pre-enrichments; tetrathionate (TT) broth and buffered-peptone water (BPW) were used to culture *Salmonella*. For TT broth pre-enrichment, 10 ml of lymph node homogenate was added into 90 ml TT broth, and incubated 48 hours at 37° C. For BPW pre-enrichment, 10 ml lymph node homogenate sample was added into 90 ml BPW, and incubated for 24 hours at 42° C. After incubation, 100 µl of TT broth and 100 µl of BPW were inoculated into appropriately labeled tubes containing 10 ml Rappaport-Vassiliadis R 10 (RV) broth, and incubated for 24 hours at 42° C. After incubation, the RV tubes

were vortexed and streaked for isolation of *Salmonella* onto Xylose-Lysine-Tergitol-4 (XLT-4) agar and Brilliant Green Sulfa (BGS-N) plates, and incubated for 48 – 72 hours at 37° C. Suspected *Salmonella* colonies were inoculated into Triple Sugar Iron (TSI) agar and Lysine Iron agar (LIA), and inoculated for 24 hours at 37 °C. Agglutination tests were carried out on positive samples using *Salmonella* O antiserum poly A-I and Vi (Figure 1). The results of culture were classified as positive or negative, without any quantification of the number of *Salmonella* recovered.

For quality assessment and control, the sets of lymph node homogenate samples were concurrently cultured with one known positive sample containing with *Salmonella enterica* 1 colony forming units (cfu)/g, one negative sample, and one unknown sample either inoculated with *Salmonella enterica* 1 cfu/g or not-inoculated. During the period of the study the results of the quality control for known and unknown samples was 100% correctly identified. Bacterial culture procedure was associated with a quality control measures. In a quality control studies conducted by the laboratory 6 months prior to the initiation of this study, the detection limit of the reference culture method for *Salmonella enterica* was 1 cfu/g in ground pork. Ground pork was used for these baseline studies as large quantities of lymph nodes were not readily available. The *Salmonella* used for the detection limit study was obtained from Curt Thompson of Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University. The minimum detection limit for bacterial culture protocol was 1 cfu/ml.

After random selection of homogenates for analysis using the index test, these samples were submitted and evaluated for *Salmonella*. Real-time PCR was conducted by

a molecular microbiologist trained by a PhD in microbiology, blinded to the culture test results and the prevalence of positive samples in the sample set.

Total genomic DNA from mesenteric and sub-iliac lymph node homogenate samples prior to enrichment was extracted by Ambion[®] MagMAX[™] isolation kit according to the manufacturer's instructions (Cat. AM1840; Austin, Texas). Briefly, lymph node homogenate samples were centrifuged at 100 x g for 1 minute at 4° C to remove tissue debris. A 175 µl volume of supernatant was transferred to a bead tube containing 235 µl of lysis binding solution. Then, the samples were bead-beated for 5 minutes at 20 Hz on a tissue lyser (Qiagen Valencia, CA) to lyse the bacteria to release nucleic acid by physical disruption with the zirconia beads. Later, the beads were pelleted by centrifugation at 16,000 x g for 6 minutes. After centrifugation, 115 µl of each sample was used for subsequent nucleic acid purification. Nucleic acid purification procedure, which involved different washing, binding and elution steps, was performed on 96-well plates using a KingFisher[®] 96 Magnetic Particle Processor (Thermo Scientific, Waltham, MA).

Real time PCR for the detection of *Salmonella* in swine lymph node samples collected at the abattoir were carried out according to the method described previously (19). This assay targeted *Salmonella* enterotoxin *stn* gene. Cutoff value and threshold level were set at 40 cycles and 0.1, respectively. Minimum detection limit was 50 cfu/ml for real-time PCR.

Approximately, 400 samples are required from both populations to be tested with 95% probability that *Salmonella* would be detected in sub-iliac lymph nodes if the true prevalence is greater than or equal to 1%, and in mesenteric lymph nodes if the true

prevalence is greater than or equal to 42% (Table 1). Sample size calculations were performed using the free source software (1) based on the sensitivity and specificity of culture method at 93% and 99% respectively, and sensitivity and specificity for real time PCR expected to be at 99% respectively. Preliminary data showed that the *Salmonella* prevalence in mesenteric lymph nodes to be 42%. *Salmonella* prevalence in sub-iliac lymph nodes was expected to be 1% (personal communication Dr. Annette O'Connor and Dr. James McKean)

For the statistical analysis, Bayesian modeling approaches were used to estimate diagnostic sensitivity and specificity of the reference and index test. Two models were constructed.

The 1st analysis involved a test for sensitivity and specificity using data from two populations (mesenteric and sub-iliac lymph nodes) without a gold standard test assuming dependence between the outcomes of the index and reference tests (10). The analysis assumed that the index and reference outcomes for a given sample are correlated because both tests were testing for similar biological phenomenon. Further, this analysis assumed that sensitivity and specificity of PCR and culture remains the same in both lymph node populations.

The models were run using the freeware program WinBUGS (22). Posterior estimates were obtained on 100,000 iterations of the Gibbs sampler with a burn-in phase of 500 iterations. The median of the posterior distribution was used for the point estimate with 95% probability intervals. Assessment of convergence was determined by examining trace plot and history plot to allow using the posterior distribution produced by Gibbs sampling.

We refer to index test (real-time PCR) as T_1 and reference test (bacterial culture) as T_2 . The formulas used for determination of sensitivity and specificity are provided below.

Let T^+ and T^- denote positive and negative test results, respectively, and let D^+ and D^- denote infected and non-infected animals, respectively. Sensitivity and specificity of test be designated by Se and Sp , respectively, and prevalence is denoted by π .

The model includes two prevalence (one for each population), $(Se_{11}, Se_{12}, Se_{21}, Se_{22})$ and $(Sp_{11}, Sp_{12}, Sp_{21}, Sp_{22})$, where

$$Se_{11} = P(T_1^+, T_2^+ | D^+)$$

$$Se_{12} = P(T_1^+, T_2^- | D^+)$$

$$Se_{21} = P(T_1^-, T_2^+ | D^+)$$

$$Se_{22} = P(T_1^-, T_2^- | D^+)$$

and

$$Sp_{11} = P(T_1^+, T_2^+ | D^-)$$

$$Sp_{12} = P(T_1^+, T_2^- | D^-)$$

$$Sp_{21} = P(T_1^-, T_2^+ | D^-)$$

$$Sp_{22} = P(T_1^-, T_2^- | D^-)$$

Therefore, the conditional correlation between the two test outcomes for an infected and non-infected animal is given by,

$$\rho_{D^+} = \frac{Se_{11} - Se_1 Se_2}{\sqrt{Se_1(1 - Se_1)Se_2(1 - Se_2)}}$$

and

$$\rho D = \frac{Sp_{22} - Sp_1 Sp_2}{\sqrt{Sp_1(1 - Sp_1)Sp_2(1 - Sp_2)}}$$

respectively. Accounting for the correlation between test results for a given animal, estimates of the Se_1 , Se_2 , Sp_1 , Sp_2 are;

$$Se_1 = Se_{11} + Se_{12}$$

$$Se_2 = Se_{11} + Se_{21}$$

$$Sp_1 = Sp_{22} + Sp_{21}$$

$$Sp_2 = Sp_{22} + Sp_{12}$$

For the Bayesian analysis, beta prior distributions were constructed using beta buster software (17). The mode of the beta distribution is given by the formula $(a - 1) / (a + b - 2)$, where, for example if an experiment resulted in “s” number of test-positive animals in “n” number of truly infected animals, use of a beta (a, b) distribution with $a = s + 1$ and $b = n - s + 1$ is recommended as an appropriate choice to model the uncertainty in that parameter (17).

Estimates of the sensitivity and specificity of the index and reference tests for the beta prior distributions based on previous published data or expert opinion. *Salmonella* prevalence in mesenteric lymph nodes (population 1) has been estimated in a previous study to be 9.1% (95% CI 5.8 to 12.4) (18), therefore, the best value of 0.1, and we assumed that it should not be more than 0.5 with 95% certainty was used. Prevalence estimate for *Salmonella* in sub-iliac lymph nodes believed to be considerably lower than the mesenteric lymph nodes based on expert opinion. Therefore, the most probable value of 0.01 and 95th percentile of 0.3 was used (Personal communication Dr. James McKean and Dr. Annette O'Connor). Sensitivity and specificity for bacterial culture were

constructed based on a previous study that has used the culture protocol used in the laboratory. If the most probable value for sensitivity at 1 cfu/g level was set at 0.95 and more than 0.7 with 95% certainty, and prior mode for specificity be of 0.98 and 5th percentile of 0.7 (21). As the real-time PCR was a new test and lack of knowledge about parameters, we expected at least sensitivity and specificity that match with bacterial culture, therefore, diffuse beta distributions were used. Prior mode for PCR sensitivity was 0.9 and specificity was 0.95 with 5th percentile of 0.1 for both parameters (Table 2).

If the model for two dependent tests for two populations without a gold standard test estimated no correlation within the infected and/or non-infected sub-populations, as shown by a correlation measure with a 95% probability interval that included zero, then a second analysis was conducted assuming independence. The secondary analysis assumed, the two test outcomes for a given sample were independent (11).

The influence of the prior distributions on the posterior estimates of the model parameters were assessed by using relatively non-informative priors for the culture sensitivity and specificity. Thus, for sensitivity and specificity of the bacterial culture, uniform beta prior distributions (1) were used. Finally, influence analysis was conducted with perturbed data to determine the impact of analysis on the inference.

The results of the models were reported as sensitivity, specificity of the index and reference tests, expected prevalence in the two populations and corresponding 95% probability intervals.

Reproducibility of real-time PCR assay was evaluated by testing half of the samples listed under each cell of the 2 x 2 table that describes the cross-classified test results from bacteriology culture and initial real-time PCR (Table 3). Real-time PCR was

conducted by the same molecular microbiologist using the same protocol that used for the initial PCR assay. Results of the first PCR assay were blinded to the microbiologist.

RESULTS

It was not possible to obtain the desired sample size, and only 170 samples were used for the study. Cross classified data for study one are given in a contingency table (Table 3). In total, 168 MLN and 169 SILN were tested for *Salmonella* using bacterial culture and in-house real-time PCR methods. Two MLN samples and one SILN sample were missing and therefore, excluded from PCR assay. Cross-classified results of initial PCR and second PCR are given in Table 4. For sub-iliac lymph nodes, of the 20 samples that were positive for the initial PCR only two samples gave positive results on re-test, and whereas of 65 samples that were negative by the initial PCR only 51 samples were negative results by the re-test. For the mesenteric lymph nodes, out of 22 samples positive for first PCR (7 culture positive and 15 culture negative) only 7 were positive by the second PCR while of the 59 that were negative by the initial PCR (13 culture positive and 46 culture negative) only 44 samples were detected as negative on re-test.

The dependence model estimated negligible correlation between the index and reference tests (less than 0.2) for positive samples -0.08 ($-0.42, 0.23$) and for negative samples 0.02 ($-0.04, 0.16$), therefore, the secondary analysis assuming independence was conducted. The parameter estimates for sensitivity and specificity of bacterial culture and real-time PCR were consistent between dependence model and independence model. Better convergence was observed in the independence model. This determination of better convergence was based on trace plot and probability interval for culture sensitivity

in two independent tests was slightly narrower than the two dependant tests. The estimated *Salmonella* prevalence in mesenteric lymph nodes was similar in both models i.e. 0.29 (0.21, 0.39) and 0.29(0.21, 0.41). The estimated *Salmonella* prevalence in sub-iliac lymph node was 0.002 (0.0002, 0.02) in two dependent tests while it was negligible or zero in independence model 0.0 (0.0, 0.01).

Based on the test results obtained for 166 samples selected for the second PCR, Bayesian estimates of sensitivity and specificity were, 0.37 (0.18, 0.59) and 0.76 (0.68, 0.82), respectively, for the initial real-time PCR assay, and 0.42 (0.23, 0.64) and 0.79 (0.72, 0.86), respectively, for the re-test PCR assay. The level of agreement between initial and re-test PCR were 0.6 for both sub-iliac lymph nodes and mesenteric lymph nodes.

DISCUSSION

Reproducibility of the results of real-time PCR was poor, and this in turn adversely influence the diagnostic test accuracy of this PCR. Although the estimates of the sensitivity and specificity were reasonably similar for the two PCR runs, the individual samples showed little agreement ($\kappa=-0.1$). This made it impossible to conduct a meaningful statistical analysis as the results were not reproducible, an essential element in any diagnostic test evaluation. The study shows the value of following STARD guideline to assess a diagnostic outcome.

The reasons the PCR was not repeatable may be due to two fold. False positive results may be due to cross reactions and can be prevented by application of appropriate

laboratory operation procedure and optimization of the assay. False negative results were of special concern during this study, may be due to potential inhibitory substances.

The study design could have been improved by changing the protocol used for the sample preparation and testing procedure for culture and PCR. Since the study was designed to test the samples once for culture and PCR, no samples left for re-testing both culture and PCR assay. Therefore, it was able to re-test only PCR assay. If the study design included blinded multiple testing of same samples at once or re-testing for both tests later, we would be able test the repeatability of both tests.

In conclusion, test sensitivity and specificity estimates of bacterial culture and PCR assay to detect *Salmonella* in field samples may not be valid since it was unable to reproduce both tests.

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Table 1. Sample size estimates for two populations with different prevalence levels

Prevalence in population one	Prevalence in population two		
	5%	1%	0.5%
50%	413	409	409
40%	407	401	401
30%	375	364	362

Table 2. Beta-prior distributions obtained from beta-buster software for parameters in studies one and two

Parameter	Median (95% probability intervals)
<i>Study 1</i>	
P1	0.01 (0.003 ; 0.353)
P2	0.1(0.018 ; 0.564)
Se1	0.95 (0.652 ; 0.990)
Sp1	0.98 (0.647 ; 0.995)
Se2	0.9 (0.059 ; 0.978)
Sp ₂	0.95 (0.058 ; 0.979)
<i>Study 2</i>	
P3	0.2 (0.054 ; 0.551)
Se1	0.95 (0.652 ; 0.990)
Sp1	0.98 (0.647 ; 0.995)
Se3	0.98 (0.647 ; 0.995)
Sp3	0.98 (0.647 ; 0.995)

(Prevalence of *Salmonella* in sub-iliac lymph nodes (P1) and mesenteric lymph nodes

(P2), and sensitivity (Se1) and specificity (Sp1) of bacterial culture, and sensitivity (Se2)

and specificity (Sp2) of real-time PCR in study one. Prevalence (P3) of *Salmonella* in

belly flaps skin, and sensitivity (Se1) and specificity (Sp1) of bacterial culture, and

sensitivity (Se3) and specificity (Sp3) of BAX-system PCR in study two).

Table 3. Cross-classified test results from bacteriology culture and real-time PCR for *Salmonella* in swine carcass lymph nodes (Study 1)

	Sub-iliac lymph node	
	PCR Positive	PCR Negative
Culture Positive	0	0
Culture Negative	40	129
	Mesenteric lymph node	
	PCR Positive	PCR Negative
Culture Positive	16	29
Culture Negative	29	94

Table 4. Cross-classified test results to illustrate the reproducibility of real-time PCR with 166 samples for *Salmonella* in swine carcass lymph nodes (Study 1)

	Sub-iliac lymph node	
	PCR1 Positive	PCR1 Negative
PCR2 Positive	2	14
PCR2 Negative	18	51
	Mesenteric lymph node	
	PCR1 Positive	PCR1 Negative
PCR2 Positive	7	15
PCR2 Negative	15	44

Figure 1. Flow diagram of the study to illustrate the bacterial culture protocol and PCR procedure for study 1

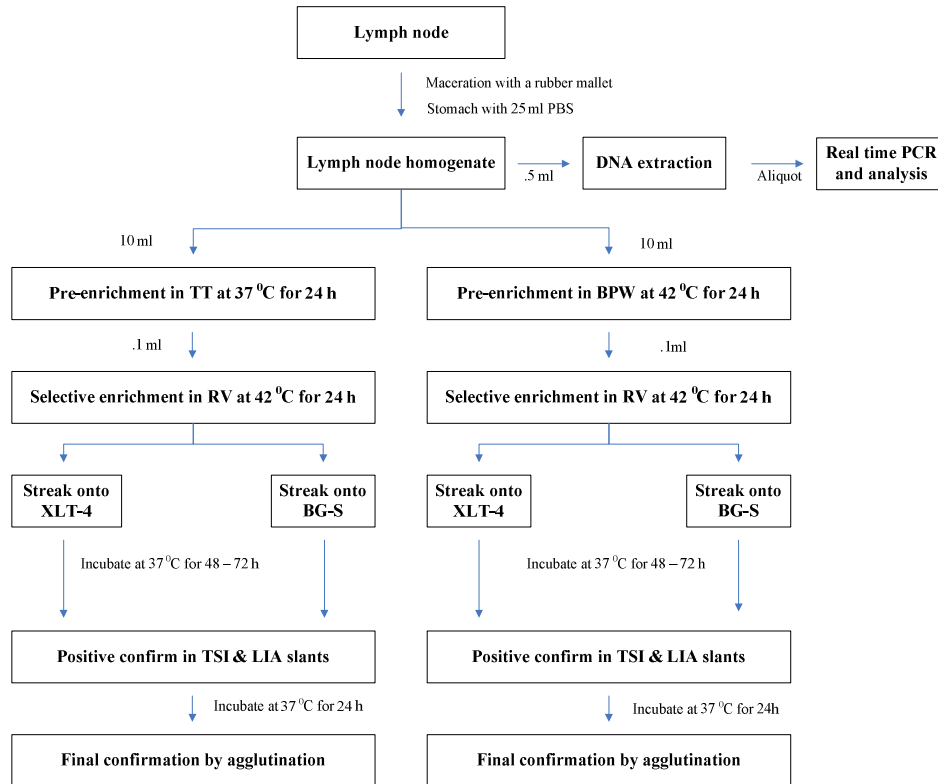
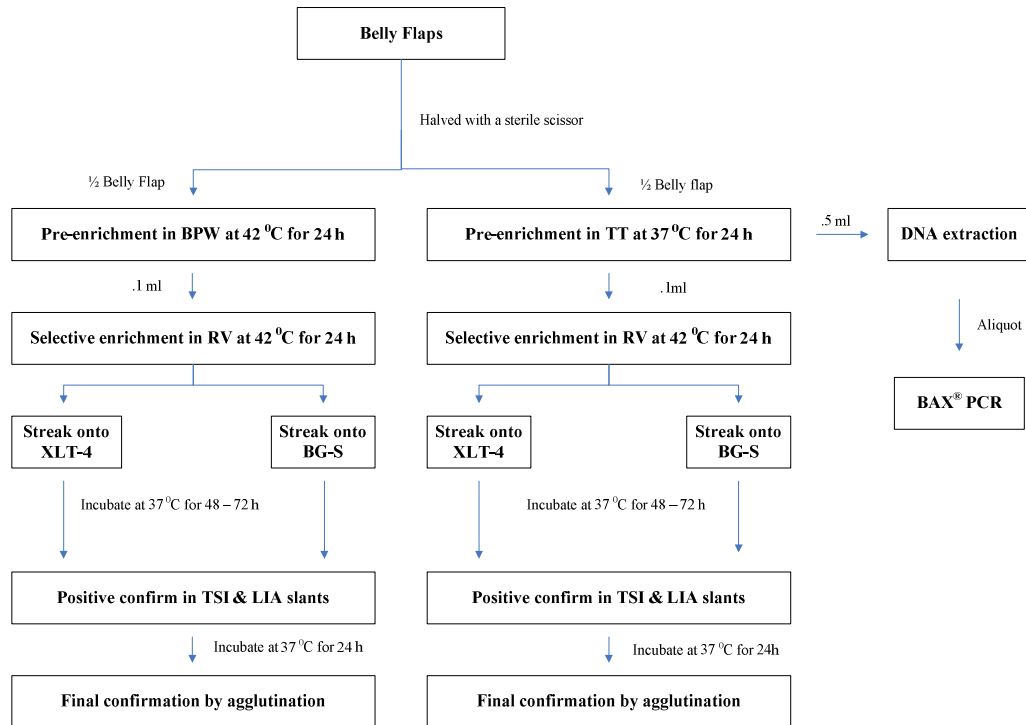


Figure 2. Flow diagram of the study to illustrate the culture protocol and PCR procedure for study 2



GENERAL CONCLUSION

Evidence-based medicine (EBM) is an approach in human medicine that requires the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients. Although this concept has been used in human medicine over the last two decades, its application to veterinary science is still in infancy. A tremendous effort has been put forth towards controlling *Salmonella* in animal production systems as a food-borne pathogen. However, no evidence-based approach has been applied to evaluate the best available control and intervention strategies to control *Salmonella* in swine. In this dissertation, an evidence-based approach was pursued to study food safety issues associated with *Salmonella* in swine.

The 1st study in the thesis evaluated using EBM concepts vaccination as a method to control *Salmonella* in swine. To achieve this objective, a systematic review was conducted on available literature to select high quality studies. However, only a qualitative analysis could be carried out due to the lack of quantitative data. This study indicated that the quality of available research is poor. The 2nd study applied EBM principles to determine whether antibacterial therapy has any effect on control of *Salmonella* in swine by applying a quantitative analysis on available research related to the subject. The analysis of binary and continuous data of studies showed no similarity of efficacy of antibiotics. Specifically, this study tried to apply the concept of meta-analysis and evaluate varying outcomes to determine efficacy. The third study was aimed was to identify a rapid and sensitive detection method for *Salmonella* screening in swine using field samples. Here, the in-house *Salmonella* culture protocol was compared with

two PCR assays conducted by two laboratories; a real-time PCR method and a BAX PCR method. Results obtained with in-house culture method was compared with different PCR assays in two different studies using the Bayesian methodology to determine the sensitivity and specificity of PCR assays without a gold-standard test. Interestingly, the culture method showed the same sensitivity and specificity in both studies indicating it as a robust diagnostic approach. Further, BAX system PCR gave promising sensitivity and specificity, but the sensitivity and specificity of real-time PCR was low.

Overall, the work conduct for this thesis identified problems during application of evidence-based medicine to veterinary science and food safety aspects. Most studies are poor in quality and fell into the lower levels of the evidence pyramid. Randomized-clinical trials, the best study design for evidence-based medicine, are not widely carried out in veterinary research due to the constraints such as financial restrictions, practical issues and other logistics. Although in human medicine, evidence-based medicine is applied to make decisions about individual patient outcomes, this study was conducted to make informed decisions on *Salmonella* control in swine on population basis with the aim of reducing food-borne salmonellosis in humans but EBM principles could not be directly applied.

This thesis highlighted difficulties of applying EBM to food safety. If EBM was applied it in purest form, the thesis would conclude that no high quality field studies existed that describe vaccination and sub-therapeutic antibiotic use to reduce *Salmonella* in the pre-harvest area of swine production. However, decisions do need to be made, even in the absence of high quality information, and it is difficult to “throw out” all the

information available. The dilemma for EBM proponents is whether to dismiss all evidence that is not high quality and risk appearing irrelevant, or to lower the standards and abandon the idea that some information is better quality. In the short term, lowering the standards and presenting the result of lower quality studies as the best available seems an easier path, however, will this really more food safety and veterinary science. In human medicine, the quality of reporting has been improved in many areas because studies are excluded when poorly conducted and reported, this change in quality is obviously needed in food safety and will only occur if authors feel pressured to improve. Reporting the summarized results from poorly reported studies in systematic reviews will not apply similar pressure to food safety researchers.

Despite this challenge, the conclusion reached is that application of evidence-based medicine to veterinary science has its own merits, but is an evolving area and direct application of all topics from human EBM to veterinary / food safety EBM will require more experience. Evidence-based approach for veterinary science and food safety interventions are useful and we should strive to give consumers the same level of scientific confidence in food safety related decisions as patient treatment decisions because this methodology assures that on the science about a topic is transparently presented, and ensures up to date and reliable information for researchers, veterinarians, producers, and finally, for consumers. Decision makers can then consider societal and economics issues, as well as scientific findings, to make the final determination of what is best.

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