THE APPLICATION OF RESEARCH SYNTHESIS AND BAYESIAN METHODS TO EVALUATE THE ACCURACY OF DIAGNOSTIC TESTS FOR *SALMONELLA* IN SWINE

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Abstract

This thesis presents the results of three complementary studies which were carried out to evaluate the accuracy of diagnostic tests for *Salmonella* in pigs. First, a research synthesis method approach, which included a systematic review, meta-analysis and meta-regression, was used to map out existing primary research investigating the accuracy of bacterial culture, antibody or antigen -capture ELISA, and PCR for *Salmonella* in pigs under field conditions.. Large statistical variability, limited methodological soundness and reporting precluded a quantitative synthesis of findings from multiple studies. The meta-regression identified significant factors, such as variations in test protocols, which explained much of the variability of reported estimates of test accuracy. The need for consistent use of a standard reference test is essential to ensure comparability of results generated in future studies.

In the second study, the accuracy of a bacterial culture, real-time (RT) PCR, and a mix-ELISA for *Salmonella* in were evaluated in western Canadian nursery and grow-finish pigs using traditional and Bayesian statistical methods. Ten farrow-to-finish pig farms from Alberta and Saskatchewan were purposively selected based on their presumptive *Salmonella* status. Bacteriological culture, RT-PCR and a mix-ELISA were performed on feces and blood samples collected from grow-finish (n=294) pigs and pens. Bayesian estimates of test sensitivity (Se) and specificity (Sp) at the individual pig level were similar to traditional statistical estimates. Sensitivity of culture and RT-PCR ranged from 65-75%, PCR Sp was 98-99% and ELISA Se and Sp at a cutoff of OD≥20% ranged from 59-63% and 84-87%, respectively. In the third study, *Salmonella* serovar distribution and risk factors for *Salmonella* shedding were investigated in breeding, nursery, and grow-finish pigs using the same 10 herds. Among 418 *Salmonella* isolates, most common serovars were Derby (28.5%), Typhimurium, var. Copenhagen (19.1%), and Putten (11.8%). More *Salmonella* were detected in pooled pen than individual pig samples,

confirming that the use of pooled samples is more effective for detecting the full range of serovars that may be present on Canadian pig farms. Sows shed significantly more *Salmonella* than nursery or grow to finish pigs, suggesting that the breeding herd is an important source of *Salmonella* persistence. Pelleted feed and nose-to-nose pig contact through pens were also associated with increased *Salmonella* prevalence, indicating that these factors are relevant as control targets.

The main advantages of research synthesis methods are increased power and precision in effect estimates and identification knowledge gaps and areas requiring further research. Bayesian methods for evaluating test accuracy are useful when there is no known "gold standard", which is often the case for zoonotic and food-borne pathogens. Both research synthesis and Bayesian methods are valuable tools for evaluating diagnostic test accuracy and should be more frequently used when developing monitoring and control programs in food safety.

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Dedication

This thesis is dedicated to three people who are more important to me than life itself: To my daughters, Toni and Jessica, thank you for putting up with having a perpetual student for a mother. You are each beautiful and brilliant - don't follow in my footsteps; blaze a new and better trail! To Jim, my soul mate and best friend, thank you for finding me and allowing me to be part of your life. Together, our two incomplete families have made a whole.

ORIGINAL CONTRIBUTION

Three separate studies comprise the body of this thesis. The first study, a systematic review/meta-analysis of the accuracy of tests for *Salmonella* in pigs, was conducted in collaboration with the Policy Advice and Effectiveness Program, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, which provided funding for access to the web-based systematic review data management program (SRS 3; TrialStat Corporation, Ottawa, ON) and for personnel to assist with the systematic review process. As project manager, my responsibilities involved coordinating and conducting all aspects of the review, including: project design, development and design of relevance screening, quality assessment and data extraction tools, literature searching, primary reviewer for all aspects of the review process, data management, and all the data analysis described in this thesis.

The second study, evaluating bacterial culture, RT-PCR, and ELISA for detecting *Salmonella* in pigs in western Canada, as well as the third study investigating the epidemiology throughout all phases of pig production, were conducted in collaboration with the Food Safety Division (FSD), Alberta Agriculture and Rural Development, Edmonton, AB. I was involved in developing an Agreement in Principle with this agency. I was also involved in the project design and in submitting grant proposals to other agencies. Funding obtained from sources other than FSD was used to offset costs associated with on-farm sample collection. I was responsible for recruiting 7 of the 10 herds included in this study, and personally collected all required samples and information from these farms. The other 3 herds were recruited by Dr. Leigh Rosengren, whose project "Antimicrobial resistance of *Salmonella*, *Escherichia coli*, and *Campylobacter* from pigs on-farm in Alberta and Saskatchewan, Canada" was carried out concurrent with this

project. Dr. Rosengren also collected all samples and information from these 3 farms. I did not perform any of the laboratory analysis. The FSD provided funding for and conducted all bacterial culture, RT-PCR, and ELISA assays. Serotyping and phagetyping were performed by the Office International des Épizooties Reference Laboratory for Salmonellosis in Guelph, ON. I conducted all of the statistical analyses related to this project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFLB	Agri-Food Laboratories Branch
ab	Antibody
ag	Antigen
BG	Brilliant green
BPW	Buffered peptone water
CQA	Canadian Quality Assurance
CCC	Concordance correlation coefficient
CI	Confidence interval
cov	Covariance
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FN	False negative
FP	False positive
FSD	Food Safety Division
GNH	GN-Hajna
g	Gram
HRP	Horseradish peroxidase
ICEPT	International Centre for Enteric Phage Typing
LPS	Lipopolysaccharide
Max	Maximum
m	Metre
µl	Microlitre
ml	millilitre
Min	Minimum
MSRV	Modified semisolid Rappaport Vassiliades
NLEP	National Laboratory for Enteric Pathogens
N/A	Not applicable
NR	Not reported
N, n	Number
OD	Optical density
OD%	Optical density % cut-off value
PBS	Phosphate-buffered saline
PCR	Polymerase-chain reaction
QA	Quality assessment
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
RAM	Rambach
RV	Rappaport-Vassiliadis
RT	Real-time
ROC	Receiver operating characteristic
REML	Restricted maximum likelihood
S.	*Salmonella*

S/P	Sample to positive ratio
SE	Selenite
Se	Sensitivity
Sp	Specificity
s.d.	Standard deviation
s.e.	Standard error
STARD	Standards for Reporting Diagnostic Accuracy
SROC	Summary receiver operating characteristic
TAGS	Test accuracy in the absence of a gold standard
TMB	Tetramethylbenzidine
TT	Tetrathionate
TN	True negative
TP	True positive
vs.	Versus
OIÉ	World Organisation for Animal Health (Office International des Épizooties)
XLD	Xylose lysine deoxycholate
XLT4	Xylose lysine tergitol4

CHAPTER 1

INTRODUCTION

Salmonella is an important food safety issue and one of the main causes of food-borne illness[1-4]. Among 4093 reported foodborne disease outbreaks from 1988 to 2007 for which a report was publicly available, nearly half were found to be attributable to *Salmonella*[5]. The number of countries reporting data on the economic costs associated with this issue is limited; however, there is little doubt that salmonellosis causes a significant public health burden and considerable costs worldwide. Monetary costs, societal burden, increased public awareness and international trade issues have prompted a number of countries to implement, or consider implementing, *Salmonella* surveillance or control programs[6-14].

Non-typhoidal *Salmonella* species (spp.) cause an estimated 1.4 million infections, 168,000 physician visits, 15,000 hospitalizations and account for an estimated 31% of all food-borne illness related deaths within the United States each year[4,15]. The associated total cost are estimated at 3 billion US$ a year[16]. In 2004, the number of reported cases in Canada was 16 per 100,000 inhabitants, but reported cases only represent "the tip of the iceberg" and non-reported cases bring the actual number of salmonellosis cases much higher[4]. Denmark has estimated that the cost of foodborne salmonellosis within that country is 15.5 million US$ annually, based on an estimated incidence of 54.6 cases per 100,000[6]. Internationally, the proportion of cases that may be attributable to pork consumption, based on available outbreak data, is estimated to be 3.0-3.6%[5,17], although in some countries this may be as high as 20%[18]. However, these estimates are frequently generated based on available outbreak data and for this reason their precision is limited. Different patterns of pork consumption among countries may be one reason for

differences in cases attributable to pork; for example, some European countries such as Austria, Belgium and Denmark the pork consumption is almost twice as much pork per capita than in countries such as Canada and the USA[19]. Differences in overall incidence in cases attributable to pork may also potentially be explained by different laboratory and epidemiological methods for estimating incidence and attribution, as well as in standards or culture of in food handling, preparation and storage, or due to geographic differences in *Salmonella* prevalence and serovar distribution.

Salmonella spp. are ubiquitous throughout the world, although serovar distribution can vary between regions or countries[20]. Over 2500 different *Salmonella* serovars have been identified to date. Taxonomically, these are divided into two species: *S. enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies: *enterica*, *salamae, arizonae, diarizonae, indica, and houtenae,* also known as subspecies I, II, IIIa, IIIb, IV, and VI, respectively. *S. enterica* subsp. *enterica* are infectious to mammals and birds, and some serovars have adapted to a single species, e.g. *S.* Typhi in primates, *S.* Dublin in cattle, and *S.* Choleraesuis in pigs[21]. Although many serovars are capable of causing disease in humans, including other host adapted species, only a handful of serovars are consistently associated with human disease. In 2002, *S.* Enteritidis accounted for 65% of all isolates recovered from humans globally, followed by *S.* Typhimurium (12%); *S.* Newport, *S.* Heidelberg and *S.* Infantis representing the top 5 serovars isolated from humans in 2002. These serovars were also the most common isolates from non-human sources (food, animal, environmental, and feed)[20].

In many industrialized countries there was a remarkable increase in the incidence of salmonellosis which started in the 1980's and in some areas continued until 1992. In Canada, peak incidence occurred in 1987 with approximately 10,700 isolates from human cases[23] and has decreased steadily since then to less than 5000 reported cases in 2004[24]. Although *S.* Typhimurium remained the top serovar isolated from humans, during this time there has been an increased prevalence of other serovars isolated from humans, in particular *S.* Hadar, *S.* Enteritidis and *S.* Heidelberg, which coincided with a parallel increase in isolates from non-human sources[23-25].

Eggs and poultry products account for the largest portion of food-borne *Salmonella* infections, causing 27% and 14% of reported foodborne outbreaks, respectively[5, 26, 27]. Pork, on the other hand, accounts for only 3.5% of reported outbreaks, and overall accounts for fewer salmonellosis outbreaks than several other food groups besides eggs and poultry, such as beef, dairy, and bakery products[5]. However, public awareness of *Salmonella* as a food safety issue in pork was sparked in 1993, when pork was associated with an outbreak of human salmonellosis involving over 500 registered cases caused by food-borne *S.* Infantis in Denmark (20 cases per 100,000 inhabitants)[28]. Subsequent to this, Denmark implemented a comprehensive national control program to reduce *Salmonella* contamination in pigs and pork both on-farm and at slaughter[6, 29, 30]. The introduction of this program and the European Union (EU) funded project "*Salmonella* in pork" (SALINPORK)[31] stimulated extensive research in order to better understand the most effective ways to monitor *Salmonella* status in pigs and to identify interventions and control options for *Salmonella* reduction at different stages of pig production[32].

In 2004, the EU introduced the EU Directive 2003/99/EC and EU Regulation No. 2160/2003 aimed at controlling food-borne zoonoses within member states, including specific targets for *Salmonella* monitoring and control[12]. A number of food animal species targets, including pigs, were established consecutively and phased in from December 2004 to December 2008. Member states are not forced to adhere to a fixed format for monitoring and control programs, and may submit their own programs (including existing ones) to the European Commission for approval. These regulations, along with additional regulations on trade, have provided further impetus for other countries outside of the EU to implement or consider implementing *Salmonella* monitoring and control programs of their own. This also contributed to increased research efforts worldwide, as various countries began to undertake studies to establish baseline information on the prevalence, distribution and epidemiology of *Salmonella* within their borders.

Clinical salmonellosis in pigs is almost always caused by either, *S.* Choleraesuis var. Kunzendorf or *S.* Typhimurium[33]; the host-specific serovar *S.* Choleraesuis infections usually manifest as septicemia whereas *S.* Typhimurium, which is not host-specific, causes enterocolitis in the pig[33]. While clinical disease is important with respect to pig health and production, it is the apparently healthy sub-clinically infected pig that presents the more important risk to food safety. A wide range of different serovars can be isolated from the gastrointestinal tract, lymph nodes and other tissues of healthy animals[34-40]. With the exception of *S.* Typhimurium and *S.* Choleraesuis, these serovars infrequently cause clinical disease in pigs; however, many are capable of causing gastrointestinal disease in humans.

Contaminated environments and infected animals are the primary sources of infection for other pigs; transmission is primarily fecal-oral, but can also occur via nose-to-nose or aerosol transmission[33, 41, 42]. Infection can be swift, with *Salmonella* being found in non-alimentary tissues within 2-3 hours of infection[43-45], or in gastrointestinal tracts within 30 minutes[46]. Rapid infection during transport and holding pens at the abattoir are known to result in an increased number of *Salmonella*-infected pigs at slaughter when compared to pigs on-farm[40, 47-51]. These sub-clinically infected animals cause contamination and cross-contamination of carcasses during the slaughter process[52-57]. *Salmonella* has been found in up to 48% of pork carcasses and 30% of retail pork products[33], presenting a significant risk to public health and food safety.

S. Typhimurium is consistently the most common serovar isolated from clinically healthy pigs or carcasses in Europe[35, 52, 58, 59]. Within North America, *S.* Typhimurium is usually the most frequent isolate from pigs, although some studies have found *S.* Derby (and occasionally other serovars) to be more prevalent[34, 36-39, 60, 61]. Serovar type and prevalence can vary substantially between regions and over time. Considerable research has been undertaken over the last 10-15 years, to understand the regional and country specific epidemiology of *Salmonella* in pigs. This has become a research priority for many countries, as the development and implementation of *Salmonella* control programs requires knowledge of the baseline prevalence and serovar distribution in targeted pig populations within a specific region.

Existing monitoring and control programs in the pig industry are designed to detect infected pigs and classify pig farms according to the level of infection[62, 63]. Critical to the conduct of these programs is the application of diagnostic procedures, such as bacterial culture[64] or

ELISA (enzyme-linked immunosorbent assay)[29, 65], through which the *Salmonella* status of pigs and herds is evaluated. Accordingly, the evaluation of the accuracy of these tests has been a significant component of research efforts involving *Salmonella* in pigs. There are a number of reasons for varying test accuracy; for example, ab-ELISA sensitivity may vary with factors such as prevalent serovars, stage of infection or immune status of the animal[66]. Culture sensitivity can vary because of a number of factors including differences in protocols between laboratories or due to different levels of expertise among laboratory workers. For these reasons, tests must generally be validated and evaluated in the population and laboratory in which they will be applied. Studies conducted in different geographic locations and swine populations can result in conflicting findings and recommendations. Together with the daunting task of sorting through the large quantities of research published on the subject, this makes it difficult for researchers and policy makers to integrate existing information efficiently in order to facilitate rational decision making.

One potential solution to this problem is to apply research synthesis methods such as systematic reviews. A systematic review is a review of a clearly formulated question that uses explicit and transparent methods to identify, select, and critically appraise all available relevant research. If sufficient quality research is identified, meta-analysis or meta-regression methods may be used to analyze and summarize the results of the included studies. Internationally, there has been increased momentum for wider use of these methods to synthesize and summarize the large volumes of information generated in the health care arena. Systematic reviews of diagnostic and screening test evaluations are carried out for the same reasons as systematic reviews of the effectiveness of interventions: to generate summary estimates of test performance based on all

available evidence, to evaluate the quality of published studies, and to account for variation in findings between studies[67]. To date this approach has rarely been used for diagnostic test questions in the areas of food safety and veterinary public health. A systematic review of studies which evaluate the accuracy of tests for *Salmonella* in pigs may potentially provide summary estimates of test accuracy, or identify reasons for variability between studies, identify gaps in current knowledge and provide direction for future research.

Traditionally, the evaluation of the sensitivity and specificity of diagnostic tests are done by comparison to a gold standard, a test (or tests) which accurately determines the true disease state of an animal[66]. The lack of a gold standard test is known to be a complicating factor in the evaluation of new or existing tests for *Salmonella* in pigs. Although notoriously imperfect, microbiological culture has long been used as the "gold standard" diagnostic test for *Salmonella*[68]. The use of an imperfect reference test makes it difficult to assess the true sensitivity and specificity of the test being evaluated. This lack of a true gold standard test is problematic in many areas of veterinary medicine, veterinary public health and food safety; for this reason, the Bayesian approach to test evaluation in these sectors has gained popularity in recent years. A Bayesian approach to test evaluation offers an alternative approach in which none of the competing tests are treated as the gold standard and the diagnostic error rates are estimated for all studied tests[69]. With respect to tests for *Salmonella* in pigs, Bayesian methods have recently been used to evaluate the accuracy of pooled samples using culture[70], two different serological assays[71], culture and PCR[72], and herd-level accuracy of culture and ELISA using different sampling methods[73]. Although methods are readily available which permit the

evaluation of all three of these tests (bacterial culture, serology and PCR) in the same Bayesian model[74], this strategy has not yet been reported in the literature for tests for *Salmonella* in pigs.

Both systematic review and Bayesian statistical methods offer new approaches for evaluating the accuracy of diagnostic tests for *Salmonella* in pigs, a critical component of food safety program development. Therefore, the first objective of this thesis was to evaluate the existing global knowledge-base on the accuracy of selected diagnostic tests for *Salmonella* in swine through systematic review, meta-analysis and meta-regression methods (Chapters 2 and 3). The second objective was to use Bayesian methods to examine the accuracy of a bacterial culture protocol, a broth-enriched real-time PCR and an antibody-capture ELISA, which were being considered for potential use in future monitoring and control programs for *Salmonella* in pigs in western Canada (Chapter 4). The final objective of this thesis was to describe the prevalence of and risk factors for shedding *Salmonella* throughout all phases of pig production (breeding, nursery and grow-to-finish) in order to further our understanding of the epidemiology of *Salmonella* in pigs in western Canada, using data collected from the same herds enrolled in the test evaluation study (Chapter 5).

Since a large portion of this manuscript is devoted to the conduct and results of a systematic review of the existing literature on the evaluation of diagnostic tests for detecting *Salmonella* infection in pigs, a traditional literature review would have been redundant and therefore has not been included in this thesis. Chapters 2 - 5 in this thesis were each written and formatted as independent papers intended for publication in scientific journals. As such, there is unavoidably some repetition of information between chapters.

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CHAPTER 2

EVALUATING THE GLOBAL KNOWLEDGE-BASE ON THE DIAGNOSTIC ACCURACY OF SELECTED TESTS FOR SALMONELLA SPP. IN SWINE: A SYSTEMATIC REVIEW META-ANALYSIS APPROACH

2.1 Introduction

The control of *Salmonella* species in the pork production chain is an important industry challenge due to potential public health and international trade implications. *Salmonella* is one of the main causes of food-borne illness worldwide[1-4], and pork, after poultry and poultry products, is known to be an important source of human salmonellosis in European countries[5-7]. Serious efforts to control *Salmonella* in pork began in the 1990's with the introduction of the Danish *Salmonella* control program[8] and the EU funded project "*Salmonella* in pork" (SALINPORK)[9]. Since then, considerable primary research has been conducted to better understand the most effective ways to monitor *Salmonella* status in pigs and to identify interventions and control options for *Salmonella* reduction at different stages of pig production[10]. The evaluation of both microbiological[11] and serological[12] tests to detect *Salmonella* in pigs and pork products has been a major component of these research efforts.

In a diagnostic accuracy study, the test under evaluation, otherwise known as the index test, is applied to a set of subjects or samples. Ideally, the results obtained with the index test are compared with a "gold standard", a test which has perfect sensitivity (Se) and specificity (Sp), which is applied to the same set of subjects or samples. In reality, a perfect gold standard test is difficult to identify, and this is particularly true when testing for *Salmonella* in pigs. Instead, index tests are evaluated by comparison to existing imperfect tests; this imperfect test is more accurately referred to as a "reference standard", rather than a "gold standard". The Se and Sp of

the index test are the "apparent" Se and Sp, since they are calculated relative to the results of the reference standard rather than the true (but usually unknown) status of the samples or subjects. Measures of agreement between index and reference tests, such as Cohen's Kappa statistic and correlation between tests, can also be calculated.

Accurate detection of *Salmonella*, *Salmonella* DNA, or antibodies to *Salmonella* in pig populations is vital for effective monitoring and control of this organism both at farm and slaughter levels. Most *Salmonella* infections in pigs are sub-clinical, resulting in challenges in the interpretation of the *Salmonella* status of a pig or herd. Bacterial culture is frequently the reference standard against which other tests are compared; while this test is highly specific, the sensitivity is highly variable and is prone to false-negative results[11]. The use of serological tests at slaughter has been incorporated, with varying success, into some national surveillance programs since these tests are rapid, less costly and more suitable to automation than culture. There has also been interest in other rapid tests such PCR (polymerase chain reaction) and ag-ELISA (antigen-capture enzyme-linked immunosorbent serologic assay) tests for detecting *Salmonella* in pigs both on farm and at slaughter[13-16]. Although PCR and ag-ELISA are reported to perform comparably with culture, these tests have yet to be used to a larger extent in surveillance programs.

A large number of studies have been published evaluating the accuracy of tests in different geographic locations and swine populations, often resulting in conflicting findings and recommendations. In order to evaluate the existing global knowledge-base on the accuracy of selected diagnostic tests for *Salmonella* in swine, a systematic review approach was undertaken.

Systematic reviews employ explicit methods to methodically search, critically appraise and synthesize the available literature to minimize bias. This type of review provides a consistent and transparent approach to summarizing evidence from the existing body of literature. A systematic review of diagnostic tests may result in the identification of effective tests, as well as identify gaps in existing research and knowledge and areas requiring further research. In this review, the term "diagnostic test" is used in its general context and does not differentiate between screening (detection in asymptomatic subjects) and diagnostic (confirmation in symptomatic subjects) tests[17]. To the best of the author's knowledge, no other systematic review of diagnostic tests used to detect *Salmonella* infection in pigs has been reported.

The objective of this study was to identify, appraise, and summarize scientific literature supporting either the individual- or herd-level accuracy of selected diagnostic tests for *Salmonella* in pigs under field conditions, using systematic review methodology. Random-effects pooled estimates of test accuracy parameters, primarily test Se and Sp, obtained through meta-analysis, were reported.

2.2 Review Approach

2.2.1 The review question

This review addressed the question "What is the accuracy of selected diagnostic tests used for detecting *Salmonella* in pigs under field conditions?" In order to answer this question, a systematic review-meta-analysis approach was used to: 1. identify, appraise and summarize or synthesize primary research investigating the Se and Sp of selected diagnostic tests for

Salmonella in pigs at either the individual- or herd-level, including post-hoc estimation of test accuracy data from primary research where two or more of these tests were used simultaneously on the same population; and, 2. compare the agreement or correlation between tests for *Salmonella* in pigs when applied simultaneously to the same sample populations. A test was defined as: i. bacterial culture conducted on feces, tissue, or carcass swab samples; ii. antibody or antigen capture ELISA for antibodies in blood or "meat juice" (tissue fluid) or *Salmonella* antigens in feces, tissue, or carcass swabs; or, iii. PCR assay to detect *Salmonella* DNA in feces, tissue, or carcass swabs.

2.2.2 Literature search

Literature searches were performed in ten different electronic databases through the University of Saskatchewan Library Server. These databases included Agricola, CAB Abstracts, MEDLINE (PubMed interface), BIOSYS Previews, Web of Science, Food Science and Technology Abstracts (FSTA), CISTI, Scopus, Dissertation and Theses (ProQuest) and Theses Canada Portal. The initial searches were conducted between June 12 and June 26, 2006; an update search was conducted on June 26, 2007. The searches were limited to publications from January, 1980 until June, 2007. No language or publication type restrictions were imposed for the searches. Conference proceedings from the International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (2001, 2003, and 2005) were scanned by two independent reviewers to identify potentially relevant abstracts. Web pages from the Inventory of Canadian Agri-Food Research ICAR (http://www.icar-irac.ca/client/qt_e.aspx) and the National Pork Board (Pork Checkoff) (http://www.pork.org/PorkScience) were searched for reports on current or otherwise unpublished research. Finally, reference lists of the 5 most

recent review articles[12,15,18-20] identified via the initial literature search, were checked for articles that had not been identified by the other searches. The final reference list was then forwarded to 6 topic-experts who were asked to evaluate the list for missing references.

Keyword combinations used to conduct the searches are presented in Table 2.1. The search strategy was intended to be sensitive rather than specific, thus a number of different keywords related to the population of interest and diagnostic test outcomes were used. The keyword combination used to identify publications reporting diagnostic test evaluation was adapted from a previously published search strategy[21]. The literature search was designed to identify all relevant publications reporting the evaluation of tests used to detect *Salmonella* infection in domestic pigs as well as identify studies of other designs employing two or more tests simultaneously.

All references and respective abstracts were entered into a bibliographic management software program (EndNote v7.0, Thomson Scientific, Carlsbad, CA) and checked using the deduplication tool within the software. Manual deduplication was also done by the primary investigator (WW). References identified as theses or proceedings abstracts were also manually checked to verify that the contents were not duplicated in other publications. In the event of duplication, peer-reviewed journal publications were given preference. The final reference list was uploaded into a web-based systematic review data management program (SRS 3; TrialStat Corporation, Ottawa, ON).

2.2.3 Relevance screening

Relevance screening of the retrieved citations was performed based on the title and abstract using a pre-designed, pre-tested template. Each citation was screened independently by two reviewers, with all disagreements being resolved by consensus. Relevance was based on two criteria: i. reporting of either the evaluation of serology/ELISA, bacterial culture or PCR, or the use of two or more of these tests applied simultaneously to the same sample population, for detecting *Salmonella* infection in pig feces, blood, tissue, carcass swabs or pork "meat juice"; ii. primary research in English (including conference proceedings and theses). Language restriction was imposed as resources were not available for translation services.

The following studies were not considered for inclusion in the review and were excluded during relevance screening: challenge trials evaluating the use of one test in a population of artificially infected pigs; studies evaluating tests in artificially contaminated samples; studies which only examined differences due to sample type; studies pooling serology results from different age groups, or studies which pooled culture or PCR results from different sample/tissue types e.g. fecal results pooled with tissue results.

2.2.4 Primary quality assessment

Primary quality assessment (QA) was carried out using the full texts of articles passing through relevance screening, using a pre-designed, pre-tested template. Each citation was screened independently by two reviewers, with all disagreements being resolved by consensus. A previously published set of questions developed specifically for quality assessment of primary research in systematic reviews on diagnostic test accuracy[22] was utilized in the development of

the quality assessment form, with some modifications due to the differences between agri-food public health[23] and human health research. The final set of QA assessment questions was developed in an iterative fashion by the investigators until consensus on the final draft was achieved. The primary QA tool incorporated only questions considered essential for study inclusion; the remaining QA questions, which were used to assess overall study quality rather than as inclusion/exclusion criteria, were used as secondary QA items in conjunction with data extraction.

The primary QA tool consisted of 4 essential criteria that the study had to meet to be included in the review. The essential criteria were: 1) estimates of test accuracy, or raw data sufficient for post-hoc calculation of test accuracy, must be reported; 2) the time period between tests must be short enough to be reasonably sure that the subject's *Salmonella* status did not change between the two tests; 3) samples must be stored appropriately (refrigerated or held on ice) and processed/tested within a reasonable period of time after collection; and, 4) each test protocol had to be reported sufficiently so as to allow appropriate categorization of the test. Only those studies that met all 4 essential criteria on methodological soundness were included in the subsequent data extraction process.

2.2.5 Data extraction, including secondary QA

Pre-designed templates were used to assess the methodological soundness (secondary QA) of included primary research and to retrieve data on study characteristics, which was done by two independent reviewers. This included general information on each study, study quality related items (described in Table 2.2), details of test protocols and details of the population

tested. Details regarding test evaluation and results, such as type of sample tested and raw data and/or reported estimates of test accuracy, were entered into a spreadsheet by the primary investigator and then double-checked by a second reviewer. All disagreements were resolved by consensus. If studies reported herd-level testing, extracted herd-level observations were limited to those defining a *Salmonella*-positive status as at least one positive sample per herd. Wherever possible, raw data were captured in a 2-by-2 contingency table format in order to facilitate post-hoc analysis. Because it was uncertain which parameter(s) of test accuracy were most likely to be available, the following measures of test accuracy were extracted: Se, Sp, correlation, kappa statistic, or percent agreement, along with associated p-values, if available.

Data related to test results and the evaluations of test accuracy were entered into a spreadsheet (Microsoft Office Excel 2003, Microsoft Corporation, Redmond, WA, USA). A separate record was created for each unique test evaluation. A test evaluation was considered unique if different test protocols (within same test category) were used, if tests of different types were being compared, or if results were available for different populations or time periods. Raw data did not necessarily have to be reported in a 2-by-2 contingency form; in some cases the data required to create such a table were not readily available but could be determined from information in the results or discussion sections of the manuscript.

2.2.6 Data analysis

For the purposes of comparison, tests applied within each study were designated as the index test (test being evaluated) or as the reference test (test assumed to be the "gold standard"). Observations were grouped for analysis according to the type of index test evaluated and the type

of reference test against which the index test was compared. Because inhibitory compounds and competitive organisms in feces can interfere with tests such as bacterial culture and PCR, observations evaluating these tests, as compared to culture, were further sub-grouped according to fecal and non-fecal sample matrices. With respect to ab-ELISAs, the apparent Se/Sp will differ depending on the ELISA cut-off value specified[24-26] and definition of herd-positivity [26]. We therefore grouped observations for test comparisons involving culture and ab-ELISAs according to the level of sampling (individual or herd). For individual-level results, observations were further sub-grouped based on ab-ELISA cutoff values; however, herd-level observation were not sub-grouped by ab-ELISA cutoff values due to the small number of observations at the herd-level.

All estimates were first logit-transformed and the standard errors of the logit estimate were then computed as follow:

$$\text{logit p} = \ln\left(\frac{p}{1-p}\right) \quad \text{and} \quad S.E. = \sqrt{(1/n \times p \times (1-p))} \qquad (2.1; 2.2)$$

where n is the sample size and p is test Se, Sp, kappa statistic or correlation estimate. Data were summarized via descriptive statistics using a commercial statistical software package (Stata/SE v9.2, StataCorp LP, College Station, TX, USA). Within this software, the META command was also used to generate summary (pooled) estimates and to evaluate heterogeneity between studies, using both fixed-effects and random-effects analysis. Heterogeneity was considered significant if the associated Q-statistic was found to be significant ($P<0.10$); because the statistical power of the heterogeneity test is typically low, a more liberal criteria of $P<0.10$ was used rather than the standard $P<0.05$[27]. Random-effects estimates were reported where significant heterogeneity was detected.

2.3 Results

2.3.1 Identification and description of relevant studies

The results, presented according to each step involved in the systematic review process, are outlined in Figure 2.1. After removal of duplicate articles, 2360 references were evaluated for relevance; 137 were found relevant. No additional relevant references were identified by either the 6 consulted experts, website searches or by checking the reference lists of the 5 most recent review articles. Thirty-seven relevant references were identified by hand-searching conference proceedings. Of the relevant references, 99 were subsequently excluded during QA. The majority of the included references in this review were journal articles (n=34) while the remainder were conference proceedings (4). The primary purpose of these studies were test evaluation (n=34), prevalence assessment (2), vaccine trial (1), or pathogenesis study (1). Two studies were published in the 1980's, 4 in the 1990's, and the remaining 32 studies were published from 2000 – 2007.

Five categories of test comparisons, grouped first according to the type of index test and then by the type of reference test used, were evaluated (Table 2.3). The number of different test comparisons (observations) extracted ranged from 1 to 21 per reference. Se and Sp were most frequently reported; however, these were only reported 53% and 42% of the time, respectively. Kappa statistic, correlation or percent agreement were reported for 24%, 9% and 7% observations, respectively. Raw data were also extractable in 49% of the observations. Since Se can be calculated from raw data when it is available in a 2-by-2 contingency table format, this particular measure of diagnostic accuracy was thus available for 90% of the observations. For this

reason and because test Se/Sp is the most recognizable measure of diagnostic test accuracy, our analytical approach primarily focused on studies that either reported those estimates or data for post-hoc calculations. Kappa statistics were either reported or calculable for 117 test comparisons; correlations were reported for 18 test comparisons. One reference reported only percent agreement between tests[28].

Characteristics of the included references are outlined in Tables 2.4-1 – 2.4-5. Eighteen references reported culture vs. culture test results, 11 reported ab-ELISA vs. culture results, 5 reported ag-ELISA vs. culture results, 5 reported ab-ELISA vs. ab-ELISA results, and 7 reported PCR vs. culture results. Further results for each of these categories are reported below.

2.3.2 Methodological soundness of studies included in the review

Both primary and secondary QA items, and the number of responses to each item, are outlined in Table 2.2. Of the 99 references excluded during primary QA, 85 were excluded because they did not adequately report estimates of test accuracy or raw data sufficient for post-hoc analysis; an additional 14 references were subsequently excluded because they lacked sufficient detail describing the test protocol.

A number of QA items were poorly addressed in the 38 references included in this review. Just one study reported the use of blinding when evaluating test results. Only 6 studies reported the use of random sample selection, 9 indicated that convenience sampling was used, while the remaining studies did not report on this aspect. Study population selection criteria were equally poorly reported, with only 3 studies reporting clear inclusion or exclusion criteria.

Another 13 studies made brief, albeit unclear, comments regarding selection criteria. Twenty-one studies failed to provide sufficient information in order to determine whether samples were stored appropriately and processed/tested within a reasonable period of time after collection.

2.2.2 Se and Sp of culture (index) as compared to culture (reference):

Description of included studies

One hundred and twenty nine observations on the Se / Sp of bacterial culture, as compared to other culture, were extracted from 18 references (Table 2.4-1). The number of samples tested (n) ranged from 15 to 644 (mean 176, median 100). The number of observations extractable ranged from 2 to 21 per reference. There were 32 observations reporting Se and Sp, 27 observations reporting Se alone, and 70 observations reporting neither Se nor Sp but with extractable 2-by-2 data. Confidence intervals or standard errors were reported for only 5 observations.

Samples were collected from pigs either on farm (65 observations) or at slaughter (64 observations). Twelve observations reported the use of lymph nodes and 117 used fecal material. Most of the fecal samples were from individual pigs, taken either from finisher pigs at slaughter (46 observations) or from pigs of various age on farm (44 observations). Pen fecal samples were also evaluated (25 observations). Two observations from one study [29] combined both cecal samples and pen samples/pit sludge. Culture protocols varied considerably, differing in type and weight of sample tested, enrichment/culture media, incubation temperatures, and reference standard. Some of the commonly reported components of culture protocols used for isolating *Salmonella* that varied are listed in Table 2.5.

Sensitivity/ Specificity

Wherever possible, raw data were used to calculate test Se. We did not calculate Sp, as it is assumed that a *Salmonella*–positive culture is truly *Salmonella* positive. Thus, the Sp for all culture vs. culture test protocols is assumed to be 100% for the purposes of this review. To calculate Se from raw data, we used as the "reference standard" the combined positive culture results from both the test being evaluated and the reference test. Both 2-by-2 data and reported Se were reported for 11 observations; our calculated Se agreed with the reported Se in all but one observation, indicating that our definition reference standard was consistent with standard industry practice. The distribution of culture Se is shown in Figure 2.2; descriptive data are presented in Table 2.6. Analyses were carried out for all observations combined, as well as separately for protocols using either fecal samples or lymph nodes. Tests for heterogeneity showed significant Q-statistics for Se in all analyses. The associated P-values, random-effects pooled estimates and associated 95% confidence intervals are also shown in Table 2.6.

2.3.4 Se and Sp of ab-ELISA (index) as compared to culture (reference):

Description of included studies

Thirty-one observations on the Se / Sp of, ab-ELISA as compared to bacterial culture, were extracted from 7 references (Table 2.4-2). Twenty-four observations were at the individual level, whereas 7 observations were at the herd level (herd defined as ELISA-positive if at least one positive ab-ELISA result was found, culture-positive if at least one positive culture was found). The number of individual samples tested (n) ranged from 15 to 2403 (mean 695, median 600). For herd-level testing, the number of herds tested ranged from 40 to 67 (mean 56, median 60). There were 19 observations reporting both Se and Sp, 6 observations reporting Se alone, and

6 observations reporting neither but having extractable 2-by-2 data. Confidence intervals or standard errors were reported for 8 observations.

Samples were either collected on farm (17 observations) or at slaughter (14 observations). Seventeen observations compared ab-ELISA of sera to bacterial culture of fecal material, 8 observations compared ab-ELISA of meat juice to culture of fecal material, and 6 observations compared ab-ELISA of sera to culture of lymph tissue.

Variations in culture protocols were similar to those reported for culture vs. culture test comparison (Table 2.5). ELISAs were described as "in-house" (10 observations) or 1 of 3 test kits, Salmotype (Labordiagnostik, Leipzig, Germany; 9 observations), HerdChek (IDEXX Laboratories, Schiphol-Rijk, Noord-Holland, The Netherlands; 11 observations), or Svanovir (Svanova Biotech, Uppsala, Sweden; 1 observation). All observations determined ab-ELISA cut-off values at optical densities (OD) of 10, 20, 30 or 40%, with the exception of the observation using the Svanovir test. This observation reported cutoff values using sample to positive (S/P) ratios rather than OD%, with positive defined as S/P≥45.

Sensitivity/Specificity

Raw data and reported Se were available for 5 observations, and both calculated and reported Se agreed in all 5 cases. Where Se or Sp of ab-ELISA was not reported, these values were calculated from the raw data with culture being used as the reference standard. The distributions of individual- and herd-level Se and Sp are shown in Figures 2.3-1 through 2.4-2. Because apparent Se/Sp will change with different ab-ELISA cutoff values, observations were

also analyzed according to OD% cutoff. Descriptive data are presented in Table 2.6. Tests for heterogeneity showed a significant Q-statistic for overall Se and Sp for individual-level and herd-level observations. Sub-grouping individual-level observations by reference ELISA cut-off values also resulted in significant heterogeneity at all cut-off values. The associated *P*-values, random-effects pooled estimates and associated 95% confidence intervals are shown in Table 2.6.

2.3.5 Se and Sp of ag-ELISA (index) as compared to culture (reference):

Description of included studies

Ten observations on the Se / Sp of ag-ELISA, as compared to culture, were extracted from 5 references (Table 2.4-3). The number of observations per reference ranged from 1 to 4. The number of samples tested (n) ranged from 11 to 362 (mean 172, median 142). Se and Sp were reported for 8 observations, while the remaining 2 observations had 2-by-2 data only. Confidence intervals or standard error were reported for 2 observations only.

The types of samples reported for each observation included carcass swabs (2 observations), pooled pen feces (2 observations), individual feces (4 observations), or both individual and pen feces results (2 observations). Variations in culture protocols were similar to those described above. Five observations reported the use of VIDAS-SLM ag-ELISA (bioMérieux, Marcy l'Etoile, France), 1 reported a modification of VIDAS-SLM, and while the remaining 4 observations used other or “in-house” ag-ELISAs.

Sensitivity/Specificity

Of those observations reporting Se and Sp, the reference standard most commonly used was a combination of several culture protocols or all positive results from both culture and ag-ELISA or Se and Sp were calculated using TAGS, a procedure which assumes neither test to be a gold standard[31]. Two observations used a single culture protocol as the reference standard. For the 2 observations reporting 2-by-2 data, the data were reported as ag-ELISA as compared to all positive results obtained from 5 different culture protocols. The distributions of Se and Sp are shown in Figure 2.5-1 and 2.5-2. Examination of heterogeneity showed significant Q-statistics. Descriptive data and random-effects pooled estimates are shown in Table 2.6.

2.3.6 Se and Sp of ab-ELISA (index) as compared to ab-ELISA (reference):

Description of included studies

Only 5 references reported studies comparing 2 or more different ab-ELISA protocols. The characteristics of these studies are presented in Table 2.4-4. Due to the small number of studies included in this category of test comparisons and the wide range of different ELISA assays reported, review of these studies is limited to qualitative description only.

Wiuff et al. (2000) reported Se and Sp of the "novel" or "AQ-mix" ELISA, as compared to their in-house ELISA, for 40 sera samples from multiplier pigs; kappa statistic and correlations were also either extractable or calculated post-hoc. Agreement between another novel ELISA, designated a "trimix" ELISA, and a Danish-mix ELISA for meat juice from finishers was reported by Clouting (2001). Mejia et al. (2005) reported the correlation between results from two ELISA kits, Svanovir and Salmotype, on sera from 361 pigs (194 fattening pigs and 167

sows). The OD cutoff value used for the Svanovir kit was 40%; the cutoff value for the Salmotype ELISA was described as "calculated from a linear regression equation", but the value used was not specified. Farzan et al. (2007) reported on the agreement between the Salmotype (cutoff 20% OD) and HerdChek (cutoff 10% OD) ELISA kits using sera collected from 600 finisher pigs on-farm at both the individual and farm levels, where a positive farm was defined as having at least 5 ELISA- positive pigs. The agreement between two in-house ELISAs, described as a "simple" and a "complete" ELISA, for sera from 600 finishing pigs was reported by Proux et. al (2000).

2.3.7 Se and Sp of PCR (index) as compared to culture (reference):

Description of included studies

Seventeen observations on the Se / Sp of PCR, as compared to culture, were extracted from 7 references (Table 2.4-5). The number of samples tested (n) ranged from 11 to 337 (mean 102, median 54). The number of observations extracted ranged from 1 to 8 per reference. Se and Sp were reported for 6 observations; confidence intervals were reported for 3 of these. All 17 observations had extractable 2-by-2 data.

Samples tested included carcass swabs (4 observations), individual fecal material (8 observations), pooled pen feces (2 observations), or lymph tissue (3 observations). Variations in culture protocol were similar to those described above. PCRs were described either as "PCR" (13 observations) or "rt-PCR" (4 observations). Two studies used PCR kits (iQ-Check Assay, Bio-Rad Laboratories, Hercules, CA, USA; BAX system, Qualicon Ltd., Warwick, UK), while the other 15 were developed in-house.

Sensitivity/Specificity

Se and Sp for PCR were calculated from 2-by-2 data using culture as the reference standard and are shown in Figure 2.6-1 and 2.6-2. An examination of heterogeneity for overall Se and Sp showed significant Q-statistics. The associated P-values, random-effects pooled estimates and associated 95% confidence are presented in Table 2.6.

2.3.8 Agreement between tests

The kappa statistic was reported or calculable for the following test comparisons: culture as compared to other culture (44 observations), ab-ELISA as compared to culture (16 observations), culture as compared to ag-ELISA (14 observations), culture as compared to PCR (17 observations), and ab-ELISA as compared to other ab-ELISA (5 observations). Correlations were extracted for 13 ab-ELISA vs. culture test comparisons and 3 ab-ELISA vs. ab-ELISA test comparisons. The test of heterogeneity for both kappa and correlation values showed significant Q-statistics for all analyses. Summary statistics, P-values, random-effects pooled estimates and associated 95% confidence limits for these observations are outlined in Table 2.7. The test of heterogeneity for both kappa and correlation values showed significant Q-statistics for all analyses.

2.4 Discussion

2.4.1 Diagnostic accuracy

Measures of test accuracy were most frequently reported as Se and Sp. These measures rely on a single cut-point or criteria for classification and are highly correlated with each other. Irwig et al. (1994) advises that it is therefore imperative that Se and Sp be considered jointly and

be reported as a range of pairs e.g. as a receiver operating characteristic (ROC) curve. When using a meta-analytic approach, a summary ROC (SROC) may be useful. Still, in our review some studies reported only the Se for a given test or, as in the case of culture vs. culture, Sp was assumed to be 100% and thus plotting SROC curves was impractical. Furthermore, regression methods which allow joint evaluation of Se and Sp require data in a 2x2 contingency table format, which were only available for half the observations in this review. We chose to summarize results for Se and Sp independently for all tests in order to maximize the use of available data.

The largest proportion of tests evaluated in this review involved the comparison of culture protocols, which were primarily used on feces. Estimates for culture Se varied widely, ranging from 17 to 100%. This variation in Se is directly related to false negative (FN) culture results, which have been reported to range from 10 to 80%[11]. Though FN results were not reported for many of the studies included in this review, this range of FN results corresponds closely with the range of Se estimates which were either reported or calculated post-hoc. There are a number of possible reasons for variability in the accuracy of culture of *Salmonella*. The pooled Se estimated for culture of lymph tissue was larger than that obtained for culture of feces indicating that sample type may be an important factor. This observation is most likely explained by the large number of competing organisms found in feces, but not present in lymph tissue, which would hinder the culture and isolation of *Salmonella*. Certain enrichment media have been found to be better for culturing *Salmonella* from feces due to inhibition of the competing organisms[35-38], and recovery of specific serovars can vary depending on the enrichment media used[35,39-41] and the incubation temperature used[42]. Culture Se has also been shown to increase with increased fecal

sample size[36,43]. Moreover, there is little doubt that increased diagnostic efforts, such as more intensive sampling or the use of multiple enrichment broths or plating media, will result in increased detection[36]. Studies evaluating protocols with multiple culture media or testing in parallel would therefore be expected to report higher Se than studies that do not use these approaches. This has particularly important implications with respect to the use of reference standards. That is, the relative Se of a given culture protocol may differ dramatically depending on whether a reference standard consists of a single or serial pre-enrichment/enrichment steps or multiple and parallel pre-enrichment/enrichment steps. In the former case, the reference standard will likely detect fewer positive samples and thus the relative Se of the index test will be higher, while in the latter case the reference likely detect more positive samples and the relative Se of the index test will be lower.

The studies included in this review reported a variety of selective media, protocol variations and reference standards that were utilized in a large array of combinations, each which could have affected culture performance in some fashion. Sub-group analysis has suggested sample type may be an important source of variability between studies; however, this is just one of many differences that could potentially be examined. Direct comparison of results from studies using dissimilar protocols is difficult, if not impossible, particularly when the reference standards are also dissimilar. The interpretation of any summary estimates, therefore, is very limited recognizing the wide variation in reported protocols. The use of a single pooled estimate which disregards the potential impact of sample type, enrichment media used, reference standard, or other equally important variables which could affect culture Se/Sp, is not

recommended. Rather, the relationship between reported differences in culture protocols and culture Se needs to be examined within the context of a meta-regression analysis.

Evaluation of ab-ELISA via comparison with culture is commonly done even though the interpretation of results is difficult. Whether or not these two tests should even be directly compared is a matter of some debate. Primarily, the difficulty arises because the two tests are measuring different physiologic states. In the case of culture, current shedding of the organism is being detected; ab-ELISA, on the other hand, is detecting previous exposure to *Salmonella* as indicated by seroconversion. Still, the comparison of these two tests is often reported in the literature, and has therefore been included in this review. Since these test comparisons involve the use of culture, the same problems with varying culture protocols outlined above also apply here. Comparison of ab-ELISA and culture is further complicated due to variability introduced due to differing thresholds defining a positive result. At the individual level, interpretation of culture is clear-cut: either the animal is *Salmonella*-positive or it is not. With ab-ELISA the individual is also classified as either positive or negative, but the interpretation differs depending on the cut-off value specified for the ab-ELISA.

These different interpretations of test positivity cause significant variability in estimates of test accuracy both within a single test and between different tests, making pooling of results from different studies difficult. We attempted to deal with the problem of varying thresholds by stratifying the data according to ELISA cutoff value. While the test for heterogeneity was significant for all sub-groups comparisons as well as overall, substantial differences in the random-effects pooled estimates were seen between sub-groups. At the individual level, the Se

of ab-ELISA at OD10% was almost double (76%) as compared to OD40% (40%), while the overall correlation between ab-ELISA and bacterial culture was low (0.33). Defining a pig or herd as *Salmonella*-positive at lower antibody titres would result in infections being identified earlier, which is advantageous in *Salmonella* control programs. The general consensus of researchers in this area is that that ab-ELISA is best applied as a herd level test, to screen for on farm exposure to *Salmonella*. Although there were insufficient observations on herd-level correlation to compare with individual-level correlation, our finding that overall ab-ELISA Se is much higher at the herd level than at the individual level supports this logic.

A major advantage of using ab-ELISA is that it is more rapid and less costly than culture; furthermore, Chow et al. (2004) reported excellent agreement between manual and robotized assays, demonstrating that the test is well suited for high throughput automated screening e.g. for abattoir collected samples. Another advantage is that the ab-ELISA reflects on farm *Salmonella* exposure, whereas due to cross-contamination during transport and lairage the culture of abattoir-collected samples may overestimate on farm *Salmonella* prevalence[44]. As discussed above, the sensitivity of bacterial culture varies widely, and its usefulness for accurately identifying individual animals as *Salmonella*-positive is consequently limited; therefore, culture is best applied on farm for herd-level investigations of *Salmonella* prevalence and serovar distribution.

There were insufficient observations available for ab-ELISA (index) as compared to ab-ELISA (reference) to be able draw any conclusions regarding differences in test accuracy or to recommend any one ab-ELISA over others. While there are a number of reasons for variability in

ab-ELISA sensitivity, such as prevalent serovars, stage of infection or immune status of the animal[17], most ab-ELISAs used in the studies included in this review incorporated O-antigens 1, 4, 5, 6, 7 and 12, and should equally be able to detect serovars from serogroups B, C1 and D. Still, correlations reported between different ab-ELISAs were variable, indicating that these assays are not equal or interchangeable. Mejia et al.[34] found that heat-treatment of sera changed the ability of the Salmotype test to detect positive samples but did not affect the sensitivity of the Svanovir test. IgM is the first immunoglobulin to appear after *Salmonella* infection[45] and the HerdChek test cannot bind to IgM, unlike the Salmotype test against which it was compared in a study by Farzan et el. [26]. Therefore, the Salmotype test could be expected to detect more recent *Salmonella* infections than the HerdChek test. Farzan et al.[26] also reported that the antigens used in these assays represented only 48% of the serovars isolated in their study, which draws into question the practicality of using these tests in that particular population of pigs. Though there is no evidence at this time to support the use of one ab-ELISA test over another, it is evident that prior to the widespread implementation of any large-scale serological screening program, comprehensive bacteriological studies to determine the serovar distribution in the geographical area of interest must be carried out.

On average, the agreement between either ag-ELISA or PCR and culture was good to excellent. Given that relatively few observations were available for PCR and ag-ELISA, the associated confidence intervals for the pooled estimates of Se and Sp were accordingly rather wide, which in turn limits the usefulness of this information. Still, the pooled estimates demonstrate that these tests perform fairly well when compared to culture. PCR and ag-ELISA have an advantage over culture in that they are both faster and more cost-efficient[13,15], with the

ability to analyze large numbers of samples at one time[46]. Like culture, caution should be used when considering summary estimates of Se/Sp for these tests. PCR performance, for example, is known to suffer in the presence of inhibitory factors found in feces[47]. The effect of this is evident in this analysis, where the Se and Sp of PCR were higher for carcass swab and lymph tissue than for feces. A recent systematic review on rapid tests for *Salmonella* in feces and food reported somewhat better PCR accuracy, with pooled estimates of 85% and 98% for Se and Sp, respectively; however, no population or sample matrix was defined, and sample types were described only as '[mostly] food or veterinary samples'[48]. A number of studies included in that review examined *Salmonella* that had already been isolated by other methods, which could explain the difference between the results of that review and those obtained here. It is evident from the results of our review that the PCR performs best when used in non-fecal sample matrices. These tests may be an attractive alternative to culture-based detection techniques, particularly where rapid tests are necessary to keep *Salmonella* from entering the food chain.

2.4.2 Quality assessment of studies included in the review

As indicated by the number of studies excluded during QA, the quality of studies reporting the evaluation of diagnostic tests in this area is limited. While studies had to meet the first two quality criteria of sufficient details of test protocol and sufficient data in order to be included in the review, the two other essential quality items related to time between tests and handling/storage of samples were often unanswered. However, none gave a negative response and consequently none were excluded based on these criteria. Had failure to address these items been used as reason for exclusion, half of the studies used in this review would have been excluded.

According to Deeks (2001), the blinding of results of experimental and reference tests and the consistent use of a single good reference standard are important aspects of study quality[49]. Notably, only one study included in our review reported the blinding of test results. A metaregression analysis of a wide range of tests has demonstrated that test review bias, the bias that occurs when the investigator knows the results of the new diagnostic test when the reference standard is interpreted, resulted in an exaggeration of measures of diagnostic accuracy[50]. Thus, it is important that the readers of tests be blinded to the status of the subjects or samples tested, which includes blinding to the results of reference standard.

The inconsistent use of reference standards among studies included in this review makes pooling or comparison of these studies problematic. Ideally, one would use a "gold standard" reference test, one known to be 100% accurate. As in many cases, the difficulty is that there is no true "gold standard" for detection of *Salmonella* infection in pigs. This problem could be partly addressed by the consistent use of a single (albeit imperfect) reference test, thus allowing comparison of diagnostic accuracy across different studies. However, in the studies included in our review, the use and definition of the reference standard varied considerably throughout each group of test comparisons. Researchers usually attempted to cope with the lack of a gold standard in one of two ways: first, by using a reference standard (which was inconsistent across studies) comprised of a single test known to be imperfect, or second, by creating a reference standard consisting of a combination of all positive test results (equivalent to testing in parallel). This latter strategy was typically used when both the index test and reference test were targeting the pathogen itself (e.g. culture vs. culture, ag-ELISA vs. culture, PCR vs. culture). This approach, while valid if assuming that false positive test results do not occur, increases the complexity of

trying to categorize test comparisons according to the reference test used. In addition, the incorporation of index test results into the reference standard can result in an overestimation of the test's accuracy (incorporation bias)[17].

It is evident that the single most deficient component in the area of evaluating diagnostic tests for *Salmonella* in pigs is the identification of a single good reference standard. This observation is supported by the results of a recent meta-analysis investigating factors influencing the prevalence of *Salmonella* in pigs, which found that diagnostic procedure accounted for a significant proportion of variability between studies[51]. While research frequently focuses on balancing test accuracy against costs and time, trying to maximize accuracy while minimizing the time required to achieve results and the cost of running the tests, more effort needs to be placed on identifying a valid and reliable reference standard that can be used universally for evaluating new diagnostic test protocols. The consistent and universal application of this reference standard in future studies evaluating these tests needs to be implemented.

There are a number of other sources of variability and bias that can limit the validity of accuracy studies[17, 52, 53]. Animal age and stage of disease cause variability in test results, particularly with serological tests. Selection bias can occur if the study population is not representative of the population in which the test will be applied; experimental conditions can lead to an overestimation of test accuracy. Bias has also been associated with lack of information on index and reference test protocols and the exclusion of intermediate results from analysis. Lack of blinding, inconsistent reference standards, lack of randomization and failure to explain selection criteria were study defects common to most studies included in this review. Failure to

address these issues during the design and conduct of studies of diagnostic test accuracy negatively affects both internal and external validity.

The overall limited quality of studies in this review is not a surprising finding; however, whether this deficiency is due to poor study design or poor reporting is impossible to determine in most cases. A recent systematic review evaluating rapid tests for bacterial intestinal pathogens in food and feces also reported overall limited quality of studies included in that review[48]. Other researchers have concluded that the conduct of systematic reviews and meta-analysis for the evaluation of diagnostic tests in other areas has been hampered by the poor quality of reporting of diagnostic studies[54-56].

Better quality of reporting has been found in manuscripts of longer length[57], demonstrating that quality of reporting can be directly affected by limitations on manuscript size. Unfortunately, this variable may be out of the hands of the authors, as size restrictions are frequently imposed by the publishing journal. Efforts have been made in recent years to encourage a standardization of methods for reporting studies, via projects such as the STARD initiative (Standards for Reporting Diagnostic Accuracy)[54] and the development of the QUADAS tool (Quality Assessment of Diagnostic Accuracy Studies)[58]. Even within length restrictions imposed by publishing journals, the items listed within these tools need to be addressed. While these tools were developed for application in the human health fields, researchers in the areas of veterinary and agri-food public health should be encouraged to adopt this methodology as well. The guidelines provided by these tools should also be considered during the design and conduct of studies of diagnostic accuracy.

2.4.3 Review limitations

The literature search strategy used in this systematic review was designed to be as sensitive as possible, encompassing many electronic databases and sources of "grey literature"; however, not all this literature could be retrieved and thus it is possible that some relevant data from conference proceedings have been missed. Still, since proceedings tended to be of insufficient quality to pass through to data extraction, it is unlikely that this omission had much impact on the findings of this review.

Whiting et al.[58] developed the QUADAS checklist as a quick and simple tool for evaluating the quality of studies of diagnostic test accuracy. The tool does not incorporate a quality score for several reasons, as outlined by the developers. Primarily, the authors argued that "choices on how to weight and calculate quality scores are generally fairly arbitrary thus it would be impossible to produce an objective quality score[58]". The authors later evaluated 5 different schemes for weighting items from the same QA tool to produce quality scores, and found that conclusions regarding the effects of quality on estimates of diagnostic accuracy differed depending on the method used to produce the quality score[59]. This reinforced their earlier assertions cautioning against the use of quality scores in diagnostic reviews. Accordingly, the use of quality scores was not incorporated into the current systematic review. Rather, studies were included or excluded based on the essential quality criteria, and all studies satisfying these criteria were included in the review. Further investigation into the association of individual quality items with estimates of diagnostic accuracy, as recommended by Whiting et al.[59], is necessary; however, the meta-regression analysis required is beyond the scope of this paper.

Significant statistical heterogeneity was detected in all categories of test comparisons as well as all sub-groups examined in this study. For this reason, meta-analysis forest plots and reported pooled estimates were presented only for visual evaluation and as a summary of available information. Most test comparisons involved the use of bacterial culture; consequently, the heterogeneity in the culture protocols would impact the related summary estimates. Furthermore, the meta-analysis approach used in this review, while weighting each observation according to study size, does not take into account the fact that multiple observations were extracted from single references. A multi-level meta-regression approach[60-62] to account for dependence between observations from the same reference is needed to examine the association of test-level variables with estimates of diagnostic accuracy[27].

2.5 Conclusion

The results of this systematic review demonstrate that there is considerable variability in the accuracy of bacterial culture, antibody and antigen immunology assays and PCR used for detecting *Salmonella* organism, antibodies and DNA in pigs. The wide range of Se and Sp reported by studies included in this review is evidence of the need for caution in using summary estimates of test accuracy. To further complicate this issue, individual studies use many variations to test protocols, such that tests that may appear similar may vary on important factors that may affect performance. The use of varied reference standards makes direct comparison between studies tenuous, particularly with respect to culture. The overall quality of existing primary research evaluating the accuracy of diagnostic tests for *Salmonella* in pigs is limited, due to study design and reporting. Future studies in this area should follow guidelines such as the STARD checklist and QUADAS tool when designing and implementing studies and reporting

their results. Additionally, the identification and consistent use of a single reference standard in each test category in future research is essential to ensure comparability of results across studies. This would enhance transfer of research findings into evidence-based policy development. Further investigation using meta-regression methods to evaluate the impact of study quality items on test Se and Sp and to examine the association between quality related and test-level variables on these parameters is required.

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Table 2.1: Keyword combinations used in database searches

Terms specific to:	Keywords:
Population	grow-finish* or grower-finish* or farrow-finish* or finisher or gilt* or barrow* or suckling or nursery or sow* or boar* or swine or porcine or pig or pigs or piglet* or hog*
Diagnostic Tests	roc or roc analys* or roc area or roc auc or roc characteristic* or roc curve* or roc curve method or roc estimated or roc evaluation or likelihood ratio* or sensitivity and specificity or standards or sensitivity or specificity or screening or false positive or false negative or accuracy or predictive value* or reference value* or reference standard* or PCR or rPCR or rtPCR or r-PCR or rt-PCR or ELISA* or serology or diagnostic or culture or bacterial culture or meat juice or comparison or correlation or agreement or evaluation
Outcome	salmonell* or *enterica*

Table 2.2: Questions included in the quality assessment of studies evaluating diagnostic tests for detecting *Salmonella* infection in pigs

Primary Quality Assessment (inclusion/exclusion criteria)		Response		Consequence
Q1.	Were the estimates of test accuracy reported, or is a sufficient amount of raw data presented for post-hoc analysis?	Yes No	(52/137) (85/137)	Included Excluded
Q2.	Was the test protocol(s) described in sufficient detail to permit replication of the test?	Yes No	(38/52*) (14/52*)	Included Excluded
Q3.	If 2 or more tests are being used/compared, is the time period between tests short enough to be reasonably sure that the subject's *Salmonella* status did not change between the two tests? OR For challenge trials: Was the time from challenge administration to measurement of outcome sufficient to have the outcome of interest?	Yes No NR‡	(36/38†) (0/38†) (2/38†)	Included Excluded Included
Q4.	Were samples stored appropriately AND processed/tested within a reasonable period of time after collection?	Yes No NR	(17/38†) (0/38†) (21/38†)	Included Excluded Included
Secondary Quality Assessment§				
Q1.	Was the sample population representative of the population that will receive the test in practice?	Yes No	(38/38) (0/38)	
Q2	Were criteria for selecting the sample population clearly described?	Yes No/NR	(3/38) (35/38)	
Q3.	Were the same test(s) applied to ALL samples or a random selection of the samples?	Yes No/NR	(38/38) (0/38)	
Q4	Were the same tests applied to all subjects and all samples, regardless of the result of one or more tests?	Yes No/NR	(36/38) (2/38)	
Q5.	Were the tests independent of each other (one test did not form part of another test being used)?	Yes No/NR	(24/38) (14/38)	
Q6.	Were test results interpreted without knowledge of the results of the other test(s) (was blinding reported)?	Yes No/NR	(1/38) (37/38)	
Q7.	Were uninterpretable/intermediate test results reported?	Yes No/NR	(38/38) (0/38)	
Q8.	Were withdrawals or losses (subjects AND samples) from the study, if any, explained?	Yes No/NR	(38/38) (0/38)	
Q9.	Was the study population randomly selected?	Yes No/NR	(6/38) (32/38)	

* This question was applicable only to references not excluded by Q1 (n=52)
† This question was applicable only to references not excluded by Q2 (n=38)
‡ Not Reported
§ Applies only to references not excluded during Primary QA (n=38)

Table 2.3. Categories of test comparisons, grouped by index and reference test, examined in a systematic review of the diagnostic accuracy of selected tests for *Salmonella* in pigs

Index Test	Reference Test
Culture	Culture
ab-ELISA	Culture
ag-ELISA	Culture
PCR	Culture
ab-ELISA	ab-ELISA

Table 2.4-1: Characteristics of 18 references reporting observations on sensitivity (Se), specificity (Sp), kappa statistic, correlation, or extractable two-by-two contingency data for bacterial culture (as compared to culture) used to detect *Salmonella* in pigs

Author, year	Country	Index Test[*]	Reference Test	n	Sample Type[†]	Se (%)	Sp (%)	κ[‡]	ρ[§]	2x2 data available?
Bager and Petersen, 1991, J[#]	Denmark	Culture-1	Culture[¶]	373	Feces, i,f[**]	30[C††]	100[A]			No
		Culture-2	Culture	373	Feces, i,f	51[C]	100[A]			No
		Culture-3	Culture	373	Feces, i,f	88[C]	100[A]			No
		Culture-4	Culture	373	Feces, i,f	31[C]	100[A]			No
		Culture-5	Culture	373	Feces, i,f	48[C]	100[A]			No
		Culture-6	Culture	373	Feces, i,f	84[C]	100[A]			No
Botteldoorn et al., 2003, J	Belgium	Culture-1	Culture	345	Feces, i,a	74[R]	100[A]			No
		Culture-2	Culture	57	Feces, i,a	46[R]	100[A]			No
		Culture-3	Culture	345	Feces, i,a	89[R]	100[A]			No
Casey et al., 2004, J	Ireland	Culture-1	Culture-2	15	Feces, i,a	60[C]	100[A]	na[‡‡]		Yes
		Culture-2	Culture-1	15	Feces, i,a	100[C]	100[A]	na		Yes
Champagne et al., 2005, J	Canada	Culture-1	Culture	310	Feces, i,a	52[R]	100[A]			No
		Culture-2	Culture	310	Feces, i,a	84[R]	100[A]			No
		Culture-2	Culture	310	Feces, i,a	66[R]	100[A]			No
		Culture-3	Culture	310	Feces, i,a	79[R]	100[A]			No
		Culture-3	Culture	310	Feces, i,a	86[R]	100[A]			No
		Culture-1	Culture-2	310	Feces, i,a	73[C]	100[A]	0.71[R]		Yes
		Culture-1	Culture-2	310	Feces, i,a	60[C]	100[A]	0.63[R]		Yes
		Culture-1	Culture-3	310	Feces, i,a	65[C]	100[A]	0.70[R]		Yes
		Culture-1	Culture-3	310	Feces, i,a	59[C]	100[A]	0.63[R]		Yes
		Culture-2	Culture-1	310	Feces, i,a	90[C]	100[A]	0.71[R]		Yes
		Culture-2	Culture-1	310	Feces, i,a	96[C]		0.63[R]		Yes
		Culture-2	Culture-3	310	Feces, i,a	81[C]		0.84[R]		Yes
		Culture-2	Culture-3	310	Feces, i,a	89[C]		0.77[R]		Yes
		Culture-2	Culture-3	310	Feces, i,a	72[C]		0.73[R]		Yes
		Culture-2	Culture-3	310	Feces, i,a	90[C]		0.85[R]		Yes
		Culture-3	Culture-1	310	Feces, i,a	98[C]		0.70[R]		Yes
		Culture-3	Culture-1	310	Feces, i,a	97[C]		0.63[R]		Yes
		Culture-3	Culture-2	310	Feces, i,a	98[C]		0.84[R]		Yes
		Culture-3	Culture-2	310	Feces, i,a	84[C]		0.77[R]		Yes
		Culture-3	Culture-2	310	Feces, i,a	95[C]		0.73[R]		Yes
		Culture-3	Culture-2	310	Feces, i,a	92[C]		0.85[R]		Yes

Author, year	Country	Index Test*	Reference Test	n	Sample Type†	Se (%)	Sp (%)	κ‡	ρ§	2x2 data available?
Cherrington and Huis, 1993, J	The Netherlands	Culture-1	Culture-2	100	Feces, i,f	90[C]	100[A]	0.87[C]		Yes
		Culture-1	Culture-3	100	Feces, i,f	90[C]	100[A]	0.49[C]		Yes
		Culture-1	Culture-4	100	Feces, i,f	87[C]	100[A]	0.61[C]		Yes
		Culture-1	Culture-5	100	Feces, i,f	100[C]	100[A]	0.40[C]		Yes
		Culture-2	Culture-1	100	Feces, i,f	93[C]	100[A]	0.87[C]		Yes
		Culture-2	Culture-3	100	Feces, i,f	90[C]	100[A]	0.47[C]		Yes
		Culture-2	Culture-4	100	Feces, i,f	82[C]	100[A]	0.48[C]		Yes
		Culture-2	Culture-5	100	Feces, i,f	96[C]	100[A]	0.32[C]		Yes
		Culture-3	Culture-1	100	Feces, i,f	72[C]	100[A]	0.49[C]		Yes
		Culture-3	Culture-2	100	Feces, i,f	50[C]	100[A]	0.47[C]		Yes
		Culture-3	Culture-4	100	Feces, i,f	71[C]	100[A]	0.76[C]		Yes
		Culture-3	Culture-5	100	Feces, i,f	100[C]	100[A]	0.66[C]		Yes
		Culture-4	Culture-1	100	Feces, i,f	67[C]	100[A]	0.61[C]		Yes
		Culture-4	Culture-2	100	Feces, i,f	61[C]	100[A]	0.48[C]		Yes
		Culture-4	Culture-3	100	Feces, i,f	95[C]	100[A]	0.76[C]		Yes
		Culture-4	Culture-5	100	Feces, i,f	100[C]	100[A]	0.52[C]		Yes
		Culture-5	Culture-1	100	Feces, i,f	31[C]	100[A]	0.40[C]		Yes
		Culture-5	Culture-2	100	Feces, i,f	29[C]	100[A]	0.32[C]		Yes
		Culture-5	Culture-3	100	Feces, i,f	53[C]	100[A]	0.66[C]		Yes
		Culture-5	Culture-4	10	Feces, i,f	40[C]	100[A]	0.52[C]		Yes
Davies et al., 2000, J	USA	Culture-1	Culture-2	136	Feces, i,f	37[C]	100[A]	0.30[C]		Yes
		Culture-2	Culture-1	136	Feces, i,f	84[C]	100[A]	0.30[C]		Yes
Erdman and Harris, 2003, J	USA	Culture-1	Culture-2	51	Feces, i,f	100[R]	100[A]	0.94[R]		Yes
		Culture-2	Culture-1	51	Feces, i,f	91[C]	100[A]	0.94[R]		Yes
		Culture-1	Culture-2	118	Feces, p,f	100[R]	100[A]	0.86[R]		Yes
		Culture-2	Culture-1	118	Feces, p,f	78[C]	100[A]	0.86[R]		Yes
Harvey et al., 2001, J	USA	Culture-1	Culture-2	557	LN‡‡, i,a	74[C]	100[A]	0.39[C]		Yes
		Culture-2	Culture-1	557	LN, i,a	64[C]	100[A]	0.39[C]		Yes
		Culture-1	Culture-2	644	Feces, i,a	82[C]	100[A]	0.25[C]		Yes
		Culture-2	Culture-1	644	Feces, i,a	40[C]	100[A]	0.25[C]		Yes
Hoorfar and Baggesen, 1998, J	Denmark	Culture-1	Culture	100	Feces, p,f	77[R]	100[A]			No
		Culture-2	Culture	100	Feces, p,f	72[R]	100[A]			No
		Culture-3	Culture	100	Feces, p,f	45[R]	100[A]			No

Author, year	Country	Index Test*	Reference Test	n	Sample Type†	Se (%)	Sp (%)	κ‡	ρ§	2x2 data available?
		Culture-4	Culture	100	Feces, p,f	71[R]	100[A]			No
		Culture-1	Culture-2	100	Feces, p,f	84[R]	100[A]	0.47[C]		Yes
		Culture-1	Culture-3	100	Feces, p,f	87[R]	100[A]	0.20[C]		Yes
		Culture-1	Culture-4	100	Feces, p,f	85[R]	100[A]	0.50[C]		Yes
		Culture-2	Culture-1	100	Feces, p,f	78[R]	100[A]	0.47[C]		Yes
		Culture-3	Culture-1	100	Feces, p,f	51[R]	100[A]	0.20[C]		Yes
		Culture-4	Culture-1	100	Feces, p,f	78[R]	100[A]	0.50[C]		Yes
Hoorfar and Visby Mortensen, 2000, J	Denmark	Culture-1	Culture	183	Feces, p,f	86	100[A]			No
		Culture-2	Culture	183	Feces, p,f	80	100[A]			No
		Culture-3	Culture	190	Feces, p,f	57	100[A]			No
		Culture-4	Culture	100	Feces, p,f	72	100[A]			No
		Culture-5	Culture	100	Feces, p,f	77	100[A]			No
		Culture-5	Culture	190	Feces, p,f	64	100[A]			No
		Culture-6	Culture	190	Feces, p,f	32	100[A]			No
		Culture-1	Culture-2	183	Feces, p,f			0.76[C]		Yes
		Culture-4	Culture-5	190	Feces, p,f			0.45[C]		Yes
Jensen et al., 2003, J	Denmark	Culture-1	Culture	396	Feces , p,f	86[C]	95.5[R]	0.77[C]		Yes
		Culture-2	Culture	396	Feces , p,f	79[C]	100[R]	0.77[C]		Yes
Korsak et al., 2004, J	Belgium	Culture-1	Culture[##]	78	Feces, i,a	50[R]	100[A]	0.45[R]		Yes
		Culture-2	Culture[##]	78	Feces, i,a	65[R]	100[A]	0.53[R]		Yes
Michael et al., 2003, J	Brazil	Culture-1	Culture	126	Feces, i,f	36[R]	100[R]			No
		Culture-1	Culture	126	Feces, i,f	71[R]	100[R]			No
		Culture-2	Culture	126	Feces, i,f	32[R]	100[R]			No
		Culture-2	Culture	126	Feces, i,f	71[R]	99[R]			No
		Culture-3	Culture	126	Feces, i,f	92[R]	98[R]			No
		Culture-3	Culture	126	Feces, i,f	86[R]	100[R]			No
		Culture-4	Culture	126	Feces, i,f	81[R]	87[R]			No
		Culture-4	Culture	126	Feces, i,f	86[R]	100[R]			No
		Culture-5	Culture	126	Feces, i,f	40[R]	96[R]			No
		Culture-5	Culture	126	Feces, i,f	43[R]	100[R]			No
		Culture-6	Culture	126	Feces, i,f	17[R]	91[R]			No
		Culture-6	Culture	126	Feces, i,f	29[R]	97[R]			No

Author, year	Country	Index Test*	Reference Test	n	Sample Type†	Se (%)	Sp (%)	κ‡	ρ§	2x2 data available?
Nollet et al., 2001, P	Belgium	Culture-1	Culture	75	Feces, i,a	59[R]	100[A]	0.49[R]		No
		Culture-1	Culture	75	LN, i,a	89[R]	100[A]	0.91[R]		No
		Culture-1&2	Culture	75	Feces, i,a	88[R]	100[A]			No
		Culture-1&2	Culture	75	LN, i,a	98[R]	100[A]			No
		Culture-1&3	Culture	75	Feces, i,a	98[R]	100[A]			No
		Culture-1&3	Culture	75	LN, i,a	98[R]	100[A]			No
		Culture-1&4	Culture	75	Feces, i,a	97[R]	100[A]			No
		Culture-1&4	Culture	75	LN, i,a	98[R]	100[A]			No
		Culture-2	Culture	75	Feces, i,a	70[R]	100[A]	0.61[R]		No
		Culture-2	Culture	75	LN, i,a	86[R]	100[A]	0.88[R]		No
		Culture-2&3	Culture	75	LN, i,a	98[R]	100[A]			No
		Culture-2&3	Culture	75	Feces, i,a	99[R]	100[A]			No
		Culture-2&4	Culture	75	Feces, i,a	98[R]	100[A]			No
		Culture-2&4	Culture	75	LN, i,a	98[R]	100[A]			No
		Culture-3	Culture	75	LN, i,a	89[R]	100[A]			No
		Culture-3	Culture	75	Feces, i,a	96[R]	100[A]	0.94[R]		No
		Culture-3&4	Culture	75	LN, i,a	99[R]	100[A]	0.91[R]		No
		Culture-3&4	Culture	75	Feces, i,a	100[R]	100[A]			No
		Culture-4	Culture	75	LN, i,a	89[R]	100[A]	0.91[R]		No
		Culture-4	Culture	75	Feces, i,a	93[R]	100[A]	0.90[R]		No
Osumi et al., 2003, J	Japan	Culture-1	Culture	348	Feces, i,f	98[C]	100[A]	0.97[C]		Yes
		Culture-2	Culture	348	Feces, i,f	47[C]	100[A]	0.49[C]		Yes
Rostagno et al., 2005, J	USA	Culture-1	Culture	100	Feces, p,a	82[R]	100[A]	na	na	Yes
		Culture-2	Culture	100	Feces, p,a	94[R]	100[A]	na	na	Yes
		Culture-3	Culture	100	Feces, p,a	95[R]	100[A]	na	na	Yes
		Culture-1	Culture	100	Feces, p,a	78[R]	100[A]	na	na	Yes
Vassiliadis et al., 1981, J	Greece	Culture-1	Culture	100	Feces, i,a	38[C]	100[A]	0.43[C]		Yes
		Culture-2	Culture	100	Feces, i,a	49[C]	100[A]	0.54[C]		Yes
		Culture-3	Culture	100	Feces, i,a	56[C]	100[A]	0.61[C]		Yes
		Culture-4	Culture	100	Feces, i,a	67[C]	100[A]	0.71[C]		Yes
		Culture-5	Culture	100	Feces, i,a	95[C]	100[A]	0.96[C]		Yes
Vassiliadis et al.,	Greece	Culture-1	Culture	117	Feces, i,a	83[C]	100[A]	0.89[C]		Yes

Author, year	Country	Index Test*	Reference Test	n	Sample Type†	Se (%)	Sp (%)	κ‡	ρ§	2x2 data available?
1987, J		Culture-2	Culture	117	Feces, i,a	72[C]	100[A]	0.74[C]		Yes
		Culture-3	Culture	117	Feces, i,a	49[C]	100[A]	0.51[C]		Yes

* Index and reference test protocols are numbered to differentiate between different test protocols within the same study; two or more number appearing together indicates testing in parallel

† When two sample types are listed, the first refers to the index test evaluated, the second refers to the reference test; otherwise, the same sample was used for both index and reference tests

‡ Kappa statistic,

§ Correlation coefficient

J=Journal article, P=Proceedings

¶ unless otherwise noted by numbers, reference consists of combination of all culture positive samples, from ≥2 culture protocols

** i=individual level; h=herd-level; p= pooled or pen-level; f=samples collected on-farm; a=samples collected at abattoir

†† C=post-hoc calculated value, R= reported value, A=specificity assumed 100% for culture (index) vs. culture (reference)

‡‡ na=cannot be calculated due to zero values in 2x2 table

§§ LN=Lymph nodes

Analysis performed using TAGS software

Table 2.4-2: Characteristics of 11 references reporting observations on sensitivity (Se), specificity (Sp), kappa statistic, correlation, or extractable two-by-two contingency data for evaluating diagnostic tests for ab-ELISA (as compared to culture) used to detect *Salmonella* in pigs

Author, year	Country	Index Test[*]	Reference Test	n	Sample Type[†]	Se (%)	Sp (%)	κ[‡]	ρ[§]	2x2 data available?
Botteldoorn et al., 2003, J[#]	Belgium	Ab-ELISA, 10	Culture	325	Meat juice, feces, i,a	80[C††]	50[C]	0.18[C]		Yes
		Ab-ELISA, 40	Culture	325	Meat juice, feces, i,a	55[C]	83[C]	0.36[C]		Yes
		Ab-ELISA, 10	Culture	67	Meat juice, feces, h,a	74[C]	63[C]	0.35[C]		Yes
		Ab-ELISA, 40	Culture	67	Meat juice, feces, h,a	37[C]	90[C]	0.29[C]		Yes
Casey et al., 2004, J	Ireland	Ab-ELISA, 40	Culture-1	15	Meat juice, feces, i,a	44[C]	17[C]	0		Yes
		Ab-ELISA, 40	Culture-2	15	Meat juice, feces, i,a	60[C]	na[‡‡]	na		Yes
Chow et al., 2004, J	Canada	Ab-ELISA, SP	Culture	66	Sera, feces, i,f	89[R]	98[R]	0.87[R]	0.89[R]	Yes
Daniels et al., 2001, P	USA	Ab-ELISA, 20	Culture	148	Sera, feces, i,f	92[R]	69[A]			No
		Ab-ELISA, 20	Culture	142	Sera, feces, i,f	60[R]	59[A]			No
		Ab-ELISA, 30	Culture	148	Sera, feces, i,f	69[R]	63[A]			No
		Ab-ELISA, 30	Culture	142	Sera, feces, i,f	57[R]	84[A]			No
		Ab-ELISA, 40	Culture	148	Sera, feces, i,f	45[R]	62[A]			No
Davies et al., 2003, J	UK	Ab-ELISA, SP	Culture	421	Sera ,carcass swab, i,a			0.20[R]		No
		Ab-ELISA, SP	Culture	418	Sera, feces, i,a			0.14[R]		No
		Ab-ELISA, SP	Culture	369	Sera, feces, i,a			0.44[R]		No
		Ab-ELISA, SP	Culture	421	Meat juice, carcass swab, i,a			0.10[R]		No
		Ab-ELISA, SP	Culture	418	Meat juice, feces, i,a			0.42[R]		No
Davies et al., 2004, J	UK	Ab-ELISA, 10	Culture	2403	Meat juice, feces, i,a	52[R]	61[R]	0.17[C]		Yes
		Ab-ELISA, 40	Culture	2403	Meat juice, feces, i,a	29[R]	89[R]	0.20[C]		Yes

Author, year	Country	Index Test*	Reference Test	n	Sample Type†	Se (%)	Sp (%)	κ‡	ρ§	2x2 data available?
Farzan et al., 2007, J	Canada	Ab-ELISA-1, 10	Culture	40	Sera, feces, h,f	100[R]	28[R]			No
		Ab-ELISA-2, 20	Culture	40	Sera, feces, h,f	100[R]	10[R]			No
		Ab-ELISA-1, 10	Culture	600	Sera, feces, i,f	53[R]	72[R]	0.05[R]		No
		Ab-ELISA-2, 10	Culture	600	Sera, feces, i,f	94[R]	49[R]			No
		Ab-ELISA-1, 20	Culture	600	Sera, feces, i,f	41[R]	86[R]			No
		Ab-ELISA-2, 20	Culture	600	Sera, feces, i,f	65[R]	79[R]	0.10[R]		No
		Ab-ELISA-1, 30	Culture	600	Sera, feces, i,f	29[R]	91[R]			No
		Ab-ELISA-2, 30	Culture	600	Sera, feces, i,f	59[R]	86[R]			No
		Ab-ELISA-1, 40	Culture	600	Sera, feces, i,f	29[R]	93[R]			No
		Ab-ELISA-2, 10	Culture	600	Sera, feces, i,f	35[R]	90[R]			No
Kolb, 2003, P	USA	Ab-ELISA, 40	Culture	8	Sera , LN[§§], p,a				0.67[R]	No
Nollet et al., 2005, J	Belgium	Ab-ELISA, 10	Culture	1821	Sera, LN, i,a	83[R]	30[C]	0.14[R]		Yes
		Ab-ELISA, 20	Culture	1821	Sera, LN, i,a	60[R]	53[C]	0.12[R]		Yes
		Ab-ELISA, 40	Culture	1821	Sera, LN, i,a	35[R]	79[C]	0.09[R]		Yes
		Ab-ELISA, 10	Culture	60	Sera, LN, h,a	100[R]		na		No
		Ab-ELISA, 20	Culture	60	Sera, LN, h,a	98[R]		0.25[R]		No
		Ab-ELISA, 40	Culture	60	Sera, LN, h,a	84[R]		0.15[R]		No
Proux et al., 2000, J	France	Ab-ELISA-2, 40	Culture	600	Sera, LN, i,a				0.45[R]	No
		Ab-ELISA-2, 40	Culture	120	Sera, Feces, p,a				0.17[R]	No
Sibley et al., 2003, J	Canada	Ab-ELISA, SP	Culture	67	Sera, Feces, i,f			0.65[R]		No

* Index and reference test protocols are numbered to differentiate between different test protocols within the same study; two or more number appearing together indicates testing in parallel

† When two sample types are listed, the first refers to the index test evaluated, the second refers to the reference test; otherwise, the same sample was used for both index and reference tests

‡ Kappa statistic,

§ Correlation coefficient

J=Journal article, P=Proceedings

¶ Ab-ELISA positive cutoff value: 10=OD10%; 20=OD20%; 30=OD30%; 40=OD40%; SP= serum-to-positive ratio 25%

** i=individual level; h=herd-level; p= pooled or pen-level; f=samples collected on-farm; a=samples collected at abattoir

†† C=post-hoc calculated value, R= reported value, A=specificity assumed 100% for culture (index) vs. culture (reference)

‡‡ na=cannot be calculated due to zero values in 2x2 table

[§§] LN=Lymph nodes
[##] Analysis performed using TAGS software

Table 2.4-3: Characteristics of 5 references reporting observations on sensitivity (Se), specificity (Sp), kappa statistic, correlation, or extractable two-by-two contingency data for ag-ELISA (as compared to culture) used to detect *Salmonella* in pigs

Author, year	Country	Index Test[*]	Reference Test	n	Sample Type[†]	Se (%)	Sp (%)	κ[‡]	ρ[§]	2x2 data available?
Cherrington and Huis, 1993, J[#]	The Netherlands	Ag-ELISA-1	Culture	100[i]	Feces, i,f	56[C]	98[C]	0.60[C]		Yes
		Ag-ELISA-2	Culture	100[i]	Feces, i,f	65[C]	86[C]	0.52[C]		Yes
Korsak et al., 2004, J	Belgium	Ag-ELISA-1	Culture[##]	78	Feces, m,f	100 [R]	86 [R]			NA
		Ag-ELISA-2	Culture[##]	78	Feces, m,f	34 [R]	98 [R]			NA
Uyttendaele et al., 2003, J	Belgium	Ag-ELISA	Culture	11	Carcass swab, i,a	100[C]	100[C]	1[C]		Yes
Wegener and Baggesen, 1997, J	Denmark	Ag-ELISA-1	Culture	362	Feces, i,a	90[R]	99[R]			No
		Ag-ELISA-2	Culture	362	Feces, i,a	77[R]	89[R]			No
		Ag-ELISA-1	Culture	189	Feces, p,f	98[R]	99[R]			No
		Ag-ELISA-2	Culture	189	Feces, p,f	71[R]	92[R]			No
Yeh et al., 2002b, J	Taiwan	Ag-ELISA	Culture	257	Carcass swab, i,a	100[R]	98[R]	0.89[R]		Yes

[*] see Table 4-1 for footnote descriptions

Table 2.4-4: Characteristics of 5 references reporting observations on sensitivity (Se), specificity (Sp), kappa statistic, correlation, or extractable two-by-two contingency data ab-ELISA (as compared to ab-ELISA) used to detect *Salmonella* in pigs

Author, year	Country	Index Test[*]	Reference Test	n	Sample Type[†]	Se (%)	Sp (%)	κ[‡]	ρ[§]	% agree	2x2 data available?
Clouting et al., 2001, J[#]	UK	Ab-ELISA-1, 40[¶]	Ab-ELISA-2, 40	40	Meat juice, i,a					0.91[R]	No
Farzan et al., 2007, J	Canada	Ab-ELISA-1, 20	Ab-ELISA-2, 10	600	Sera, i,f			0.56[R]		0.83[R]	No
		Ab-ELISA-1, 20	Ab-ELISA-2, 10	40	Sera, h,f			0.80[R]		0.93[R]	
Mejia et al., 2005, J	Spain	Ab-ELISA, 40[¶]	Ab-ELISA[‡‡]	361	Sera, i,f[**]			0.19[C]	0.55[R]		Yes
Proux et al., 2000, J	France	Ab-ELISA-1, 40	Ab-ELISA-2, 40	600	Sera, i,a				0.63[R]		No
Wiuff et al., 2000, J	Denmark	Ab-ELISA-1, 10	Ab-ELISA-2, 10	40	Sera, i,f	100[R]	93[R]	0.95[C]			Yes
		Ab-ELISA-1, 40	Ab-ELISA-2, 40	40	Sera, i,f	100[R]	86[R]	0.85[C]	0.86[R]		Yes

[*] Index and reference test protocols are numbered to differentiate between different test protocols within the same study; two or more number appearing together indicates testing in parallel

[†] When two sample types are listed, the first refers to the index test evaluated, the second refers to the reference test; otherwise, the same sample was used for both index and reference tests

‡ Kappa statistic,

[§] Correlation coefficient

[#] J=Journal article, P=Proceedings

[¶] Ab-ELISA positive cutoff value: 10=OD10%; 20=OD20%; 30=OD30%; 40=OD40%; SP= serum-to-positive ratio 25%

[**] i=individual level; h=herd-level; p= pooled or pen-level; f=samples collected on-farm; a=samples collected at abattoir

[††] C=post-hoc calculated value, R= reported value, A=specificity assumed 100% for culture (index) vs. culture (reference)

[‡‡] OD cut-off calculated from a linear regression equation constructed with a set of five control sera provided by the manufacturer.

Table 2.4-5: Characteristics of 7 references reporting observations on sensitivity (Se), specificity (Sp), kappa statistic, correlation, or extractable two-by-two contingency data for PCR (as compared to culture) used to detect *Salmonella* in pigs

Author, year	Country	Index Test[*]	Reference Test	n	Sample Type[†]	Se (%)	Sp (%)	κ[‡]	ρ[§]	2x2 data available?
Bohaychuk et al., 2007, J[#]	Canada	PCR	Culture	287	Feces, i,f[**]	99[R††]	97[R]	0.96[R]		Yes
		PCR	Culture	249	Carcass swab, i,a	100[R]	98[R]	0.95[R]		Yes
		PCR	Culture	337	Feces, i,a	100[R]	82[R]	0.94[R]		Yes
Feder et al., 2001, J	USA	PCR	Culture	92	Feces, i,f	77[R]	96[R]	0.71[R]		Yes
		PCR	Culture	34	Feces, i,f	55[R]	39[R]	0.06[R]		Yes
Oliveira et al., 2006, J	Brazil	PCR	Culture	90	Feces	77[C]	98[C]	0.78[C]		Yes
		PCR	Culture	48	LN, i,L	94[C]	90[C]	0.83[C]		Yes
		PCR	Culture	45	Feces, i,L	71[C]	86[C]	0.57[C]		Yes
		PCR	Culture	12	Tonsil, i,L	100[C]	100[C]	1[C]		Yes
		PCR	Culture	12	Ileum, i,L	100[C]	100[C]	1[C]		Yes
		PCR	Culture	12	Feces, i,L	100[C]	100[C]	1[C]		Yes
		PCR	Culture	108	Feces, i,L	83[C]	92[C]	0.75[C]		Yes
		PCR	Culture	54	Feces, i,L	88[C]	86[C]	0.71[C]		Yes
Sibley et al., 2003, J	Canada	PCR	Culture	67	Feces, i,f	95[R]	93[R]	0.94[R]	0.94[R]	Yes
Uyttendaele et al., 2003, J	Belgium	PCR	Culture	11	Carcass swab, i,a	100[C]	78[C]	0.56[C]	.	Yes
Wu et al., 2003, J	Taiwan	PCR	Culture	230	Carcass swab, i,a	100[C]	97[C]	0.72[R]		Yes
Yeh et al., 2002a, J	Taiwan	PCR	Culture	50[i]	Carcass swab, i,a	100[C]	100[C]	1[C]		Yes

[*] Index and reference test protocols are numbered to differentiate between different test protocols within the same study; two or more number appearing together indicates testing in parallel

[†] When two sample types are listed, the first refers to the index test evaluated, the second refers to the reference test; otherwise, the same sample was used for both index and reference tests

‡ Kappa statistic, [§] Correlation coefficient [#] J=Journal article, P=Proceedings

[**] i=individual level; h=herd-level; p= pooled or pen-level; f=samples collected on-farm; a=samples collected at abattoir; L=laboratory based study

[††] C=post-hoc calculated value, R= reported value, A=specificity assumed 100% for culture (index) vs. culture (reference)

Table 2.5: Variable components of culture protocols for isolating *Salmonella* and frequency of reporting in 129 observations (test comparisons) of culture, as compared to other culture

Protocol step	Protocol variable	#of observations reporting
Pre-enrichment	BPW[a]	99
	GNH[b]	4
	TT[c]	9
	Other	8
	None	11
Enrichment	RV[d]	48
	TT	37
	MSRV[e]	31
	SE[f]	14
	Diasalm	10
	Other	6
Selective agar	BG[g]	85
	XLD[h]	47
	XLT4[i]	23
	Other	5
Enrichment incubation temperature	37±2C	31
	42±2C	96
Reference standard	Single-branched[†]	76
	Multi-branched[††]	55

[a]Buffered peptone water; [b]GN-Hajna broth; [c]Tetrathionate broth; [d]Rappaport-Vassiliadis broth; [e]Modified semisolid Rappaport Vassiliades agar; [f]Selenite broth; [g]Brilliant green agar; [h]Xylose lysine deoxycholate agar; [i]Xylose lysine tergitol4 agar.

[†]single or serial pre-enrichment/enrichment steps

[††]multiple and parallel pre-enrichment/enrichment steps

Table 2.6.Sensitivity and specificity of diagnostic tests examined in a systematic review of the diagnostic accuracy of selected tests for *Salmonella* in pigs

		Sensitivity:									Specificity:								
Index	Reference	N*	Mean (%)	s.d. (%)	Min (%)	Max (%)	Median (%)	$P_{Q\text{-}stat}$ †	Pooled†† Se (%)	CI_{95}	N*	Mean (%)	s.d. (%)	Min (%)	Max (%)	Median (%)	$P_{Q\text{-}stat}$ †	Pooled†† Sp (%)	CI_{95}
Culture	Culture																		
Overall		129	75	21	17	100	82	0.000	79	(75,82)	na**								
(feces)		117	73	22	17	100	80	0.000	77	(73,80)	na**								
(lymph tissue)		12	90	11	64	99	94	0.000	92	(86,95)	na**								
ab-ELISA	Culture																		
Individual																			
Overall		24	56	20	29	96	56	0.000	57	(49,65)	23	68	25	0	98	74	0.000	75	(67,82)
OD10%		5	72	19	52	94	80	0.000	76	(58,88)	5	61	23	30	98	59	0.000	54	(35,72)
OD20%		5	64	18	41	92	60	0.000	65	(53,75)	5	69	14	53	86	69	0.000	71	(54,84)
OD30%		4	54	17	29	69	58	0.000	53	(34,71)	4	81	12	63	91	85	0.000	83	(70,91)
OD40%		9	42	11	30	60	44	0.000	40	(34,46)	8	67	35	0	93	83	0.000	82	(74,88)
S/P≥45		1	---	---	96	96	---				1	---	---	93	93	---			
Herd		7	85	23	37	100	98	0.000	97	(89,99)	4	48	36	10	90	45	0.000	46	(19,75)
ag-ELISA	Culture	10	79	23	34	100	84	0.000	79	(65,89)	10	95	6	89	100	98	0.000	95	(92,98)
PCR	Culture																		
Overall		17	91	14	55	100	100	0.000	91	(82,95)	17	91	15	39	100	97	0.000	93	(87,96)
(feces)		10	85	15	55	100	86	0.000	86	(75,93)	10	88	18	39	100	92	0.000	91	(82,96)
(carcass swabs)		4	100	0	100	100	100	1	100	(99,100)	4	93	10	78	100	98	0.013	96	(86,99)
(lymph tissue		3	98	3	94	100	100	0.000	95	(84,98)	3	100	0	100	100	100	1	100	(40,100)

*number of observations (test comparisons) in each category

† Q-statistic *P*-value

†† Pooled estimate obtained via meta-analysis; where significant heterogeneity exists (Q-stat *P*-value <0.10), random effects estimates reported

**culture specificity assumed to be 100%, therefore parameters not reported

Table 2.7. Agreement and correlation between diagnostic tests examined in a systematic review of the diagnostic accuracy of selected tests for *Salmonella* in pigs

		Kappa (κ)								Correlation							
Test 1	Test 2	N*	Mean	s.d.	Min	Max	Median	$P_{Q\text{-}stat}$ †	Pooled Kappa (CI_{95})**	N	Mean	s.d.	Min	Max	Median	$P_{Q\text{-}stat}$	Pooled Corr (CI_{95})
Culture	Culture	44	0.62	0.24	0	0.94	0.68	0.000	0.66 (0.59,0.72)	na††							
ab-ELISA	Culture																
Individual																	
OD10%		4	0.13	0.06	0.05	0.18	0.15	0.000	0.13 (0.09,0.17)	0	----	----	----	----	----	----	
OD20%		2	----	----	0.10	0.12	----	0.184	0.11 (0.10,0.13)	0	----	----	----	----	----	----	
OD40%		4	0.16	0.15	0	0.36	0.15	0.000	0.19 (0.09,0.35)	1	----	----	----	----	----	----	
S/P≥25		6	0.33	0.21	0.1	0.65	0.31	0.000	0.29 (0.17,0.45)	0	----	----	----	----	----	----	
S/P≥45		1	0.87	----	----	----	----	----		1	0.89	----	----	----	----	----	
Herd		2	----	----	0.15	0.25	----	0.000	0.20 (0.12,0.31)	2	----	----	0.17	0.67	----	0.000	0.37 (0.06,0.84)
Culture	PCR	17	0.79	0.24	.05	1	0.83	0.000	0.82 (0.72,0.89)	na††							
Culture	ag-ELISA	14	0.54	0.21	0.21	1	0.51	0.000	0.52 (0.41,0.62)	na††							
ab-ELISA	ab-ELISA																
Individual		4	0.64	0.34	0.19	0.95	0.71	0.000	0.70 (0.30,0.94)	3	0.68	0.16	0.55	0.86	0.63	0.000	0.70 (0.56,0.93)
Herd		1	0.80	----	----	----	----	----		na††							

* number of observations (test comparisons) in each category

† Q-statistic P-value

** Pooled estimate obtained via meta-analysis; where significant heterogeneity exists (Q-stat P-value <0.10), random effects estimates reported

†† no observations available

Figure 2.1: Steps in conducting the systematic review

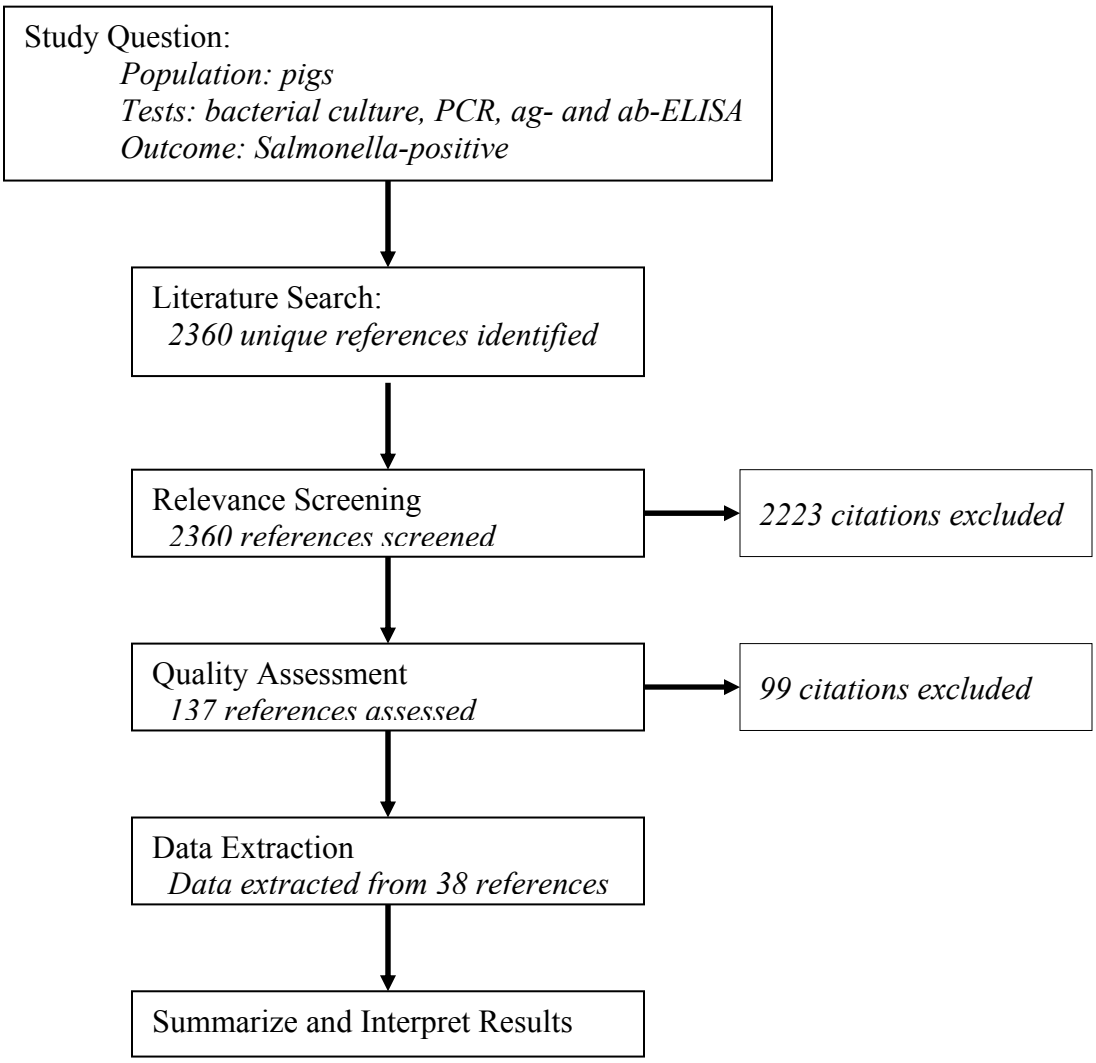


Figure 2.2: Forest plot from a random-effects meta-analysis of the relative sensitivity of bacterial culture (as compared to other culture) used to detect *Salmonella* in pigs

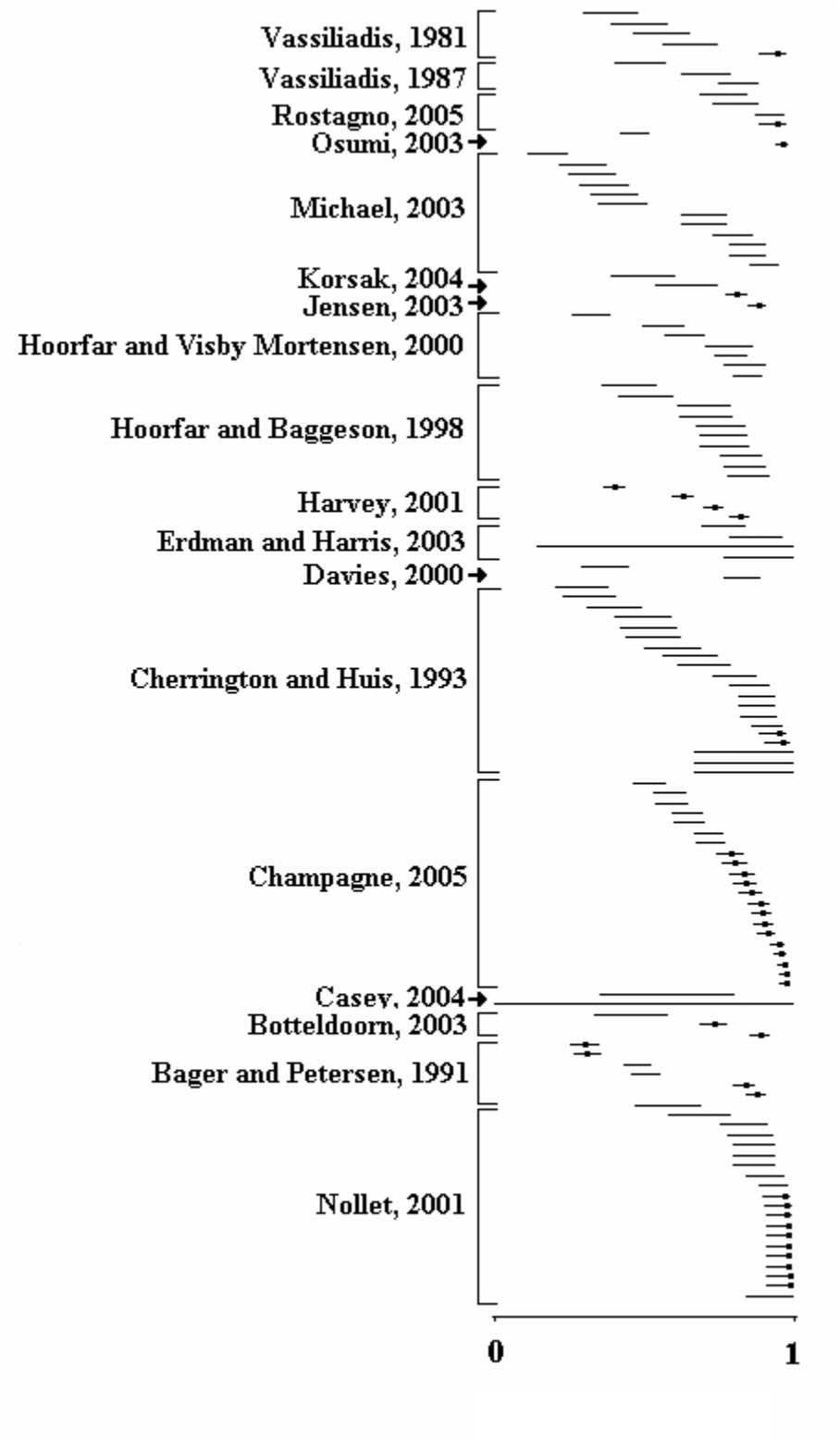


Figure 2.3-1: Forest plot from a random-effects meta-analysis of the individual-level relative sensitivity of ab-ELISA (as compared to bacterial culture), used to evaluate the *Salmonella* status of pigs

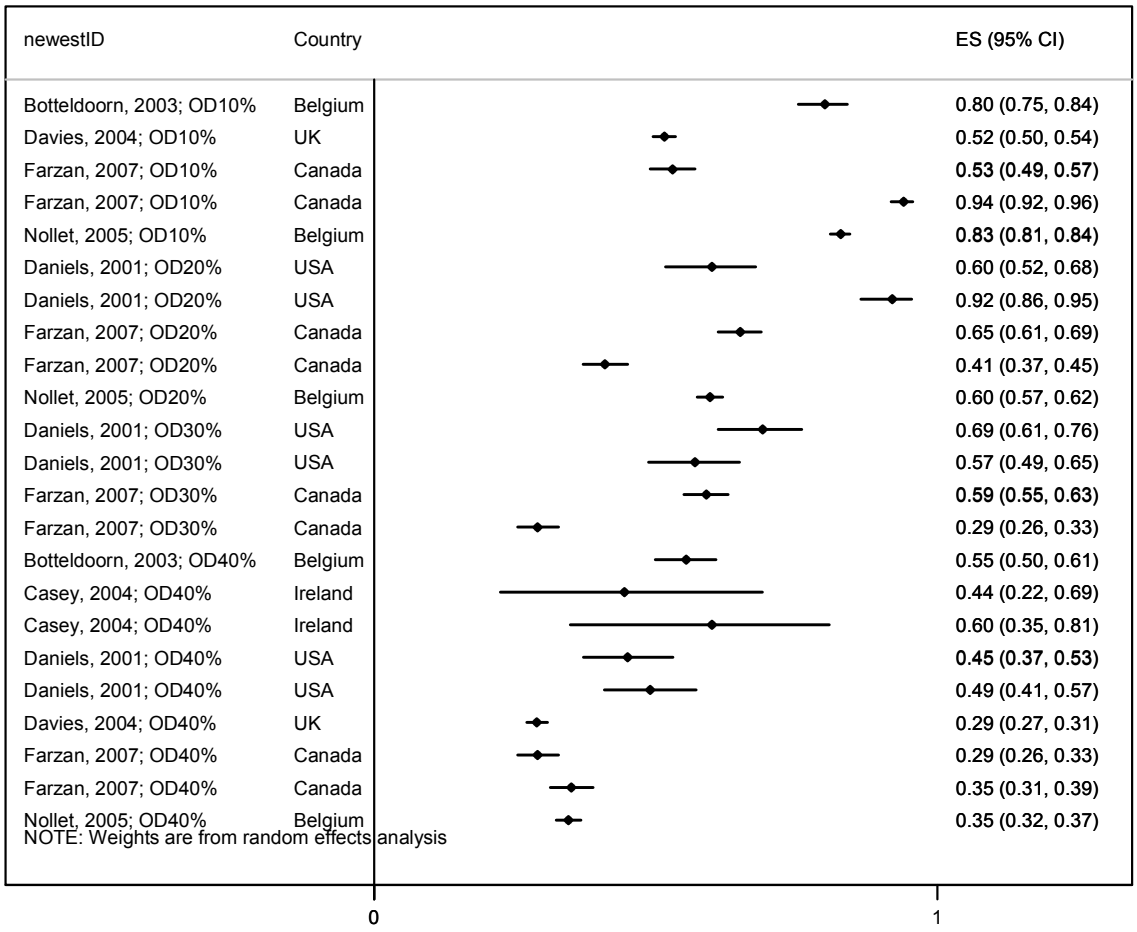


Figure 2.3-2: Forest plot from a random-effects meta-analysis of the individual-level relative specificity of ab-ELISA (as compared to bacterial culture), used to evaluate the *Salmonella* status of pigs

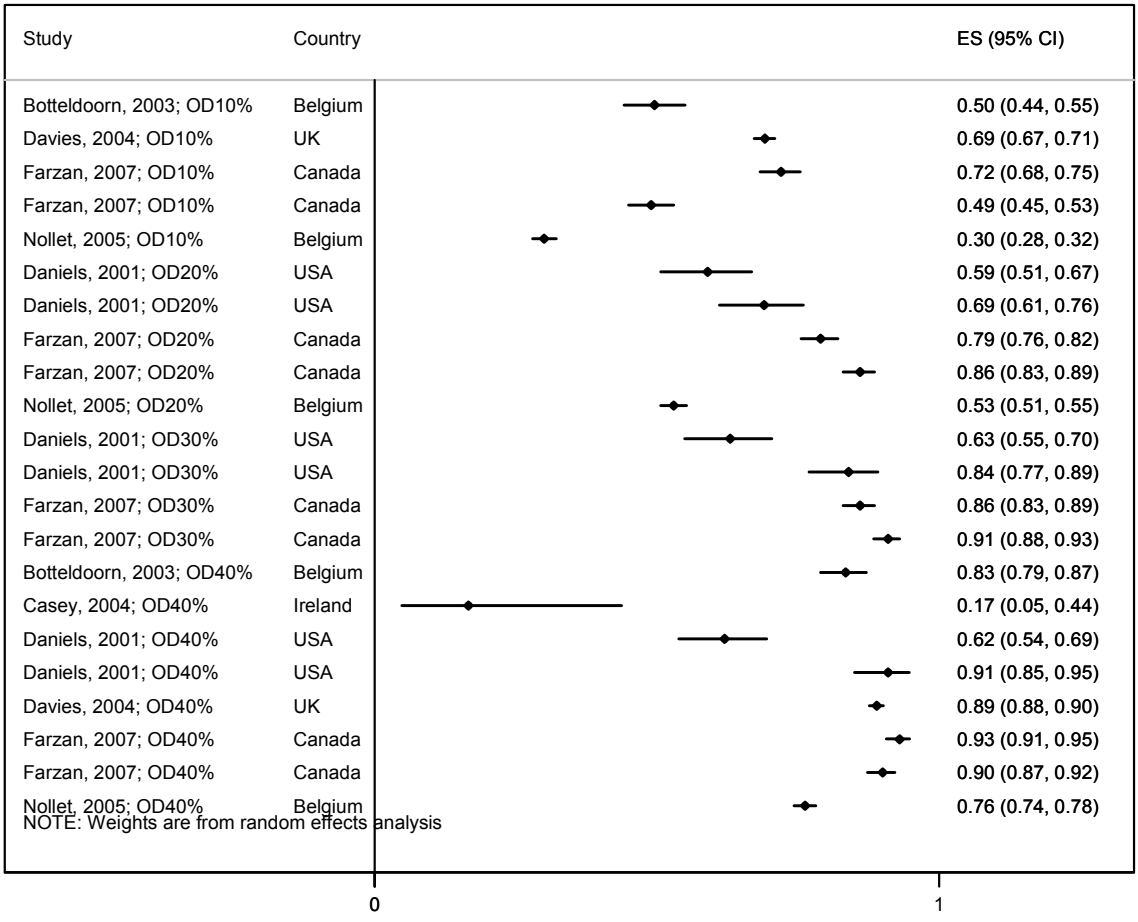


Figure 2.4-1: Forest plot from a random-effects meta-analysis of the herd-level relative sensitivity of ab-ELISA (as compared to bacterial culture), used to evaluate the *Salmonella* status of pigs

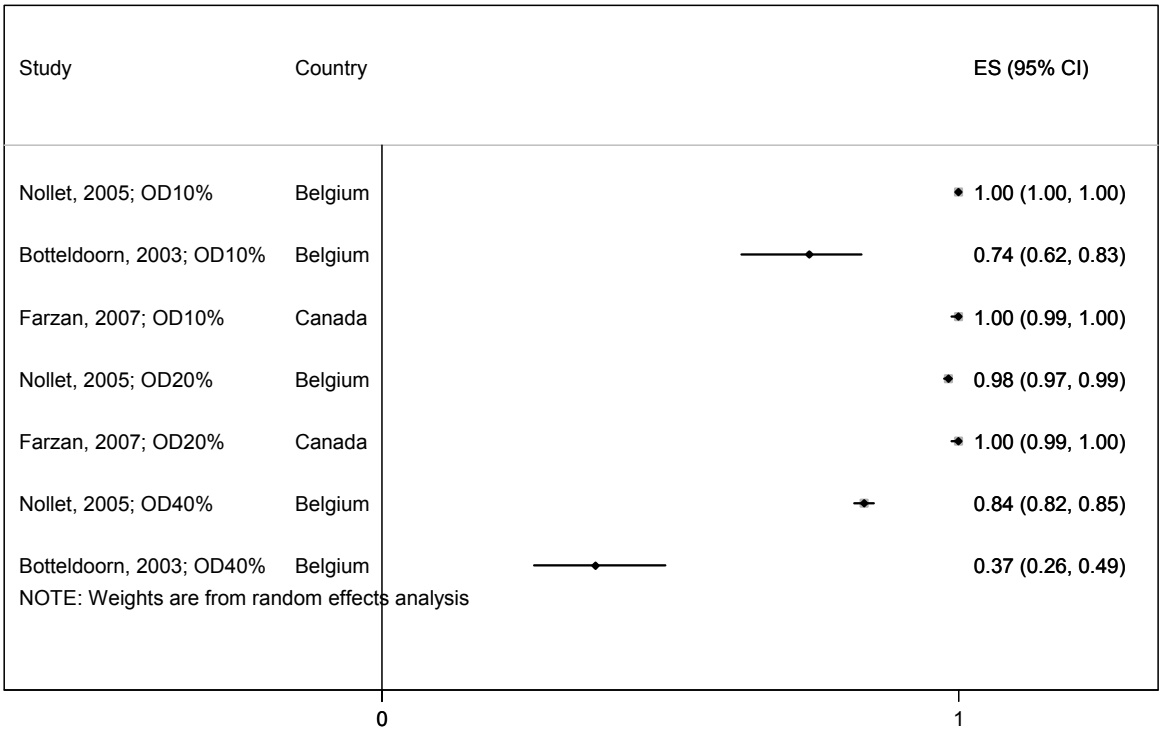
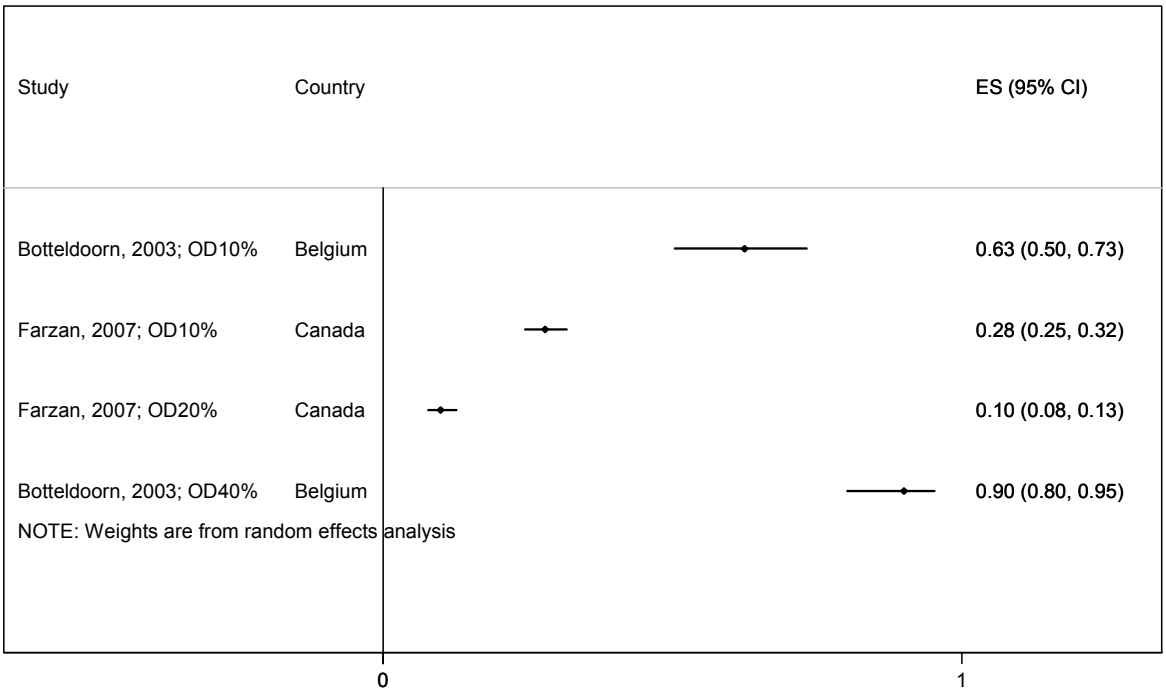


Figure 2.4-2: Forest plot from a random-effects meta-analysis of the herd-level relative specificity* of ab-ELISA (as compared to bacterial culture), used to evaluate the *Salmonella* status of pigs



* three observations did not report specificity

Figure 2.5-1: Forest plot from a random-effects meta-analysis of sensitivity of ag-ELISA (as compared to bacterial culture), used to detect *Salmonella* in pigs

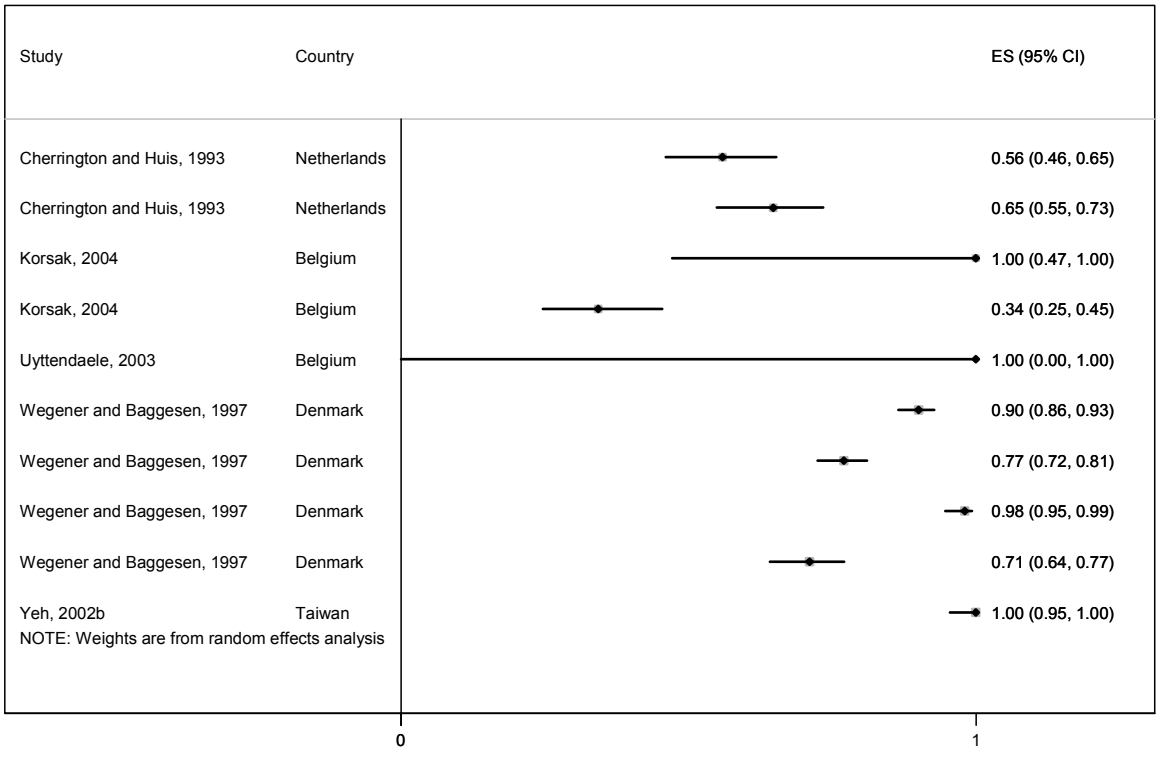


Figure 2.5-2: Forest plot from a random-effects meta-analysis of the specificity of ag-ELISA (as compared to bacterial culture), used to detect *Salmonella* in pigs

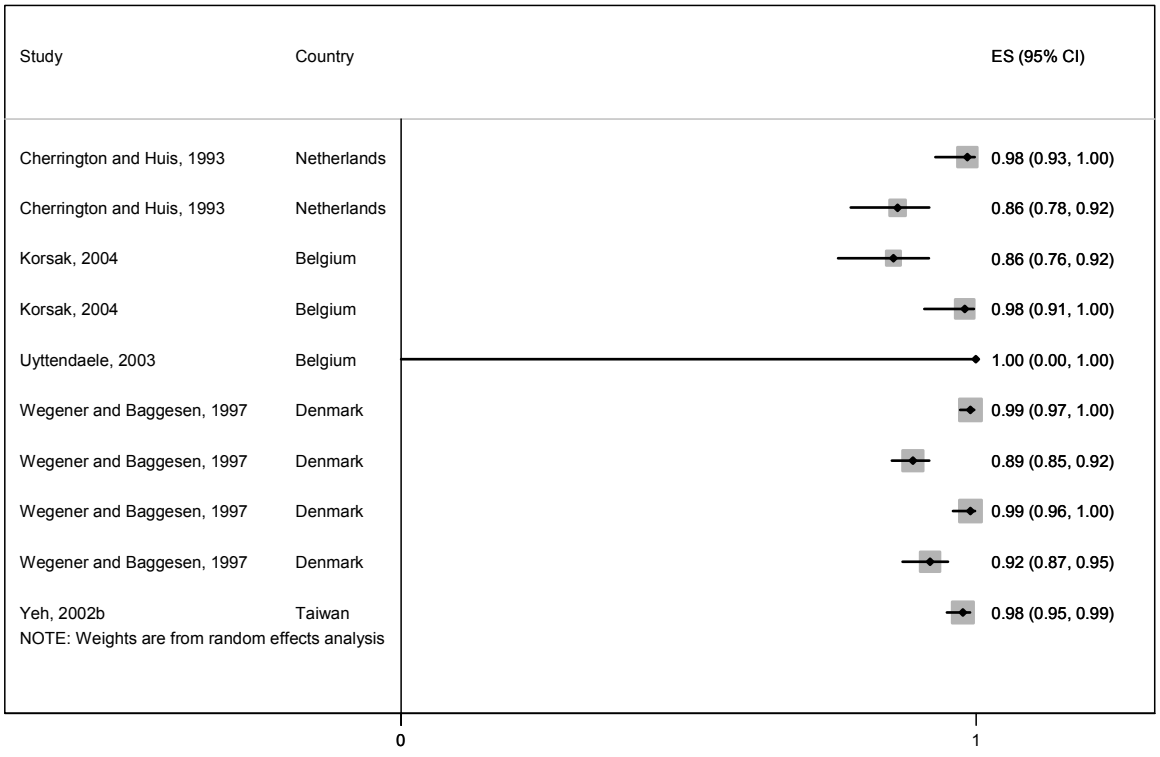


Figure 2.6-1: Forest plot from a random-effects meta-analysis of the relative sensitivity of PCR (as compared to bacterial culture), used to detect *Salmonella* in pigs

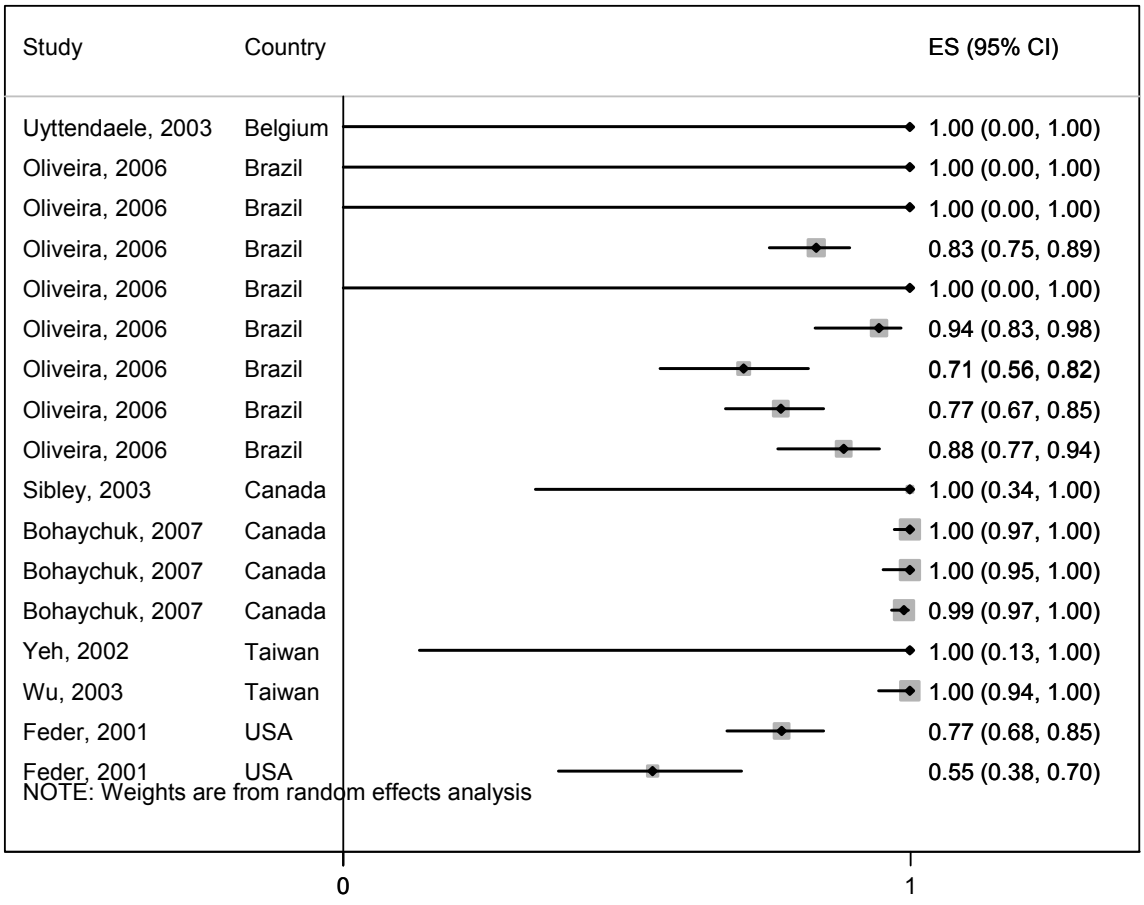
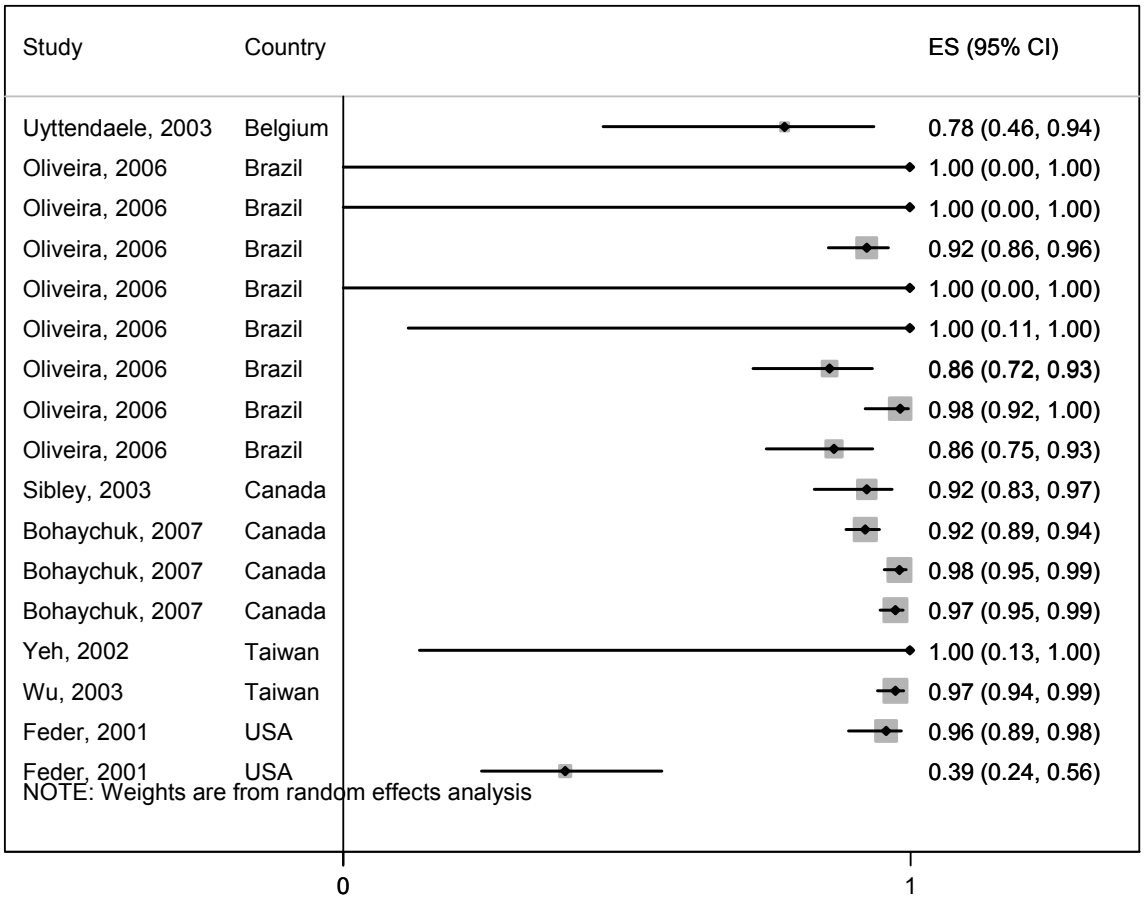


Figure 2.6-2: Forest plot from a random-effects meta-analysis of the relative specificity of PCR (as compared to bacterial culture), used to detect *Salmonella* in pigs



CHAPTER 3

A META-REGRESSION APPROACH TO INVESTIGATING FACTORS ASSOCIATED WITH VARIABILITY IN THE DIAGNOSTIC ACCURACY OF SELECTED TESTS FOR *SALMONELLA* SPP. IN SWINE

3.1 Introduction

Subsequent to the introduction of the Danish *Salmonella* control program in pigs and the EU project "*Salmonella* in pork" (SALINPORK)[1], there has been an explosion of primary research directed at better understanding the most effective ways to monitor *Salmonella* status in this animal species. A large number of studies were published evaluating the accuracy of tests in different geographic locations and swine populations, often resulting in conflicting findings and recommendations. In a recently published meta-analysis investigating factors associated with the prevalence of *Salmonella* spp. in pig farms, the use of diagnostic procedure was significantly associated with differences in estimates of *Salmonella* prevalence between studies[2]. Obvious reasons for observed variability might include use of different types of tests (e.g. bacterial culture, PCR [polymerase chain reaction] or ELISA [enzyme-linked immunosorbent assay]), sample type (feces, blood, meat juice, carcass swabs) or sampling level (individual, pooled, farm). Other important factors might include technical variation of test characteristics (different laboratories; changes over time), choice of gold standard and cut-off value for interpretation[3].

Accurate detection of *Salmonella*, *Salmonella* DNA, or antibodies to *Salmonella* in apparently healthy pigs is essential for effective monitoring and control programs. It follows, then, that the diagnostic accuracy of the screening tests used to monitor *Salmonella* in pigs must be known to correctly determine their *Salmonella* status. We recently conducted a systematic

review in order to identify, appraise, and summarize the existing published primary literature reporting the accuracy of selected diagnostic tests bacterial culture, PCR, antigen [ag-] and antibody [ab-] capture ELISA) for *Salmonella* in swine under field conditions (Chapter 2). We observed a wide range of reported sensitivity (Se) and specificity (Sp) within various test categories based on individual studies included in that review as well as statistically significant heterogeneity between studies for all test categories. Where significant heterogeneity exists between studies, pooling reported estimates of Se or Sp from multiple studies is not appropriate; rather, the use of meta-regression analysis is recommended in order to investigate the factors potentially associated with variability between studies[4]. Information obtained through this type of analyses can help identify problems with the transferability of diagnostic tests, reveal flaws in primary studies, and guide the design of future studies[5].

The primary objective of this study was to identify the study-level and test-characteristic variables that could explain the variation in test Se and Sp among studies, and to quantify the association between these variables and test Se and Sp using a meta-regression approach.

3.2 Methods

3.2.1 Review Approach

The review question was "What is the accuracy of existing bacterial culture, PCR, ag- and ab-ELISA used for detecting *Salmonella* status in pigs under field conditions?" The conduct, analysis and detailed results of the systematic review have been previously reported (Chapter 2). Briefly, ten electronic databases and grey literature sources were searched for all

potentially relevant research in which bacterial culture, antibody or antigen capture ELISA or PCR were evaluated for Sa*lmonella* in pigs, including post-hoc estimation of test accuracy where two or more of these tests were used simultaneously on the same population. Literature searches were limited to publications from January, 1980 until June, 2007. Citations were screened for relevance and study quality by two independent reviewers using pre-designed, pre-tested templates. Data from included studies were extracted by the primary reviewer; results were then verified by a second, independent reviewer.

Thirty-seven relevant studies of sufficient methodological and reporting soundness were included in the meta-regression. The main characteristics of the studies are shown in Table 3.1. For the purposes of comparison, tests applied within each study were designated as the index test (the test being evaluated) or as the reference test (the test used as the reference or "gold" standard). More than one test comparison was usually obtained from a single publication. Henceforth, each unique test comparison will be referred to as an "observation", while the publication from which the observation was extracted will be referred to as the "study". Observations were grouped for analysis according to the type of index test evaluated and the type of reference standard against which the index test was compared (Table 3.2).

3.2.2. Estimates of Se and Sp

Where raw data were available, Se and Sp were calculated from the raw data according to

$$Se = \frac{TP}{TP + FP} \quad \text{and} \quad Sp = \frac{TN}{TN + FN} \qquad (3.1;\ 3.2)$$

where TP, FP, TN and FN are the true positive, false positive, true negative and false negative test results, respectively. The apparent Se of culture (as compared to culture) was calculated

using all positive results from both the index test and the reference test or tests; culture Sp was assumed to be 100% in this category of test comparisons. For all other test comparison categories, the apparent Se and Sp were calculated relative to the results of the reference standard. Where raw data were not available, the reported Se and Sp were used in the analyses. Estimates of Se and Sp were logit-transformed and the standard errors of the logit prevalence were computed as follows:

$$\text{logit}\, p = \ln\left(\frac{p}{1-p}\right) \quad \text{and} \quad S.E. = \sqrt{(1/n \times p \times (1-p))} \qquad (3.3;\ 3.4)$$

where n is the sample size and p is test Se or Sp.

3.2.3 Predictor variables examined

The test and study characteristic variables that were selected for the meta-regression analyses are shown in Tables 3.3 to 3.5. The variables were selected for biological relevance, completeness of records (no missing data) and non-uniform responses.

3.2.4 Meta-regression

Analyses were carried out for each category of test comparison shown in Table 3.2. For each category, random-effects meta-regression models were evaluated to identify sources of heterogeneity in Se and Sp among observations, and to evaluate the association between observation- and study-level predictors and estimates of Se and Sp. These models were specified according to:

$$\text{logit}\, p_{ij} = \beta_0 + \beta X_{ij} + v_j + \mu_i + \varepsilon_{ij} \qquad (3.5)$$

where β_0 represents the intercept or the overall mean if no other predictor was included in the model, β represents the coefficient for the ith predictor in study j, v_j represents the effect of

study *j*, μ_i represents the effect of observation *i*, and ε_{ij} represents the sampling error for observation *i* within study *j*. The variance of μ_i and v_j (σ^2_μ and σ^2_v) represent the variation between observations and between studies, respectively, and were estimated using a restricted maximum likelihood (REML) algorithm (GLLAMM, Stata/SE v9.2, StataCorp LP, College Station, TX, USA). The sampling variance of the observations, σ^2_ε, was determined from the within-observation variation and sample size[6]. The proportion of the total study variance that was due to variation between observations (ρ_μ) and between studies (ρ_v) was computed as:

$$\rho_\mu = \frac{\sigma^2{}_\mu}{\sigma^2{}_\mu + \sigma^2{}_v + \sigma^2{}_\varepsilon} \quad \text{and} \quad \rho_v = \frac{\sigma^2{}_v}{\sigma^2{}_\mu + \sigma^2{}_v + \sigma^2{}_\varepsilon} \qquad (3.6; 3.7)$$

where $\sigma^2{}_\mu$ and $\sigma^2{}_v$ represent the variation between observation and between studies, respectively, and were estimated from the null model and σ^2_ε was the sampling variance. If $\sigma^2{}_\mu$ or $\sigma^2{}_v$ was found to be small, then μ_i or v_j was dropped from the regression model. Where ρ_μ and ρ_v exceeded 25%, test and study characteristic variables were then examined to determine how much of this variance was accounted for by each predictor[6]. Variables were also examined to quantify their association with test Se and Sp.

The unconditional associations between each predictor variable and test Se or Sp were first evaluated in univariable regression models. All variables with an unconditional *P*-value of less than 0.20 were evaluated for inclusion in a multivariable model using a manual forward-stepwise process. Variables with $P \leq 0.05$ were considered statistically significant. Biologically reasonable first-order interaction terms were examined where more than one significant risk factor was identified in the final main effects model. Statistically significant interaction terms were included in the final model.

3.3 Results

3.3.1 Culture (index) as compared to culture (reference)

3.3.1-1 Univariable meta-regression models

One hundred and twenty nine observations on the Se of bacterial culture (Se_c), as compared to other culture, were extracted from 18 references[7-24]. For Se_c, the proportion of total variance of that was due to the variance between observations ($Se_c \rho_\mu$) and between studies ($Se_c \rho_v$) was 36% and 11%, respectively (Eq. 3.6 and Eq. 3.7). Table 3.6 shows the proportion of $Se_c \rho_\mu$ and $Se_c \rho_v$ that was explained by fitting a meta-regression model for each individual predictor. The type of enrichment used in both the index and reference protocols accounted for the largest proportion of $Se_c \rho_\mu$, explaining 22% and 16%, respectively. Agar type (reference), reference type, agar type (index) and enrichment type (index) each explained between 48% and 80% of $Se_c \rho_v$.

3.3.1-2 Study quality variables

Random selection of sampling units was the only study quality variable examined that was significantly associated with apparent culture Se, and accounted for 12% and 76% of $Se_c \rho_\mu$ and $Se_c \rho_v$, respectively.

3.3.1-3 Multivariable meta-regression

Enrichment temperature, study population, agar type (index) and enrichment type (index and reference) were the predictors remaining significant in the final multivariable model (Table 3.7). There was a significant interaction between enrichment temperature and enrichment types (index). This model explained 54% of $Se_c\ \rho_\mu$ and 100% of $Se_c\ \rho_v$.

3.3.2 Ab-ELISA (index) as compared to Culture (reference)

3.3.2-1 Univariable meta-regression models

Twenty-four individual-level observations on the Se and Sp of ab-ELISA (Se_E and Sp_E), as compared to bacterial culture, were extracted from 7 references[9,10,25-29]; one observation was dropped because the ab-ELISA cutoff value was reported as a S/P ratio while all other observations were reported as OD% values[27]. For observations on the Se_E, σ^2_v was found to be zero, thus v_j was dropped from the regression model; the proportion of total variance that was due to the variance between observations ($Se_E\ \rho_\mu$) was 70%. For Sp_E, the proportion of total variance that was due to the variance between observations ($Sp_E\ \rho_\mu$) and between studies ($Sp_E\ \rho_v$) was 20% and 5%, respectively (25% overall). The proportion of ρ_μ and ρ_v that was explained by each individual predictor is shown in Table 3.8. Ab-ELISA cut-off value was the only variable significantly associated with Se_E and accounted for 42% of $Se_E\ \rho_\mu$. Ab-ELISA cut-off value was also significantly associated with Sp_E and accounted for 79% of $Sp_E\ \rho_\mu$. Culture sample type and study population each accounted for 100% of $Sp_E\ \rho_v$.

3.3.2-2 Study quality variables

None of the quality variables examined were significantly associated with Se_E nor did they explain any of $Se_E \rho_\mu$. Inclusion criteria was the only quality variable significantly associated with Sp_E, and accounted for 13% of $Sp_E \rho_\mu$ and 100% of $Sp_E \rho_v$.

3.3.2-3 Multivariable meta-regression

The results of the final models for Se_E and Sp_E are shown in Table 3.9. Ab-ELISA cut-off value was the only predictor variable included in the final model for Se_E, which decreased by 10-12% with each 10% increase in ab-ELISA OD. Ab-ELISA cut-off value and inclusion criteria were the variables remaining in the model for Sp_E. Ab-ELISA Sp increased with each increase in ab-ELISA, and decreased if the study reported any inclusion criteria. This model explained 79% of ρ_μ for Sp_E, but none of ρ_v.

3.3.3 PCR (reference) as compared to culture (index)

3.3.3-1 Univariable meta-regression models

Seventeen observations on the Se and Sp of PCR (Se_p and Sp_p), as compared to culture, were extracted from 7 references[30-36]. For PCR Se_p, the proportion of total variance of that was due to the variance between observations ($Se_p \rho_\mu$) and between studies ($Se_p \rho_v$) was 6% and 79%, respectively (Table 3.8). PCR type, study size, and study population were significantly associated with Se_p, and each accounted for 100% of $Se_p \rho_v$. Sample type was also significantly associated with Se_p, and accounted for 54% of $Se_p \rho_\mu$ and 45% of $Se_p \rho_v$. For Sp_p, the proportion of total variance that was due to σ^2_v was found to be zero, thus v_j was dropped from the

regression model. The proportion of total variance that was due to the variance between observations was 24% and there was no further examination of σ^2_{μ}. Study population and study size were significantly associated with Sp_p (Table 3.8).

3.3.3-2 Study quality variables

None of the study quality variables were significantly associated with Se_p or Sp_p, nor explained any of $Se_p \rho_{\mu}$ or $Se_p \rho_{v}$.

3.3.3-3 Multivariable meta-regression

Sample type and study size were found to be significantly associated with Se_p in multivariable regression (Table 3.10). Study size was the only variable significantly associated with Sp_p.

3.3.5 Ag-ELISA (reference) as compared to culture (index)

3.3.5-1 Univariable meta-regression models

Ten observations on the Se and Sp of ag-ELISA (Se_E and Sp_E), as compared to culture, were extracted from 5 references[12,24,34,37,38]. For both Se_E and Sp_E, σ^2_{v} was found to be zero, thus v_j was dropped from the regression models. For Se_E, 26% of total variance was due to variance between observations; for Sp_E, only 11% was due to variance between observations. Because there were only ten observations in this category of test comparison, only two potential predictor variables were examined: sample type (feces, n=8; carcass swab, n=2) and ag-ELISA type (SLM antigen capture, n=5; other antigen capture, n=5). Neither variable was significantly associated with either Se_E or Sp_E, nor explained any of $Se_E \rho_{\mu}$ or $Sp_E \rho_{\mu}$, therefore multivariable regression

was not attempted. The Se_E and Sp_E results from the null models were 83.2% (CI_{95} 60.3, 94.2) and 95.6% (CI_{95} 91.2, 97.9), respectively.

3.3.5-2 Study quality variables

No study quality variables were available for examination, due to uniform responses for all variables.

3.4. Discussion

3.4.1 Culture (as compared to culture)

A larger proportion of the overall variance in observations of Se_c was due to variance between observations (36%) than between studies (11%), indicating that differences in individual culture protocols are a more significant source of heterogeneity than the overall differences between studies. For example, culture of samples collected at slaughter which are subject to multiple enrichments at 42±1°C, plated on multiple agars, and compared against a reference culture protocol which uses RV for enrichment, would have an apparent Se_c of 89%. On the other hand, culture of the same samples in only RV enrichment at 37°C, plated onto only BG agar and compared against a reference culture protocol which uses RV for enrichment would have an apparent Se_c of just 40%. Culture of samples collected at slaughter was associated with an increased Se_c, as compared to samples collected on-farm. Although little can be found in the literature regarding quantitative differences in the number of colony forming units per *Salmonella*-positive pig sampled at slaughter versus those sampled on-farm, research has demonstrated that the prevalence of *Salmonella*-positive animals can increase dramatically during transport and lairage[39-41]. Along with increased prevalence, pigs likely shed greater

numbers of *Salmonella* organisms at slaughter and more viable organisms in the sample material translates into a greater probability of obtaining a positive culture result. Therefore, the Se_c will be higher for samples obtained from pigs at slaughter than on-farm. This suggests that enrichment type, agar type, enrichment incubation temperature and study population need to be considered when test protocols are used for diagnostic investigation or monitoring at various stages of pork production.

It has long been recommended that the incubation temperature for RV media be 43C, rather than 37C[42], yet more than a third (15/41) of these protocols used an incubation temperature of 37C. Existing research on the growth of *Salmonella* in RV media report contradictory results, with some studies reporting that RV media is best used at 42C[43], and others finding that increased temperature did not increase the Se of RV media[44]. The incubation of TT enrichment broth (index) at 37C was marginally associated with decreased Se_c, as compared to enrichment in RV; however, this association was reversed when incubation temperature was 42C. It has been suggested that 37C is the optimal temperature for enrichment, but that for highly contaminated samples incubation at 40-43C will inhibit competing organisms[45]. This is supported by studies which have found the Se of TT media for isolating *Salmonella* from "highly contaminated foods" was better at 43C than at 35C, but for "foods with a low microbial load" 35C was preferable[43,46]. The choice of incubation temperature appears to be a crucial factor when attempting to isolate *Salmonella*. Our results confirm that, for the highly contaminated samples typically examined for *Salmonella* in pigs, higher incubation temperatures should be used with most enrichment media. The exception is with SE media where, similar to the current analyses, decreased Se at higher incubation temperatures has been reported[45].

Increased diagnostic efforts, such as more intensive sampling or the use of multiple enrichment broths or plating media, will result in increased detection of *Salmonella*[13]; our results demonstrate just how significant this difference can be. According to these results, the use of multiple enrichment media and multiple agars would result in a Se_c of 65%, whereas single enrichment in RV media and culture on BGA would have a Se_c of just 27%. In reality, more intensive diagnostic efforts translate into increased costs for both labor and materials, and researchers must weigh the increased cost against the expected gain in Se_c. It would therefore be misguided to make a blanket recommendation that all investigators should use a multiple-enrichment, multiple-agar protocol. Still, the use of a wide array of culture protocols which are compared against an equally wide array of reference protocols makes comparison of results from different studies difficult. This problem is highlighted in the current analysis, where the apparent Se_c varied when different enrichment media were used in the reference protocol. In order to ensure comparability among future studies evaluating Se_c, it will be necessary to adopt a common reference standard. This reference standard will need to incorporate multiple selective enrichments, and possibly multiple agars, in order to ensure the highest possible accuracy.

3.4.2 Comparisons of ab-ELISA to bacterial culture

When ab-ELISA and culture were compared, the cutoff value of the ab-ELISA was the only test-characteristic variable associated with either Se_E or Sp_E, and explained a large proportion of the variance between observations. Numerous studies have reported varying test accuracy or test agreement with varying ab-ELISA cutoff values[25,28,29,47]. The meta-regression results show that the difference in ab-ELISA accuracy is large and predictable when ab-ELISA cutoff is changed, with Se_E increasing linearly by approximately 12% with each 10% decrease in

OD value. Decreasing the ab-ELISA cutoff value will result in pigs with a lower antibody titre, such as those in earlier stages of infection or exposed to lower levels *of Salmonella*, being classified as *Salmonella*-positive. This has important implications for surveillance and control programs such as the Danish program, where the number of positive samples doubled when the cut-off value for *Salmonella*-positive status was lower from 40% to 20% OD[48].

Although various types of ab-ELISA's have been reported to perform variably when compared against other ab-ELISA's[29,49-51], ab-ELISA type had no discernible impact on the Se_E or Sp_E when ab-ELISA was evaluated relative to culture. Given the small number of available observations it was not feasible to evaluate "culture type" in the meta-regression due to the large variability in culture protocols (as discussed above) and the number of additional variables that would have had to been considered. The only test-characteristic variable examined related to culture was the type of sample cultured. It has been suggested that, at least at the herd level, the correlation between ab-ELISA and culture of lymph nodes is better than that of culture of feces[25], presumably because recent or transient infection during transport and lairage would not result in colonization of lymph nodes. However, poor correlation between culture of lymph nodes and ab-ELISA has been reported[52], as has increased *Salmonella* prevalence in lymph nodes at slaughter[39]. Unfortunately, only 3 observations reporting culture of lymph nodes were available for analysis; it is therefore possible there our analysis had insufficient power to be able to detect significant differences in test accuracy due to the type of material used for culture.

Whether or not culture and ab-ELISA should even be directly compared may be debated, given that the two tests are measuring different physiologic states. Still, the comparison of these

two tests is frequently reported in the literature. Both types of tests are valuable in their own right, potentially measuring different time points in the process of exposure, infection, and immunity within a pig or population. Culture indicates current shedding status, while serology is an indication of historical exposure; the value of these tests may not lie in their individual accuracies, but rather together in providing the most complete picture of the potential for *Salmonella* exposure in group of pigs that could be at different stages of infection.

3.4.3 PCR (as compared to culture)

PCR of enriched samples has been advocated as a fast, reliable test for detecting *Salmonella* in pigs[31,33,53]; but the sensitivity of PCR for detecting *Salmonella* without enrichment has been demonstrated to be quite poor[31,33]. All observations included in this analysis used pre-enrichment and/or enrichment procedures and, in general, the Se_p was reported to be good to excellent. Still, even when enrichment was used, the Se_p varied with different sample matrices. Previous research has shown that human fecal samples contain inhibitory substances which may interfere with PCR assays[54,55], and these substances are likely present in pig feces as well. While some commercial PCR kits, such as the QIAGEN stool kit (QIAGEN Inc., Germantown, MD, USA), may contain reagents designed to block this inhibitors, in general it appears that Se_p is best when non-fecal sample matrices are examined. Thus, when rapid tests are necessary to keep *Salmonella* from entering the food chain PCR may be an attractive alternative to culture-based detection techniques. PCR is nevertheless still useful for identifying *Salmonella* in feces. Identification of presumptive colonies of *Salmonella* is the most time, labor and cost intensive part of *Salmonella* culture; therefore, the use of broth-enriched PCR as a screening tool for pig feces may improve time and cost effectiveness, particularly when prevalence is low[56].

Study size was the only other variable found to be significantly associated with Se_p in the final meta-regression, and was the only variable associated with Sp_p. The interpretation of the effect of sample size is unclear. Publication bias and other sample size related effects are known to be a problem in studies of diagnostic accuracy[57], yet little research has been published which estimate rates of publication bias for studies of diagnostic accuracy[58]. One recent paper has reported that existing tests for publication bias that use standard errors of odds ratios are likely to be seriously misleading if applied to meta-analyses of test accuracy[57]. Consequently, no attempt to investigate this potential bias was made either in the previously reported systematic review – meta-analysis or in the current meta-regression.

3.4.4 Ag-ELISA (as compared to culture)

Given the interest in developing rapid tests for detecting *Salmonella* in pigs, it is surprising that so few observations were available for inclusion in this analysis. Since only 10 observations on the Se and Sp of ag-ELISA's were available, just two variables were selected for examination: sample type and ag-ELISA type. Neither of these variables was found to be significantly associated with either Se or Sp. It is possible that with so few observations, there was insufficient power to detect any significant differences. Half of the observations involved the use of the VIDAS assay (BioMérieux, Marcy-l'Etoile, France), a fully automated test for the detection of *Salmonella* within 24 hours[59]. This assay has only been validated in food sample matrices by the manufacturer but shows promise for use in detecting *Salmonella* in pig feces and carcass swabs as well, although complete results would not be available for three days due to selective enrichment and confirmation requirements for these sample types[24,38]. Our analysis did

not find any difference between the performance of the VIDAS assay and that of other commercially available ag-ELISA assays.

3.4.5 Summary

This analysis provides valuable insight into factors associated with variation in test Se and Sp among studies; however, these resulted should be interpreted with caution given to the limited number of observations in most categories of test comparison and the number of variables examined in each category. While every attempt was made to only include only the most relevant predictors of test accuracy, each meta-regression analysis examined more than the "one variable per 10 observations" rule of thumb that is generally accepted[60].

Various aspects of study quality, such as blinding and randomization, are known to impact estimates of test accuracy[58]. Where they were available, variables related to study quality were examined in the meta-regression, but often this was not possible due to either missing data or uniform responses across the available studies. For the most part, quality variables did not explain a substantial amount of the variance between observations or between studies. The only exceptions were random selection of study units (explaining 76% of Se_c ρ_v, for comparison among different types of culture) and reporting of inclusion criteria (explaining 100% of Sp_c ρ_v for ab-ELISA compared to culture). Study quality items were invariably applicable at the study level, rather than at the observation level; therefore, it is not surprising that these two variables explained large portions of ρ_v.

In all test comparisons, bacterial culture was used as the reference standard. While we were able to examine the association between various elements of cultures protocols and the Se of culture (as compared to other culture), the limited number of observations in the other categories of test comparison precluded the examination of the many variables that differed within the reference culture protocol. Within each category of test comparisons, the most deficient component was the lack of a single good reference standard. Research frequently focuses on maximizing accuracy while minimizing the time required and the cost of running the tests, but more effort must be placed on identifying a valid and reliable reference standard that can be used universally for evaluating new diagnostic test protocols. It is evident from our analysis that culture Se is maximized by the use of multiple enrichment/isolation steps; therefore, it is recommended that when culture is used as a reference standard – regardless of what test is being evaluated – the reference standard should consist of multiple enrichment/isolation steps in order to maximize the Se of the reference standard, and help to ensure comparability of results across studies.

3.5 References

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Table 3.1. Characteristics of 37 primary studies examined in a meta-regression of the accuracy of diagnostic tests for *Salmonella* in pigs

Author	Reference Type	Country	Types of test comparison(s) available, index[i] vs. reference[r]	Data available[a]
Bager and Petersen, 1991	Journal article	Denmark	culture[i] vs. culture[r]	Se, Sp
Bohaychuk et al., 2007	Journal article	Canada	PCR[i] vs. culture[r]	Se, Sp, κ, raw data
Botteldoorn et al., 2003	Journal article	Belgium	ab-ELISA[i] vs. culture[r] culture[i] vs. culture[r]	Se, Sp, κ, raw data
Casey et al., 2004	Journal article	Ireland	ab-ELISA[i] vs. culture[r] culture[i] vs. culture[r]	raw data
Champagne et al., 2005	Journal article	Canada	culture[i] vs. culture[r]	Se, Sp, κ, raw data
Cherrington and Huis, 1993	Journal article	The Netherlands	ag-ELISA[i] vs. culture[r] culture[i] vs. culture[r]	raw data
Chow et al., 2004	Journal article	Canada	ab-ELISA[i] vs. culture[r]	Se, Sp, κ, ρ, raw data
Daniels et al., 2001	Proceedings	USA	ab-ELISA[i] vs. culture[r]	Se
Davies et al., 2000	Journal article	USA	culture[i] vs. culture[r]	raw data
Davies et al., 2003	Journal article	UK	ab-ELISA [i] vs. culture [r]	κ
Davies et al., 2004	Journal article	UK	ab-ELISA[i] vs. culture[r]	Se, Sp, raw data
Erdman and Harris, 2003	Journal article	USA	culture[i] vs. culture[r]	Se, Sp, κ, raw data
Farzan et al., 2007	Journal article	Canada	ab-ELISA [i] vs. culture [r]	Se, Sp, κ
Feder et al., 2001	Journal article	USA	PCR[i] vs. culture[r]	Se, Sp, κ, raw data
Harvey et al., 2001[J]	Journal article	USA	culture[i] vs. culture[r]	raw data
Hoorfar and Baggesen, 1998	Journal article	Denmark	culture[i] vs. culture[r]	Se, Sp, κ, raw data

Hoorfar and Visby Mortensen, 2000	Journal article	Denmark	culture[i] vs. culture[r]	Se, Sp, raw data
Jensen et al., 2003	Journal article	Denmark	culture[i] vs. culture[r]	raw data
Kolb, 2003	Proceedings	USA	ab-ELISA[i] vs. culture [r]	ρ
Korsak et al., 2004	Journal article	Belgium	ag-ELISA[i] vs. culture[r] culture[i] vs. culture[r]	Se, Sp, raw data
Mejia et al., 2005	Journal article	Spain	ab-ELISA[i] vs. ab-ELISA[r]	ρ, raw data
Michael et al., 2003	Journal article	Brazil	culture[i] vs. culture[r]	Se, Sp
Nollet et al., 2001	Journal article	Belgium	culture[i] vs. culture[r]	Se, Sp, κ
Nollet et al., 2005	Journal article	Belgium	ag-ELISA[i] vs. culture[r]	Se, κ, raw data
Oliveira et al., 2006	Journal article	Brazil	PCR[i] vs. culture[r]	raw data
Osumi et al., 2003	Journal article	Japan	culture[i] vs. culture[r]	raw data
Proux et al., 2000	Journal article	France	ab-ELISA[i] vs. ab-ELISA[r] ag-ELISA[i] vs. culture[r]	ρ
Rostagno et al., 2005	Journal article	USA	culture[i] vs. culture[r]	Se, Sp, raw data
Sibley et al., 2003	Journal article	Canada	ag-ELISA[i] vs. culture[r] PCR[i] vs. culture[r]	Se, Sp, κ, ρ, raw data
Uyttendaele et al., 2003	Journal article	Belgium	ag-ELISA[i] vs. culture[r] PCR[i] vs. culture[r]	raw data
Vassiliadis et al., 1981	Journal article	Greece	culture[i] vs. culture[r]	raw data
Vassiliadis et al., 1987	Journal article	Greece	culture[i] vs. culture[r]	raw data
Wegener and Baggesen, 1997	Journal article	Denmark	ag-ELISA[i] vs. culture[r]	Se, Sp

Wiuff et al., 2000	Journal article	Denmark	ab-ELISA[i] vs. ab-ELISA[r]	Se, Sp, κ, ρ, raw data
Wu et al., 2003	Journal article	Taiwan	PCR[i] vs. culture[r]	κ, raw data
Yeh et al., 2002a	Journal article	Taiwan	PCR[i] vs. culture[r]	raw data
Yeh et al., 2002b	Journal article	Taiwan	ag-ELISA[i] vs. culture[r]	Se, Sp, κ, raw data

[a] Se = sensitivity; Sp = specificity; κ = kappa statisitic; ρ = correlation

Table 3.2. Categories of test comparisons, grouped by index and reference test, examined in a meta-regression of the diagnostic accuracy of selected tests for *Salmonella* in pigs

Index Test	Reference Test
Culture	Culture
ab-ELISA	Culture
ag-ELISA	Culture
PCR	Culture

Table 3.3. Test and study characteristic variables examined in a meta-regression analysis of the diagnostic accuracy of culture (as compared to culture) of *Salmonella* in pigs

Variable	Description	Categories
Test and study variables:		
Pre-enrichment type (index)	Type of pre-enrichment used in index test protocol	BPW[a]; TT[b]; other; none
Enrichment type (index)	Type of enrichment used in index test protocol	RV[d]; TT; MSRV[d]; SE[e]; other; 2 or more enrichments in parallel
Agar type (index)	Type of agar used in index test protocol	BG[f]; XLD[g]; XLT4[h]; other; 2 or more agars in parallel
Pre-enrichment type (reference)	Type of pre-enrichment used in reference test protocol	BPW; TT; other; none
Enrichment type (reference)	Type of enrichment used in reference test protocol	RV; TT; MSRV; SE; other; 2 or more enrichments used in parallel
Agar type (reference)	Type of agar used in reference test protocol	BG; XLD; XLT4; other; 2 or more agars used in parallel
Enrichment incubation temperature (index)	Temperature of incubation for enrichment step	37°C; 42±1°C
Sample type	What type of sample was cultured?	Feces; lymph tissue
Study population	Where were pigs sampled?	On-farm; at slaughter
Sampling level	What was the unit sampled?	Individual pig; pen floor
Reference type	Publication type	Journal article; conference proceeding
N	Number of individuals or pens sampled	
Study quality variables:		
Inclusion criteria	Paper describes inclusion criteria	Yes; no
Independent tests	Index and reference tests were independent of each other (one test did not form part of the other)	Yes; no
Appropriate storage and timely processing	Paper describes samples stored appropriately and processed/tested within a reasonable period of time after collection	Yes; not reported
Random selection	Paper describes random selection of sampling unit	Yes; no/not reported

[a]Buffered peptone water; [b]Tetrathionate broth; [c]Rappaport-Vassiliadis broth; [d]Modified semisolid Rappaport Vassiliades agar; [e]Selenite broth; [f]Brilliant green agar; [g]Xylose lysine deoxycholate agar; [h]Xylose lysine tergitol4 agar.

Table 3.4. Test and study characteristic variables examined in a meta-regression analysis of the diagnostic accuracy of ab-ELISA (as compared to culture) for detection of *Salmonella* in pigs

Variable	Description	Categories
Test and study variables:		
OD%	Ab-ELISA cut-off value	OD10%; OD20%; OD30%; OD40%
Sample type (culture)	What type of sample was cultured?	Feces; lymph tissue
Sample type (ELISA)	What type of sample was tested?	Sera; meat juice
Ab-ELISA type	What type of ELISA/ELISA kit was used?	Salmotype [a]; HerdChek [b]; Svanovir [c]; in-house
Study population	Where were pigs sampled?	On-farm; at slaughter
Reference type	Publication type	Journal article; conference proceeding
N	Number of individuals or pens sampled	
Study quality variables:		
Inclusion criteria	Paper describes inclusion criteria	Yes; no
Appropriate storage and timely processing	Paper describes samples stored appropriately and processed/tested within a reasonable period of time after collection	Yes; not reported

[a] Labordiagnostik, Leipzig, Germany
[b] IDEXX Laboratories, Schiphol-Rijk, Noord-Holland, The Netherlands
[c] Svanova Biotech, Uppsala, Sweden

Table 3.5. Test and study characteristic variables examined in a meta-regression analysis of the diagnostic accuracy of PCR (as compared to culture) for detection of *Salmonella* in pigs

Variable	Description	Categories
Test and study variables:		
PCR type	General description of PCR class	PCR; real-time PCR
Study size	Number of samples tested	≤108; ≥230
Study population	Where were pigs sampled?	On-farm; at slaughter
Sample type	What type of sample was cultured?	Feces; lymph tissue; carcass swab
*Study quality variables:**		
Appropriate storage and timely processing	Paper describes samples stored appropriately and processed/tested within a reasonable period of time after collection	Yes; not reported

*other quality variables not examined due to uniform response

Table 3.6. Culture (as compared to culture) sensitivity: proportion of the observation (ρ_{μ}), and study-level (ρ_{ν}) variance explained by individual predictors

Model	ρ_{μ}	ρ_{ν}
Culture, as compared to culture:		
Pre-enrichment type (index)	0.06	0.17
Enrichment type (index)	0.22	0.49
Agar type (index)	0.10	0.48
Pre-enrichment type (reference)	0.04	0.00
Enrichment type (reference)	0.16	0.00
Agar type (reference)	0.04	0.80
Enrichment incubation temperature (index)	0.05	0.30
Sample type	0.03	0.19
Study population	0.00	0.23
Sampling level	0.01	0.00
Reference type	0.00	0.73
N	0.00	0.03

Table 3.7. Culture (as compared to culture) sensitivity: Coefficients, *P*-values and impact of predictors associated with the sensitivity of culture used to detect *Salmonella* in pigs.

Variable	n	Coefficient	*P*-value	Overall *P*-value	Sensitivity[a]
Enrichment incubation °C					
37	32	Reference			
42±1	97	0.92	<0.01		47.8%
Study population					
On-farm	65	Reference			
Slaughter	64	0.59	0.03		39.7%
Agar type (index)				<0.001	
BG	51	Reference			
XLD	25	0.59	0.58		
XLT4	14	0.25	0.35		
Other	5	-1.43	<0.01		8.0%
Multiple	34	0.42	0.08		
Enrichment type (index)				<0.001	
RV	41	Reference			
TT	24	-0.78	0.08		
MSRV	24	0.14	0.83		
SE	9	0.28	0.64		
Other	13	0.42	0.26		
Multiple	18	1.22	<0.01		55.0%
Enrichment type (reference)					
RV	21	Reference		<0.001	
TT	7	0.03	0.94		
MSRV	17	0.79	0.02		44.5%
SE	5	1.73	<0.01		67.3%
Other	7	0.45	0.24		
Multiple	72	-0.22	0.41		
Interaction: Enr °C x Enr (index)				0.005	
Enr °C x RV	57	Reference			
Enr °C x TT	20	1.03	0.04		54.0%[b]
Enr °C x MSRV	22	0.08	0.90		
Enr °C x SE	7	-1.86	<0.01		15.8%[b]
Enr °C x Other	5	0.03	0.96		
Enr °C x multiple	18	na[c]			
Intercept		-1.01	0.04		26.7%

[a] value obtained by adding variable coefficient to intercept coefficient, which is then back-transformed by: Se = 1/(1+exp – (coefficient)).

[b] includes coefficients for interaction term as well as the related main effects

Table 3.8. Ab-ELISA and PCR (as compared to culture): sensitivity and specificity: *P*-values and proportion of the observation (ρ_μ), and study-level (ρ_v) variance explained by individual predictors

Model	*Sensitivity*			*Specificity*		
	ρ_μ (Se)	ρ_v (Se)	*P*-value	ρ_μ (Sp)	ρ_v (Sp)	*P*-value
Ab-ELISA, as compared to culture:						
ELISA cut-off value	0.42	na*	0.002	0.79	0.00	0.002
ab-ELISA type	0.02		0.46	0.04	0.00	0.60
Sample type (culture)	0.01		0.69	0.00	0.15	0.26
Sample type (ab-ELISA)	0.00		0.78	0.00	1.00	0.97
Study population	0.00		0.87	0.00	1.00	0.04
Reference type	0.03		0.37	0.01	0.00	0.02
N	0.04		0.37	0.00	0.00	0.87
PCR, as compared to culture:						
PCR type	0.00	1.00	<0.001	na**	na**	0.51
Study size	0.08	1.00	<0.001			0.04
Study population	0.09	1.00	<0.001			0.16
Sample type	0.54	0.45	0.02			0.31

*little or no variance detected at this level
**total variance <25%, therefore not examined further

Table 3.9. Coefficients, *P*-values and impact of predictors associated with the sensitivity and specificity of ab-ELISA (as compared to culture), used to detect *Salmonella* in pigs.

Variable	n	Coefficient	*P*-value	Overall *P*-value	Se/Sp[a]
Sensitivity:					
ab-ELISA cutoff value					
OD10%	5	reference		0.002	
OD20%	5	-0.51	0.27		65.7%
OD30%	4	-1.03	0.03		53.2%
OD40%	9	-1.52	<0.01		41.1%
Intercept	23	1.16	<0.01		76.1%
Specificity:					
ab-ELISA cutoff value				0.001	
OD10%	5	reference			
OD20%	5	0.94	<0.01		76.0%
OD30%	4	1.43	<0.01		83.7%
OD40%	9	1.66	<0.01		86.6%
Inclusion criteria					
No	18	reference			
Yes	5	-1.60	0.01		19.9%
Intercept	23	0.21	0.52		55.2%

[a] value obtained by adding variable coefficient to intercept coefficient, which is then back-transformed by: Se = 1/(1+exp – (coefficient)).

Table 3.10. Coefficients, *P*-values and impact of predictors associated with the sensitivity and specificity of PCR (as compared to culture), used to detect *Salmonella* in pigs.

Variable	n	Coefficient	*P*-value	Overall *P*-value	Se/Sp[a]
Sensitivity:					
Sample type				0.016	
Feces	10	reference			
Carcass swab	4	2.62	0.08		97.9%
Lymph tissue	3	1.68	0.02		94.9%
Study size					
≤108	13	reference			
≥230	4	3.48	0.02		99.1%
Intercept	17	1.20	<0.00		76.9%
Specificity:					
Study size					
≤108	13	reference			
≥230	4	1.27	0.04		96.6%
Intercept	17	2.07	<0.00		88.8%

CHAPTER 4

COMPARISON OF BACTERIAL CULTURE, PCR AND A MIX-ELISA FOR THE DETECTION OF *SALMONELLA* STATUS IN NURSERY AND GROW-TO-FINISH PIGS IN WESTERN CANADA USING A BAYESIAN APPROACH

4.1 Introduction

The importance of controlling *Salmonella* in pigs and pork began to grow in the 1990's with the implementation of the Danish *Salmonella* control program[1] and subsequently by the initiation of similar monitoring and control programs across Europe. These programs are mainly based on the detection of infected pigs and the further classification of pig farms according to the level of infection[2, 3]. Although many studies have been published in recent years evaluating the accuracy of tests used for the detection of *Salmonella*-infected pigs, the results are often conflicting or ambiguous[4-6].

Traditionally, the evaluations of the sensitivity (Se) and specificity (Sp) of diagnostic tests are done by comparison to a gold standard, a test (or tests) which accurately determines the true disease state of an animal[7]. Test Se and Sp can be directly estimated from observed results when a true gold standard is used. As is often the case, though, evaluation of tests for detecting *Salmonella* infection in pigs is complicated by the lack of a gold standard. Thus, when tests for the detection of *Salmonella* in pigs are evaluated, test Se and Sp are usually assessed relative to other imperfect tests, resulting in relative Se and Sp estimates. A Bayesian approach to test evaluation offers an alternative approach in which none of the competing tests are treated as the imperfect gold standard and the diagnostic error rates are estimated for all studied tests[8]. In this approach, prior information (such as that generated by previous research or expert opinion) is combined with the observed data to obtain posterior distributions of test parameters[9]. Knowledge

of the true disease or infection status of the animal is therefore not necessary, and instead this unknown information is incorporated into the model as a latent variable[10].

The advantage of latent class models is that the Se and Sp of two or more tests can be obtained in one model relative to the true, but unknown (latent) disease status. The Bayesian approach is particularly useful for evaluating tests that are measuring chronic, persistent infections and for which no gold standard exists[9]. *Salmonella* infections are prevalent in pigs, with most infections being sub-clinical[11]. Acutely infected pigs may shed *Salmonella*, but will test seronegative without sufficient time to mount an immune response. Infection may be cleared, or pigs may progress to a chronic carrier state with intermittent shedding of *Salmonella* in the feces[12, 13]. These chronic carriers then contribute to the persistence of *Salmonella* within the herd. Consequently, within a sub-clinically infected herd there will be found a mix of both acute and chronic *Salmonella* carrying or shedding pigs which may be either seropositive or seronegative[14-17]. Currently, there is no gold standard for detecting sub-clinical *Salmonella* infection in pigs.

The study objective was to evaluate the diagnostic accuracy of a bacterial culture, real-time polymerase-chain reaction assay (RT-PCR), and a mix- enzyme-linked-immunosorbent serologic assay (ELISA) for detecting *Salmonella* in western Canadian pigs under field conditions using Bayesian methods, and to compare the results of this approach to those obtained with traditional methods.

4.2 Materials and methods

4.2.1 Farm selection

The numbers of herds and samples collected were a function of both logistic and financial constraints. To ensure *Salmonella*-positive samples were obtained, farms were purposely selected based on presumed *Salmonella* status[18, 19]. Ten farrow-to-finish pig herds (herd size n>100 sows) from Alberta (7 farms) and Saskatchewan (3 farms) were selected by swine veterinarians, based on presumed *Salmonella* positive status (n=7) or *Salmonella* negative status (n=3) and the producer's willingness to participate in the study. Herds were presumed positive at the time of herd selection if either the herd veterinarian or producer observed clinical salmonellosis within the previous 12 months, if *Salmonella* species were identified during routine testing, or if replacement breeding stock were purchased from known *Salmonella*-positive farms. Herds were presumed negative if none of these criteria were met.

4.2.2 Sample collection

Each herd was visited once from May through August 2004. Samples were delivered to the laboratory either within 2 hours of leaving the farm, or held on ice overnight and delivered the following day.

Collection of individual fecal and blood samples: In the grow-to-finish area on all farms, 1 individual rectal fecal sample (minimum 10 g) was collected from each of 30 pigs, with each sample from a different pen which was selected according to a computer-generated random number list. From each of these pigs, blood was also collected via jugular venipuncture into 10

ml vacutainer tubes. In the nursery area, blood was collected from 1 pig in each of 30 randomly selected pens or from 1 pig in all pens if there were less than 30 pens on the farm. No individual fecal samples were collected from nursery pigs as most pigs were too small to collect 10 g feces directly from the rectum.

Collection of pooled fecal samples: In both the grow-to-finish and the nursery areas, 1 pooled pen floor fecal sample was collected from each pen where individual fecal or blood samples were also collected. Pooled samples consisted of a minimum of 5 g of fecal material taken from each of 5 different floor locations within each pen (minimum 25 g total).

4.2.3 Bacteriological Culture

Bacteriologic culture for *Salmonella* was performed by the Agri-Food Laboratories Branch (AFLB), Food Safety Division of Alberta Agriculture and Rural Development. All samples were refrigerated and cultured within 24-48 hours of receiving samples at the laboratory. Fecal samples were thoroughly mixed prior to culture.

Ten grams of feces were inoculated into 90 ml of buffered peptone water (BPW) and incubated at 35°C for 20-24 hours. After incubation, 0.1 ml of BPW was inoculated into 10 ml of Rappaport Vassiliadis broth (RV). The RV tubes were placed into a 42°C water bath for 30 minutes, and then placed in a 42°C incubator for 22-24 hours. Simultaneously, 1 ml of BPW was inoculated into tetrathionate broth (TT) to which 0.2 ml of iodine solution had been added just prior to use, and placed in a 35°C waterbath for 30 minutes. The TT tubes were then placed in a 35°C incubator 22-24 hours.

After incubation 10 µl of RV and TT were streaked onto XLT4 and Rambach (RAM) selective agar plates and incubated at 35°C for 18-24 hours. Plates were read after 24 and 48 hours. At the same time, 0.3 ml of TT (0.1 ml to each of 3 sites) was inoculated onto a modified semi-solid RV (MSRV) plate and incubated at 42°C for 20 – 24 hours. The halos of growth that occurred on the MSRV plates were streaked to XLT4 and RAM plates and incubated at 35°C for 24 hours. Negative plates were reincubated and read again at 48 and 72 hours.

Colonies that morphologically appeared as *Salmonella* were screened using triple sugar iron agar slants, urea agar slants, and lysine iron agar slants, and the colonies were then plated to a blood agar plate and MacConkey plate to check for purity. Suspect colonies were tested with *Salmonella* Poly O and Poly O1 antisera agglutination (Denka Seiken Co. Ltd., Japan). Unusual or atypical reacting suspect colonies were further tested using Vitek GNI or API-20E (bioMérieux Vitek, MO, USA). All isolates were frozen at –70°C.

4.2.4 Serotyping

One isolate from each *Salmonella* positive sample was sent for serotyping and phagetyping at the Public Health Agency of Canada, Laboratory for Foodborne Zoonoses, Guelph, ON. The serotyping and phagetyping techniques followed standard procedures. Briefly, the O or somatic antigens of the *Salmonella* isolates were determined using slide agglutination[20] while the H or flagellar antigens were identified using a microtitre plate technique[21]. Serovars were named according to the antigenic formulae of Le Minor and Popoff[22]. The standard phagetyping technique described by Anderson and Williams [23] was used. The phage typing scheme and phages for *Salmonella* Typhimurium[24-26] were obtained from the International Centre

for Enteric Phage Typing (ICEPT), Central Public Health Laboratories, Colindale, UK via the National Laboratory for Enteric Pathogens (NLEP), Health Canada, Winnipeg, MB. *Salmonella* isolates which did not react with any of the typing phages were designated untypeable.

4.2.5 RT-PCR

RV and TT enrichment broths (150 µl each) from each sample cultured as described above were mixed together then analyzed using a previously published RT-PCR assay[27] with primers and hybridization probes to the *Salmonella* invA gene. DNA extraction was done using a semiautomated magnetic particle processor (KingFisher mL, Thermo Electron Corporation, Vantaa, Finland) and DNA extraction kit (Magnesil KF genomic system kit, Promega, Madison, WI, USA.) according to the manufacturers' instructions. RT-PCR was performed using the LightCycler RT-PCR machine (Roche, Germany). Internal control and target DNA both were amplified by the same set of primers to monitor each PCR reaction for inhibition and accuracy of reagent preparation. Results were interpreted as positive or negative depending on the shape of the curve and the crossing point provided by the LightCycler analysis software.

4.2.6 Serology

Serum samples were analyzed using a covalent mix-ELISA (Svanovir, Svanova Biotech, Uppsala, Sweden)[28]. The assay consists of 96-well microtiter plates photochemically coated with *Salmonella* Typhimurium and *Salmonella* Choleraesuis lipopolysaccharide (LPS) O-antigen 1, 4, 5, 6, 7 and 12, and is supposed to detect *Salmonella* serogroups B, C1 and D.

In brief, controls consisted of one blank, one commercial and one in-house *S.* Typhimurium positive serum, and one commercial and one in-house negative serum. These were diluted 1:400 with a phosphate-buffered saline (PBS) Tween dilution buffer and dispensed in duplicate onto each plate. Test sera were similarly diluted and single samples were dispensed onto each plate. Plates were covered to prevent evaporation and incubated for one hour at room temperature, then washed five times with PBS Tween buffer using the Automatic Microplate Washer (BioRad, France). Plates were returned to the Biomek 2000 Worksurface where 100 µL of freshly prepared Horseradish Peroxidase (HRP) rabbit anti-swine IgG conjugate solution was added to each well. Plates were again covered and incubated for one hour at room temperature. Wells were washed and returned to the Worksurface where 100 µL of substrate solution (tetramethylbenzidine (TMB) in substrate buffer containing hydrogen peroxide) was added to each well, then incubated for 10 minutes at room temperature. Then 50 µL of stop solution (0.5 M sulphuric acid) was added to each well. The optical density (OD) of each well was read at a wavelength 450 nm using a microtiter plate reader (Vmax; Molecular Devices, CA, USA). Analysis was performed using OD cutoff values of 20% and 40%.

4.2.7 Statistical analyses

Data for grow-to-finish pigs and nursery pigs were analyzed separately. Descriptive statistics, McNemer's χ^2 test, and kappa statistics were calculated for individual- and pen-level data, and the concordance correlation coefficient (CCC) was used to compare herd-level data (Stata/SE v9.2, StataCorp, College Station, TX). The McNemer's χ^2 was used to determine whether pens or pigs were significantly more likely to be test positive on one test or the other[29]. Analyses were done separately for results at ELISA cutoff values of OD≥20% and OD≥40%, as

well for sample positive control (S/P) values, which were calculated by using the formula of (sample mean) — (negative control mean) / (positive control mean) — (negative control mean). Agreement between tests beyond what is expected by chance was assessed by interpreting the kappa coefficient: <0.2 = slight agreement; 0.2–0.4 = fair; 0.4–0.6 = moderate; 0.6–0.8 = substantial; and >0.8 = almost perfect agreement[29]. The results for culture of pooled pen fecal samples and then individual fecal samples were compared to the corresponding ELISA results for the blood sample taken from that pen or pig. Culture results were also compared to corresponding RT-PCR results for both pen and individual samples. The CCC, which is robust with as few as 10 pairs of observations[30], was used to compare the proportion of culture-positive results at the herd-level to the proportion of positive ELISA results in each herd. The CCC was calculated separately for nursery pigs, individual grow-to-finish pigs, and grow-to-finish pens at ELISA OD≥20% and at ELISA OD≥40%.

The culture and ELISA results were also examined to test the hypothesis that the ELISA would be more likely to detect some *Salmonella* serogroups than others. A generalized linear mixed model was specified using the GLLAMM command in Stata (Stata/SE v9.2, StataCorp, College Station, TX), with a random intercept to account for clustering of samples within herd, in order to determine whether significantly more ELISA-positive samples were detected in pens or pigs that were *Salmonella*-positive with isolates belonging to serogroups B, C1 or D than other serogroups.

A latent-class Bayesian approach was used to estimate the Se and Sp of bacterial culture (Se_c, Sp_c), RT-PCR (Se_p, Sp_p), and the mix-ELISA (Se_E, Sp_E)[9]. A three-test, two-population

model with dependence between culture and RT-PCR was specified using the WinBUGS software (http://www.mrc-bsu.cam.ac.uk/bugs/). Since this model lacks identifiability (degrees of freedom are less than the number of parameters being estimated), informative prior information were required for at least some of the component parameters[31]. The priors used were the mean values and standard deviations (s.d.) generated through a systematic review of primary research evaluating these tests in various pig populations (Chapter 2). Prior information for Sp_c was set at a value approaching 100%, as all isolates were confirmed by serotyping, and prior Se_c was specified as having a mode of 56% with 95% certainty value greater than 36%. It was also necessary to specify prior information for Sp_p in order to permit estimation of the covariance between culture and RT-PCR Se's and Sp's. Since preliminary analysis showed nearly perfect agreement between culture and RT-PCR, we chose a prior value for Sp_p approaching the same value as for Sp_c but with a broader confidence interval which reflected the increased uncertainty regarding this parameter; hence Sp_p was specified as having a mode of 98% with 95% certainty value greater than 90%.

Models were run for 4 datasets distinguished by the ELISA results used in each model: pen-level data at ELISA OD≥20% and at ELISA OD≥40%, and individual-level data at ELISA OD≥20% and at ELISA OD≥40%. For each dataset, an initial model was run using non-informative prior information for Se_p, Se_E, and Sp_E (Table 4.1, Model A). Two additional models were run in order to assess the potential influence of the prior distribution on posterior parameter estimates; one model specified informative prior distributions for Se_p (Model B), while the other model specified informative prior distributions for Se_E and Sp_E as well as Se_p (Model C). Because preliminary analysis showed near perfect agreement between culture and RT-PCR, the

prior used for Se_c was also specified for Se_p. For the ELISA, the priors used were Se_E: mode 48% with 95% certainty value greater than 24%; Sp_E: mode 72% with 95% certainty value greater than 30%. The values for the Se's and Sp's were used to approximate the prior mode and 95% confidence intervals required to compute β distributions using the Beta Buster software (http://www.epi.ucdavis.edu/ diagnostictests/ betabuster.html). Measures of variability were limited to ± 1 s.d.. Non-informative priors were used for the *Salmonella* prevalence in both populations 1 and 2 in all models. The results of the three-test, two-population model with dependence between culture and RT-PCR were also compared to a two-test, two population conditional independence model for culture and ELISA alone[9] (Table 4.1, Models D and E).

Some studies indicate that *Salmonella* prevalence may be associated with herd size[32, 33]. Therefore, to address the assumption of different population prevalences, herds were divided into two populations based on herd size: population 1 consisted of 5 herds with fewer than 250 breeding females per herd (mean= 195, range 130 to 240); population 2 consisted of 5 herds with more than 400 breeding females per herd (mean= 866, range 426 to 2070).

Inferences were based on 50,000 iterations after discarding an initial burn-in of 5,000 iterations. Convergence was assessed by running multiple chains from dispersed starting values[34], checking the standard errors, and visually checking of the kernel density and trace plots for each parameter. Model fit was determined by estimation of the Baye's P-value, with values distant from the extremes of 0 and 1 indicating good model fit[35]. Analyses were also run separately for each population to verify the assumption of constant Se and Sp across populations.

The relative Se of culture, as compared to the Svanovir ELISA , and the relative Se and Sp of the Svanovir ELISA, as compared to culture, were also computed using conventional methods and then compared to the above results obtained via the Bayesian method. The cross-tabulated data used in these analyses are shown in Table 4.2.

4.3 Results

4.3.1 Agreement between tests

Nursery pigs: At total of 254 pens were sampled. The mean age of pigs in these pens was 6.6 weeks (range 3-11 weeks). The proportions of samples positive by each test and serovar distribution are shown in Tables 4.2 and 4.3. The proportion positive at the pen level by either culture or the Svanovir ELISA differed significantly for both OD≥20% (P=0.02) and OD≥40% (P<0.001). There was also a significant difference in the proportion positive by either culture or RT-PCR (P=0.02); this difference was primarily due to false negative RT-PCR results for 25% (6/24) of *S.* Typhimurium var. Copenhagen and 20% (3/15) of *S.* Mbandaka culture-positive samples. The kappa test showed almost no agreement between the Svanovir ELISA and either culture (OD≥20%: κ=0.02, standard error [s.e.] = 0.03; OD≥40%: κ = 0.001, s.e. 0.02; S/P:) or RT-PCR (OD≥20%: κ = 0.02, s.e. 0.04; OD≥40%: κ = 0.004, s.e. 0.02). Results for S/P ratios were identical to results using OD≥40%. Agreement between culture and RT-PCR was excellent (κ = 0.88, s.e. 0.03). There was also no concordance between the proportions of nursery pig samples that were culture positive and samples that were ELISA positive in each herd (CCC OD≥20%: -0.011, s.e. 0.034; OD≥40%: 0.013, s.e. 0.017).

Because there was no agreement between either bacterial culture or RT-PCR and the Svanovir ELISA in samples from nursery pigs, this dataset was not examined further. All final analyses of test performance parameters were limited to data from grow-to-finish pigs only.

Grow-finishers: A total of 295 pens were sampled. The mean age of pigs in these pens was 16.5 weeks (range 8-27 weeks). The proportions of samples positive by each test and serovar distribution are shown in Tables 4.2 and 4.3. Seventy-two percent (84/117) of *Salmonella* isolates from pooled feces and 74% (55/77) of isolates from individual feces belonged to serogroups B, C1 and D. Corresponding ELISA OD≥20% results for B, C1 and D culture-positive samples were 50% (42/84) ELISA-positive for pen samples and 63% (34/54) for individual samples. ELISA OD≥40% results were 31% (10/84) and 41% (22/54) ELISA-positive for pen and individual samples, respectively. The Svanovir ELISA (at either OD≥20% or OD≥40%) was not more likely to detect seropositive pigs if the corresponding pooled or individual fecal sample was positive for serogroups B, C1 and D than if other serogroups were present (P=0.18 and P=0.22 for pooled and individual samples, respectively).

The proportion positive at the pen level by either the Svanovir ELISA or culture differed significantly (OD≥20%: P=0.02; OD≥40%: P<0.001); similar difference were found between the ELISA and RT-PCR (OD≥20%: P=0.04; OD≥40%: P<0.001). There was no significant difference in the proportion positive between culture and RT-PCR (P=0.32). The kappa statistic showed only fair agreement between the Svanovir ELISA and either culture (OD≥20%: κ=0.31, s.e. = 0.06; OD≥40%: κ = 0.26, s.e. 0.05) or RT-PCR (OD≥20%: κ = 0.31, s.e. = 0.06; OD≥40%: κ = 0.27, s.e. 0.05). Results for S/P ratios were identical to results using OD≥40%.

Agreement between culture and RT-PCR was almost perfect (κ = 0.97, s.e. 0.06). The concordance between the proportion of culture positive pen-samples and the proportion of positive ELISA results for each herd was 0.59 (s.e. 0.20, *P*=0.003) at OD≥20% and 0.49 (s.e. 0.16, *P*=0.002) at OD≥40%.

Similar results were obtained at the individual level. The proportion positive by either the Svanovir ELISA or culture differed significantly (OD≥20%: *P*=0.03; OD≥40%: *P*=0.003) and RT-PCR (OD≥20%: *P*=0.04; OD≥40%: *P*=0.002). The kappa statistic again showed only fair agreement between the Svanovir ELISA and either culture (OD≥20%: κ = 0.38, s.e. = 0.06; OD≥40%: κ = 0.36, s.e. 0.06) or RT-PCR (OD≥20%: κ = 0.39, s.e. = 0.06; OD≥40%: κ = 0.35, s.e. 0.06). Results for S/P ratios were identical to results using OD≥40%. Agreement between culture and RT-PCR was almost perfect (κ = 0.92, s.e. 0.06). Herd-level concordance between the proportions of positive individual animal culture and ELISA results was 0.64 (s.e. 0.20, *P*=0.001) at OD≥20% and 0.62 (s.e. 0.15, *P*<0.000) at OD≥40%.

4.3.2 Estimation of Se and Sp

Individual pigs were considered *Salmonella* positive if they were test positive by either culture, RT-PCR or ELISA. Division into 2 populations according to herd size resulted in apparent population prevalences of 24-36% and 41-47% for populations 1 and 2, respectively, depending on ELISA cutoff value. At the pen level, apparent population prevalences were 32-42% and 53-59% for populations 1 and 2, respectively. The results of the individual population analyses showed that the accuracy of each test was similar in the two populations examined, thus

satisfying the assumption of constant test accuracy across populations (data not shown). Posterior Bayesian estimates of population prevalences are shown in Table 4.1.

The Bayesian estimates of Se and Sp for each test were determined using the raw data summarized in Table 4.4. Estimation of Se and Sp using S/P ratios was not done, since these data were considered equivalent to data at OD≥40% (due equivalent kappa statistics). Convergence was achieved after 50,000 iterations in all models. Bayesian P-values for the goodness-of-fit statistic ranged from 0.10 to 0.36 for all models (Table 4.1), indicating that there was no substantial lack of fit in these models. A small but significant covariance (cov[Se] = 0.16-0.19, s.d. 0.023-0.046) between Se_c and Se_p was detected. No significant covariance was detected between Sp_c and Sp_p. The resulting posterior (mean) estimates from each model at both ELISA OD≥20% and ≥40% for the pen data and individual data are shown in Table 4.1. The posterior estimates for Se_c, Se_p and Sp_E were somewhat dependant on prior information since estimates differed slightly when informative priors were used for Se_p and Se_E. Posterior estimates for Se_c and Se_p decreased by 5% when informative priors were specified for Se_p, while posterior Sp_E increased by 6-8% when informative ELISA priors were used. However, posterior estimates for Sp_c, Sp_p and Se_E remain essentially unchanged between models. The influence of posterior information is most noticeable in the two-test Model D, whereby specification of an uninformative prior for Se_c resulted in posterior Se_c estimates 14-17% greater than the other models.

Relative Se_p and Sp_p, as compared to culture and estimated according to traditional methods, were excellent, ranging from 95-97% and 98-99% for Se_p and Sp_p, respectively (Table

4.5). The estimates of relative Se_c, Se_E and Sp_E were similar to results obtained from the Bayesian analysis.

4.4 Discussion

The results from the Bayesian analysis were similar to results obtained from traditional estimates of Se and Sp. One discrepancy between the two methods was seen in the resulting estimates for Se_c, with the Bayesian posterior estimates being 14-17% higher when an uninformative prior for Se_c was used in the two-test model (Model D) than that obtained via the traditional method. Another difference between the two approaches was that the Bayesian approach eliminated some of the variability in the estimates associated with the choice of ELISA cutoff value. While estimates for Se_c obtained via the traditional approach varied by about 12% depending on ELISA cutoff value, the posterior Bayesian estimates for Se_c within each model were constant regardless of cutoff value, and corresponded closely to traditional estimates at cutoff OD≥40%. This effect was also apparent in the posterior estimates for *Salmonella* prevalence in each population, where prevalences within models were constant regardless of cutoff value.

The estimates of Se for culture and the Se and Sp of serological tests for *Salmonella* in pigs as reported in the literature vary widely, and have been summarized in a recent systematic review (Chapter 2). This review provided prior estimates for these parameters, but these were rather diffuse even when limited to ±1 s.d. of the reported mean values. In general, the posterior estimates resulting from the Bayesian analysis corresponded closely with those obtained via traditional analysis with culture considered the gold standard, and were not greatly influenced by

the informative priors specified in this study. Mainar et. al.[5] also reported that posterior estimates from the Bayesian comparison of two ELISAs were not heavily influenced by the informative priors used and, although not reported in that paper, evaluation of the cross-tabulated raw data provided showed traditional estimates also corresponded closely with the Bayesian analysis. While their study applied Bayesian methods to compare the accuracy of the Svanovir and HerdCheck (Idexx Laboratories, USA) ELISAs in slaughter pigs in Saskatchewan, Canada, it is interesting to note their posterior estimate for the Se of the Svanovir ELISA in individual pigs, at a cutoff value of OD≥20% (63%), was almost identical to the results obtained in the current study. ELISA Sp was also similar to our results (89%).

Most often, the comparison of culture and serology is performed on samples taken from pigs at slaughter, and in these cases the agreement or correlation between culture and serology at the pig level has been low. This could be at least partially attributed to transient or recent infection acquired during lairage[36, 37]; however, when sampling on-farm, the lairage factor is circumvented. Farm-based studies are fewer than abattoir-based studies, and in these studies agreement or correlation has also found to be only poor to fair, particularly at the pig level[6, 14, 38]. In the current study, the agreement between bacterial culture of feces and serum ELISA at either the pig or pen level was likewise only fair, although agreement was slightly better at the individual level than at the pen level (κ = 0.36-0.38 vs κ = 0.26-0.31, respectively). Another study in Canadian finishing pigs reported better agreement between these two tests at the individual-level[39]; that study reported ELISA results as S/P ratios, which are not directly comparable to the OD% cutoff values reported here. Because only 66 samples from 2 farms were used in that study,

the expected confidence intervals for estimates of kappa, Se and Sp would, however, be wider than those for the current study.

Somewhat better agreement between culture and serology has been reported by other studies using farm- or cohort-level comparisons or with longitudinal sampling[38, 40, 41]. Farm-level correlation between culture and serology in the current study (CCC 0.49-0.64) was similar to that reported by a recent study in Alberta finishing pigs (correlation = 0.47, farm visit level)[40]. Our finding that the agreement between culture and the Svanovir ELISA was better at the farm-level than at the individual pig-level was also similar to another recent study comparing two commercial ELISAs to culture[6]. The results of this study parallels existing research which indicate serological results are not a reliable indicator of shedding or active infection in the individual pig[5, 42-44]. Rather, serology is best applied at the herd level as a tool for screening on-farm exposure to *Salmonella* infection, as is currently done in some *Salmonella* monitoring and control programs[1, 45].

The Svanovir ELISA detects antibodies against serogroups B, C1, or D, and should detect immunological response these serogroups, but in the current study this ELISA (at OD≥20%) only detected 50% of pens and 63% of pigs positive for these serogroups. Furthermore, the Svanovir ELISA detected similar proportions of pens and individuals which were culture-positive for serogroups other than B, C1 and D. Other researchers have also reported similar observations[5]. There are a number of potential reasons for these discrepancies. First, it is possible that animals were transiently, or recently, infected with serogroups B, C1 or D and consequently the Svanovir ELISA would not be expected to detect infection in these animals[46]. Secondly, infected pigs shed

Salmonella intermittently[36, 47] thus even those animals which harbor the organism and which have mounted an immune response would inconsistently be culture-positive. Thirdly, animals may have mounted an immune response against B, C1 or D and subsequently been infected with a serovar from another serogroup which was detected at the time of testing. While the Svanovir ELISA incorporates antigens which represent 90% of serovars isolated in Denmark and The Netherlands[48-50], within Canada serovar distribution can be quite variable and different than the European situation[6, 51, 52]. As shown in this study, a large portion of pigs which were culture positive for *Salmonella* from serogroups B, C1 and D may not be detected by the Svanovir ELISA. For this reason, it is important to recognize and quantify the limitations of this test in the populations to which it is applied.

The RT-PCR used in this study has been previously evaluated in a variety of different sample matrices, including the fecal samples used in the current study, and the results have been described elsewhere[27]. When compared to culture alone, the agreement of the RT-PCR was nearly perfect, and Se_p and Sp_p, calculated by traditional methods, were reported to be excellent. The excellent agreement between the two tests was confirmed in the current analysis. In the Bayesian analysis, posterior estimates of Se_p and Sp_p corresponded closely with that of culture. Although, in general, there are relatively few studies published evaluating the performance of PCR (as compared to culture) to detect *Salmonella* in pig feces, the available information indicates that PCR of pre-enriched samples tends to be quite sensitive[15, 53-55]. Specificity, however, may be more variable. PCR does not differentiate between viable and non-viable *Salmonella*, and this could potentially impact Sp_p[56]. PCR-positive samples should be verified through bacterial culture in order to confirm *Salmonella* prevalence and serotype distribution.

Identification of presumptive colonies of *Salmonella* is the most time, labor and cost intensive part of *Salmonella* culture; therefore, the use of broth-enriched PCR as a screening tool may improve time and cost effectiveness, particularly when prevalence is low[57].

Initially, the objective of this study was to evaluate culture, ELISA and RT-PCR in both nursery and in grow-to-finish pigs; however, there were not enough seropositive nursery pigs detected to allow the analysis to be done at this production level even though the prevalence based on culture positive was 31%. Maternal antibodies can be detected in piglets in the first few weeks of life[14], but this passive protection decreases after a few weeks and the piglets become susceptible to infection. Active serological response to *Salmonella* infection has been detected starting at 5-8 weeks of age, with most seroconversion occurring after 10 weeks of age[14, 38, 58]. Even though a significant portion of nursery pigs were found to be shedding *Salmonella*, it is likely that most of the nursery pigs examined in our study were too young to have been able to mount a detectable immune response. Serology for *Salmonella* is not useful in nursery pigs, and, therefore, efforts to monitor *Salmonella* status in these animals would use bacterial culture, PCR or antigen-capture ELISA assays.

This is the first study conducted in western Canada in which both traditional and Bayesian statistical approaches were used to evaluate the accuracy of culture, RT-PCR and the Svanovir ELISA to detect on-farm *Salmonella* status of pigs. The difference between Bayesian and traditional approaches may be of only theoretical interest if both approaches lead to similar findings, as was the case in this study. Still, the Bayesian approach eliminated variability in estimates for Se_c, Se_p and population prevalences associated with varying ELISA cutoff values.

Also, posterior Bayesian estimates of Se_c corresponded more closely with the higher traditional estimates, at ELISA OD≥40. The ability of the culture protocol used in this study to detect *Salmonella*-positive fecal samples was enhanced by the use of multiple enrichment and selective media steps run in parallel[59], and the use of 10 g of fecal material[60, 61]. The excellent agreement between culture and RT-PCR results, and the posterior estimates for Se_c obtained via the Bayesian analysis, provides evidence that this combination of sampling and testing protocols results maximizes that ability to identify *Salmonella* when it is present in pig feces in detectable amounts.

When considering the *Salmonella* status of pigs, pens or herds given the results of both culture (or RT-PCR) and ELISA, the latent class would be a mixture of exposure and infection. Branscum et al cautions "the latent class is unclear for an acute infectious disease", singling out salmonellosis as an acute infectious disease for which latent class analysis of antigen-antibody test comparison is inappropriate[9]. However, sub-clinical *Salmonella* infection in pigs is not an acute infectious disease, but rather a persistent, chronic infection within herds which are comprised of individual pigs with various shedding/infection status[11]. Evaluation of culture or PCR via comparison with an antigen-capture ELISA (and vice-versa) in sub-clinically infected pigs is often reported in the literature even though the interpretation of results is difficult. Primarily, the difficulty arises because the tests are potentially measuring different time points in the process of exposure, infection, and immunity for that pig. In the case of culture or PCR, current shedding of the organism is being detected; ELISA, on the other hand, is detecting previous exposure to *Salmonella* as indicated by seroconversion. Within the context of the Bayesian model which evaluates these tests together, the latent class becomes "ever exposed"

(culture, RT-PCR or ELISA positive) or "never exposed" (culture, RT-PCR and ELISA negative), and reflects the information that the pig provides regarding the potential for contamination in its environment. This has important implications for *Salmonella* monitoring and control programs which focus on identifying high-risk farms, defined by within-herd *Salmonella* prevalence. Bayesian methods provide the advantage over traditional methods by allowing estimation of test parameters without having to designate either test as the gold standard and allowing incorporation of prior knowledge of test parameters and provide a viable alternative for comparing tests for *Salmonella* in pigs. A recent study has reported the use of these methods to evaluate the herd-level Se and Sp of culture and ELISA for *Salmonella* in pigs[62]; however, to the author's knowledge, this is the first study reporting the use of Bayesian statistical methods to compare the accuracy of bacterial culture, RT-PCR and ELISA at the individual level.

The results of this study, combined with other recent research, provides evidence that, when comparing bacterial culture and the Svanovir ELISA under field conditions, the ELISA (at a cutoff of OD≥20% and for individual pigs) has a mean Se of 62-65% and a mean Sp of 80-90% within the Canadian pig population, while the Se_c of this protocol is 70-75% at the pen level and 65-71% for individuals. This has important implications for use in risk analysis and as prior information for future Bayesian approaches for studying the diagnostic accuracy of tests for *Salmonella* in pigs. The accuracy of RT-PCR, as compared to culture, was found to be excellent, further supporting the use of this test as an alternative tool for detecting or screening for *Salmonella* in pigs. Bacterial culture, however, is still an essential requirement when information on serovar distribution or the extent of antimicrobial resistance is desired.

4.5 References

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Table 4.1: Bayesian model estimates of pig-level Se and Sp of culture, RPCR and Salmotype ELISA for detecting *Salmonella* infection in grow-finish pigs from 10 farms in Alberta and Saskatchewan

	Model A[a*]		Model B[b]		Model C[c]		Model D[d]		Model E[e]	
	Estimate	(s.d.)	Estimate	(s.d.)	Estimate	(s.d.)	Estimate	(s.d.)	Estimate	(s.d.)
Pen-level:										
ELISA OD% ≥20%:										
Se_c	0.75	(0.072)	0.70	(0.062)	0.72	(0.059)	0.89	(0.085)	0.72	(0.071)
Se_p	0.74	(0.073)	0.69	(0.061)	0.70	(0.058)				
Se_E	0.47	(0.048)	0.47	(0.048)	0.46	(0.046)	0.50	(0.051)	0.48	(0.052)
Sp_c	0.99	(0.004)	0.99	(0.004)	0.99	(0.004)	0.97	(0.025)	0.97	(0.027)
Sp_p	0.98	(0.009)	0.98	(0.009)	0.98	(0.009)				
Sp_E	0.85	(0.043)	0.87	(0.045)	0.93	(0.037)	0.82	(0.037)	0.86	(0.045)
Prev Pop1 [g]	0.35	(0.065)	0.37	(0.068)	0.36	(0.060)	0.27	(0.059)	0.35	(0.072)
Prev Pop2 [h]	0.63	(0.068)	0.67	(0.067)	0.66	(0.067)	0.55	(0.068)	0.64	(0.073)
Bayes P-value	0.35		0.28		0.31		0.28		0.11	
Cov(Se)	0.17	(0.037)	0.19	(0.026)	0.19	(0.026)				
Cov(Sp)	0.006	(0.004)	0.006	(0.004)	0.006	(0.004)				
ELISA OD% ≥40%:										
Se_c	0.75	(0.069)	0.75	(0.069)	0.72	(0.060)	0.90	(0.080)	0.73	(0.071)
Se_p	0.74	(0.071)	0.74	(0.071)	0.71	(0.060)				
Se_E	0.29	(0.043)	0.31	(0.043)	0.28	(0.041)	0.31	(0.048)	0.29	(0.046)
Sp_c	0.99	(0.004)	0.99	(0.004)	0.99	(0.004)	0.96	(0.029)	0.97	(0.027)
Sp_p	0.99	(0.004)	0.99	(0.006)	0.99	(0.009)				
Sp_E	0.95	(0.025)	0.92	(0.019)	0.92	(0.025)	0.94	(0.023)	0.95	(0.025)
Prev Pop1	0.34	(0.060)	0.36	(0.062)	0.34	(0.057)	0.26	(0.058)	0.33	(0.068)
Prev Pop2	0.63	(0.070)	0.66	(0.071)	0.65	(0.070)	0.54	(0.067)	0.64	(0.077)
Bayes P-value	0.33		0.28		0.28		0.36		0.16	
Cov(Se)	0.17	(0.036)	0.19	(0.027)	0.18	(0.023)				
Cov(Sp)	0.006	(0.004)	0.006	(0.004)	0.006	(0.004)				
Individual:	Estimate	(s.d.)	Estimate	(s.d.)	Estimate	(s.d.)	Estimate	(s.d.)	Estimate	(s.d.)
ELISA OD% ≥20%:										
Se_c	0.71	(0.084)	0.65	(0.068)	0.67	(0.065)	0.82	(0.13)	0.66	(0.086)
Se_p	0.74	(0.097)	0.67	(0.070)	0.69	(0.067)				
Se_E	0.62	(0.059)	0.61	(0.060)	0.59	(0.056)	0.64	(0.072)	0.63	(0.074)

Table 4.1 (continued)

	Model A[a*]		Model B[b]		Model C[c]		Model D[d]		Model E[e]	
	Estimate	(s.d.)	Estimate	(s.d.)	Estimate	(s.d.)	Estimate	(s.d.)	Estimate	(s.d.)
Sp_c	0.99	(0.005)	0.99	(0.005)	0.99	(0.005)	0.97	(0.022)	0.97	(0.023)
Sp_p	0.98	(0.011)	0.98	(0.012)	0.98	(0.012)				
Sp_E	0.85	(0.040)	0.86	(0.041)	0.84	(0.033)	0.83	(0.044)	0.87	(0.048)
Prev Pop1 [f]	0.22	(0.059)	0.25	(0.061)	0.23	(0.053)	0.20	(0.067)	0.27	(0.074)
Prev Pop2 [g]	0.43	(0.062)	0.48	(0.060)	0.45	(0.059)	0.37	(0.071)	0.43	(0.071)
Bayes P-value	0.13		0.10		0.10		0.33		0.19	
Cov(Se)	0.16	(0.049)	0.19	(0.028)	0.18	(0.029)				
Cov(Sp)	0.004	(0.003)	0.004	(0.003)	0.004	(0.003)				
ELISA OD% ≥40%:										
Se_c	0.71	(0.081)	0.65	(0.066)	0.68	(0.064)	0.82	(0.12)	0.67	(0.077)
Se_p	0.73	(0.093)	0.66	(0.067)	0.70	(0.065)				
Se_E	0.39	(0.057)	0.38	(0.057)	0.38	(0.054)	0.42	(0.072)	0.41	(0.071)
Sp_c	0.99	(0.005)	0.99	(0.005)	0.99	(0.005)	0.97	(0.025)	0.97	(0.024)
Sp_p	0.98	(0.012)	0.98	(0.023)	0.98	(0.012)				
Sp_E	0.95	(0.023)	0.96	(0.018)	0.93	(0.021)	0.94	(0.025)	0.96	(0.023)
Prev Pop1	0.22	(0.053)	0.24	(0.053)	0.22	(0.047)	0.19	(0.058)	0.24	(0.061)
Prev Pop2	0.44	(0.065)	0.47	(0.064)	0.45	(0.061)	0.37	(0.070)	0.43	(0.071)
Bayes P-value	0.27		0.23		0.21					
Cov(Se)	0.16	(0.046)	0.19	(0.027)	0.18	(0.030)				
Cov(Sp)	0.004	(0.004)	0.004	(0.004)	0.004	(0.004)	0.52		0.39	

*Models A, B and C specified with prior information for Se_c: mode 57% with 95% certainty value greater than 37%; Sp_c: mode 99% with 95% certainty value greater than 98%; Sp_c: mode 98% with 95% certainty value greater than 90%. Models with varying prior information for Se_E, Sp_E, and Se_p are detailed below.

[a] Model A: 3-test model with conditional dependence between culture and PCR: informative priors for Se_c, Sp_c, and Sp_p as above; other priors non-informative e.g. $\beta(1,1)$.

[b] Model B: model as in (A), but with informative prior for Se_p mode 57% with 95% certainty value greater than 37%.

[c] Model C: model as in (B), but with informative priors for ELISA - Se_E: mode 48% with 95% certainty value greater than 24%; Sp_E: mode 72% with 95% certainty value greater than 30%.

[d] Model D: 2-test conditional independence model, informative prior for Sp_c only, mode 99% with 95% certainty value greater than 98%; all other priors non-informative e.g. $\beta(1,1)$

[e] Model E: model as in (D), but with informative priors for Se_c: mode 57% with 95% certainty value greater than 37%.

[f] herds with <250 breeding females [g] herds with >400 breeding females

Table 4.2. Results of bacterial culture, RT-PCR and the Svanovir ELISA used to detect sub-clinical *Salmonella* infection in nursery and grow-to-finish pens and pigs from 10 farms in Alberta and Saskatchewan.

	ELISA 20%		ELISA 40%		RT-PCR	
	+	-	+	-	+	-
Pen-level nursery pig:						
Culture +	3	77	1	79	69	11
Culture –	5	162	2	165	2	172
RT-PCR +	3	67	1	69	-	-
RT-PCR –	5	171	2	174	-	-
Pen-level grow-finish:						
Culture +	55	57	34	78	110	3
Culture –	35	147	13	169	1	181
RT-PCR +	54	56	34	76	-	-
RT-PCR –	36	148	13	171	-	-
Individual-level grow-finish:						
Culture +	45	28	29	44	69	4
Culture –	44	176	17	203	5	216
RT-PCR +	46	28	29	45	-	-
RT-PCR –	43	176	17	202	-	-

Table 4.3.The number of nursery and grow-to-finish pens or pigs on 10 farms in Alberta and Saskatchewan, for which *Salmonella* serotypes were isolated from feces and for which the RT-PCR and ELISA (at OD≥20% and OD≥40%) were positive.

Serotypes	Serogroups	# pens or pigs +	RT-PCR +	$ELISA_{OD20}$+	$ELISA_{OD40}$+
Nursery pen samples :					
Typhimurium var. Copenhagen	B	24	18	0	0
Derby	B	6	6	1	1
Heidelberg	B	1	1	0	0
Mbandaka	C1	15	12	0	0
Ohio	C1	4	4	1	0
Infantis	C1	4	3	0	0
Livingstone var. 14+	C1	3	3	0	0
Anatum	E1	3	2	0	0
Give	E1	2	2	1	0
Putten	G2	6	5	0	0
Untypeable spp.	na	15	15	0	0
Grow-finish pen samples :					
Derby	B	48	46	26	18
Typhimurium var. Copenhagen	B	19	19	8	3
Mbandaka	C1	8	7	3	3
Ohio	C1	1	1	0	0
Infantis	C1	7	7	5	2
Enteritidis	D1	1	1	0	0
Anatum	E1	5	4	2	2
Lexington var. 15+	E1	1	1	0	0
Give var. 15+	E1	1	1	0	0
Give	E1	1	1	0	0
Rubislaw	F	1	1	1	1
Putten	G2	14	14	10	5
Untypeable spp.	na	10	10	4	3
Grow-finsih individual samples:					
Derby	B	33	31	18	12
Typhimurium var. Copenhagen	B	9	8	5	2
Typhimurium	B	3	3	3	2
Infantis	C1	6	6	6	4
Mbandaka	C1	4	3	2	2
Anatum	E1	2	1	1	1
Give	E1	1	1	1	1
Putten	G2	11	8	5	3
Untypeable spp.	na	8	8	6	4

Table 4.4. Three-way classification of test results for culture, RT-PCR and Svanovir ELISA (OD≥20% and OD≥40%) for the detection of sub-clinical *Salmonella* infection in grow-to-finish pigs from 10 farms in Alberta and Saskatchewan.

	Population1[a], $ELISA_{OD20\%}$	Population2[b], $ELISA_{OD20\%}$	Population1, $ELISA_{OD40\%}$	Population2, $ELISA_{OD40\%}$
Pen-level result[c]:				
+ + +	20	34	11	23
+ + –	16	39	25	50
+ – +	0	1	0	0
+ – –	0	2	0	3
– + +	0	0	0	0
– + –	0	1	0	1
– – +	24	11	10	3
– – –	84	62	98	70
Total	144	150	144	150
Individual result[a]:				
+ + +	16	29	10	19
+ + –	5	19	11	29
+ – +	0	0	0	0
+ – –	3	1	3	1
– + +	0	1	0	0
– + –	0	4	0	5
– – +	27	16	10	7
– – –	92	80	109	89
Total	143	150	143	150

[a] herds with <250 breeding females
[b] herds with >400 breeding females
[c] order of test results: culture; RT-PCR; ELISA

Table 4.5. Traditional estimates of pen- and pig- level Se and Sp of culture, Svanovir ELISA and RT-PCR for detecting *Salmonella* infection in grow-to-finish pigs from 10 farms in Alberta and Saskatchewan

Index test	Reference test	Se (%)	Se $CI_{95\%}$	Sp (%)	$SpCI_{95\%}$
Pen-level					
Culture	$ELISA_{OD20\%}$	61	(50, 71)	100[a]	--
Culture	$ELISA_{OD40\%}$	72	(57, 84)	100[a]	-
RT-PCR	Culture	97	(92, 99)	99	(97, 100)
$ELISA_{OD20\%}$	Culture	49	(40, 59)	81	(74, 86)
$ELISA_{OD40\%}$	Culture	30	(22, 40)	93	(88, 96)
Pig-level					
Culture	$ELISA_{OD20\%}$	51	(40, 61)	100[a]	--
Culture	$ELISA_{OD40\%}$	63	(48, 76)	100[a]	--
RT-PCR	Culture	95	(85, 98)	98	(95, 99)
$ELISA_{OD20\%}$	Culture	62	(49, 73)	80	(74, 85)
$ELISA_{OD40\%}$	Culture	40	(29, 52)	92	(88, 95)

[a] Culture Se assumed 100%

CHAPTER 5

DISTRIBUTION OF *SALMONELLA* SEROVARS IN BREEDING, NURSERY, AND GROW-TO-FINISH PIGS, AND RISK FACTORS FOR SHEDDING IN TEN FARROW-TO-FINISH SWINE FARMS IN ALBERTA AND SASKATCHEWAN

5.1 Introduction

Salmonella is an important challenge to the swine industry worldwide because of its implications for public health. Salmonellosis in humans results in high societal costs that include medical related expenses, losses associated with reduced or lost work productivity, and other costs[1, 2]. Although in North America pork is not considered a major source for human salmonellosis, *Salmonella* in pigs has become an important research priority over the past decade, primarily as a result of extensive implementation of *Salmonella* surveillance or monitoring programs in Denmark and other European countries. In Canada, Quebec has extensively investigated *Salmonella* in pigs[3, 4], and recently initiated a provincial control program for *Salmonella* in pigs. Relatively large baseline studies were conducted in Ontario and Alberta[5, 6], where approximately 40-60% of finishing pig farms were *Salmonella* positive with the overall number of positive samples ranging from 11% (individual pigs) to 14-18% (pooled samples). A national, abattoir-based baseline study reported an overall pig carcass contamination prevalence of 4.2% [7], demonstrating that *Salmonella* carcass contamination rates within Canada are low when compared to the number of positive animals. Still, further improvement is needed and additional research into the epidemiology of *Salmonella* at both the farm and abattoir levels within Canada is required.

Pig production systems differ substantially among countries[8] and within Canada, among provinces and regions[5]. In Canada, limited research has been conducted on the epidemiology of

Salmonella in pigs, and the research that has been done to date has focused primarily on the finishing pig. Farzan et el.[6] found 46% (37/80) farms in Ontario were *Salmonella*-positive. In western Canada, Rajić et al.[5] reported that among 90 Alberta pigs finishing farms producing ≥2,000 pigs, 26% to 58% percent of farms studied were *Salmonella*-positive at any given time and had low to moderate (1-4 positive samples, average 15 samples collected per farm) within-farm prevalence. Sorensen et al.[9] examined the prevalence of *Salmonella* spp. in Alberta pigs at slaughter, reporting 35% positive cecal samples and 37 different serovars. Most recently, an examination of slaughter pigs from Saskatchewan abattoirs found 13% positive cecal samples[10]. Only one study has investigated the distribution of *Salmonella* species in various pig production phases of two integrated production systems, where prevalence ranged from 17% to 66%[3]. However, no study has investigated *Salmonella* serovar distribution throughout all phases (farrow to finish) of pig production in Western Canada. The development and implementation of *Salmonella* control programs requires knowledge of the baseline prevalence and serovar distribution in targeted pig populations within a specific region, and thorough knowledge includes investigation of the breeding herd as well as finishing pigs.

Therefore, the objectives of this study were to evaluate *Salmonella* prevalence and serovar distribution in sows, nursery and grow-to-finish pigs, and risk factors for *Salmonella* shedding, using cross-sectional sampling on 10 purposively selected farrow-to-finish pig farms in Saskatchewan and Alberta.

5.2 Materials and methods

5.2.1 Farm selection

Ten farrow-to-finish pig herds (herd size n>100 sows) from Alberta (7 farms) and Saskatchewan (3 farms) were purposely selected by swine veterinarians, based on their presumed *Salmonella* positive status (n=7) or *Salmonella* negative status (n=3), and the producer's willingness to participate in the study. Purposeful herd selection was chosen to meet the objectives of a concurrent study evaluating diagnostic tests for *Salmonella* in pigs (unpublished data). Herds were presumed positive if either the herd veterinarian or producer observed clinical salmonellosis within the previous 12 months, if *Salmonella* species were identified during routine testing, or if replacement breeding stock were purchased from known *Salmonella*-positive farms. Herds were presumed negative if none of these criteria were met. The number of herds and the number of samples used in the study was a function of logistic and financial constraints.

5.2.2 Sample collection

Each herd was visited once from May through August 2004. Samples were delivered to the laboratory either within 2 hours of leaving the farm, or held on ice overnight and delivered the following day.

Collection of individual fecal samples: On each farm, feces (minimum 10 g) were collected, from each of 10 randomly selected sows, directly from the rectum or from freshly voided feces on the floor. In the grow-to-finish area, 1 individual sample (minimum 10 g) was similarly collected from 1 pig in each of 30 different randomly selected pens. No individual fecal

samples were collected from nursery pigs as most pigs were too small to collect 10 g feces directly from the rectum.

Collection of pooled fecal samples: Twenty pooled samples were taken from the breeding phase in each herd, by collecting a minimum of 5 g of feces from 5 different sows into a single container. Samples from individual sows, as described above, were not incorporated into the pooled sample. In both nursery and grow-to-finish phases, 1 pooled pen floor fecal sample was collected from each of 30 randomly selected pens or all pens on farm if there were less than 30 pens. For each pooled pen sample a minimum of 5 g of fecal material was collected from 5 different locations on the pen floor.

5.2.3 Bacteriological Culture

Bacteriologic culture for *Salmonella* was performed by the Agri-Food Laboratories Branch (AFLB), Food Safety Division of Alberta Agriculture and Rural Development. All samples were refrigerated and cultured within 24-48 hours of receiving samples and thoroughly mixed prior to culture.

Ten grams of feces were inoculated into 90 ml of buffered peptone water (BPW) and incubated at 35°C for 20-24 hours. After incubation 0.1 ml of BPW was inoculated into 10 ml of Rappaport Vassiliadis broth (RV), which were placed into a 42°C water bath for 30 minutes, then a 42°C incubator for 22-24 hours. Simultaneously, 1 ml of BPW was inoculated into tetrathionate broth (TT) to which 0.2 ml of iodine solution had been added just prior to use, and placed in a 35°C waterbath for 30 minutes, then a 35°C incubator 22-24 hours.

After incubation 10 µl of RV and TT were streaked onto XLT4 and Rambach (RAM) selective agar plates and incubated at 35°C for 18-24 hours, then read. Plates without significant growth of suspect colonies were re-incubated and read after an additional 24 hours. At the same time, 0.3 ml of TT (0.1 ml to each of 3 sites) was inoculated onto a modified semi-solid Rappaport-Vassiliadis (MSRV) plate and incubated at 42°C for 20 – 24 hours. The halos of growth that occurred on the MSRV plates were streaked to XLT4 and RAM plates and incubated at 35°C for 24 hours. Negative plates were reincubated and read again at 48 and 72 hours. Suspect colonies were screened using triple sugar iron agar slants, urea agar slants and lysine iron agar slants and plated to a blood agar plate and MacConkey plate to check for purity then tested with *Salmonella* Poly O and Poly O1 antisera agglutination (Denka Seiken Co. Ltd., Japan). Unusual or atypical reacting suspect colonies were further tested using Vitek GNI or API-20E (bioMerieux Vitek, MO, USA). All isolates were frozen at –70°C then forwarded to the Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, for confirmation by serotyping.

5.2.4 Serotyping and phagetyping

One isolate per each *Salmonella*-positive sample, or 2 isolates if they were morphologically distinct, was sent for serotyping and phagetyping at the OIE Reference Laboratory for Salmonellosis, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON. The serotyping and phagetyping techniques followed standard procedures and have been previously described (Chapter 4).

5.2.5 Data collection

During sampling, the primary investigator observed and recorded pen and pig information; sex and age of each individual pig sampled; number of pigs in pen, area and pig density; floor and wall type, and cleanliness of each pen; feed type and feeding method; nose-to-nose contact between pigs through pen separations, and feces characteristics. A list of variables and their distribution is shown in Tables 5.1 and 5.2.

5.2.6 Statistical Analysis

The pig and pen were *Salmonella*-positive if the fecal sample collected from that pig or pen tested positive. Descriptive statistics were summarized and statistical models were developed using a commercial software program (Stata/SE v9.2, StataCorp, College Station, TX). Generalized linear mixed models, with a random intercept to account for clustering of individual and pen samples within herd, were used to: (1) examine the difference in *Salmonella* shedding among production phases and the associations between pen-level variables and *Salmonella* shedding; (2) estimate the proportion of variance of *Salmonella* shedding attributable to each production level; (3) compare *Salmonella* recovery from pooled fecal vs. individual samples collected from grow-finish pigs; and (4) describe the differences in serovar-specific prevalence among the various production phases. All models used a logit link function, binomial distribution and an exchangeable correlation structure.

Risk factor analysis was limited to pooled fecal samples to minimize potential bias introduced by different sampling strategies among production phases and because individual samples were only available from two of three phases. In the first step, the unconditional

association between each potential risk factor and whether or not the pooled fecal culture was positive for *Salmonella* was evaluated. All variables with an unconditional P-value of less than 0.20 were evaluated for inclusion in a multivariable model using a manual forward-stepwise process. Risk factors were defined as confounders if removing or adding the factor changed the effect estimate by more than 20%[11]. Variables with P<0.05 were considered statistically significant. Biologically reasonable first-order interaction terms were examined where more than one significant risk factor was identified in the final main effects model. Statically significant interaction terms were included in the final model.

To estimate the proportion of variance in *Salmonella* shedding attributable to production phase, a 3-level model was developed, including a random effect for production phase nested within farm. Using pooled samples only, a model with intercept as the only fixed term (null model) was fitted to compute the proportion of the overall variance in *Salmonella* shedding accounted for at the level of production phase and then farm. The proportion of variance that was accounted for by differences between herds was estimated as:

$$\rho_h = \frac{\sigma^2{}_h}{\sigma^2{}_h + \sigma^2{}_p + \sigma^2{}_\varepsilon} \tag{5.1}$$

where $\sigma^2{}_h$ was the herd-level variance and $\sigma^2{}_p$ was the production phase variance estimated from the null model, and $\sigma^2{}_\varepsilon$ was the sampling variance estimated according to the latent variable method[12]. Likewise, the proportion of variance that was accounted for by differences between production phases was estimated as:

$$\rho_p = \frac{\sigma^2{}_p}{\sigma^2{}_h + \sigma^2{}_p + \sigma^2{}_\varepsilon} \quad (5.2)$$

To evaluate *Salmonella* recovery from different sampling procedures, the odds of obtaining a *Salmonella* positive sample from a pooled fecal sample were compared to the odds of obtaining a positive culture from an individual sample. The unconditional association between sampling strategy and whether or not the fecal sample was *Salmonella*-positive was evaluated in a model with a random intercept for herd. This analysis was restricted to samples from grow-finish pigs as this was the only production area where both pooled and individual samples were collected from the same pen.

Both pooled and individual samples were used collectively to estimate differences in serovar-specific prevalence among the different phases of production. A positive outcome was the presence of a specific serovar; any other serovar, or any *Salmonella*-negative sample, was considered a negative outcome. For each of the 5 most prevalent serovars, the association between production phase and whether or not the fecal sample was positive for each of these 5 serovars was investigated. All models were adjusted for sampling strategy (pooled vs. individual samples) by including this variable as a fixed effect in each model.

5.3 Results

5.3.1 Farm Description

Farm size ranged from 130 to 2,070 breeding females (mean 531, median 333) and the number of pigs produced for slaughter by each farm ranged from 1,100 to 27,000 animals

annually (mean 8,332, median 4,300). Three herds primarily produced breeding stock but finished the barrows and cull gilts. Seven herds produced hogs for slaughter only.

5.3.2 *Salmonella* prevalence (both pooled and individual samples)

Salmonella was isolated from all 10 study farms. Based on total numbers of positive samples, prevalence within presumed-negative herds ranged from 20 to 56%, while prevalence within presumed-positive herds ranged from 2 to 79%. Across all production phases there were 407/1143 (35.6%) positive fecal samples (Table 5.3). Four farms accounted for 70% (284/407) of all positive samples (Table 5.3). The highest proportion was found in the breeding sows, with 38% (38/99) and 51% (102/200) of individual and pooled samples, respectively, positive for *Salmonella*. In the grow-finish population, 25% (73/294) of the individual samples and 38% (113/295) of the pooled pen samples tested positive. In the nursery, 32% (81/255) of all pooled pen samples were positive. The occurrence of *Salmonella* positive samples varied significantly among all production phases for the pooled samples ($P<0.001$) and between the breeding sows and grow-finish population for the individual samples ($P=0.002$).

5.3.3 Risk factors for shedding *Salmonella* (pooled sample results)

Risk factor variables that were unconditionally associated ($P\leq0.20$) with *Salmonella* shedding in the pooled samples were summarized in Table 5.4. Several management factors were specific and uniform to the breeding herd on the farms studied; for example, all breeding females were, naturally, “sex = female”, and most breeding females were housed in gestation stalls or farrowing crates. The variable “sex” was therefore perfectly correlated with “production phase – sows” and the variables “number of pigs in pen”, and “pig density” were also found to be highly

correlated with this production phase. Consequently, these 3 variables were not included in the initial model. A second model was developed to assess the significance of these variables in nursery and grow-finish pigs only.

Only the variables "fed pelleted feed", "production phase", and "nose-to-nose contact" were found statistically significant ($P<0.05$) in either model; thus, the estimates are reported for a single model including these 3 variables and applied to data from all production phases (Table 5.5). In this model, the odds of a positive pooled *Salmonella* culture remained different across the different production phases (Table 5.5). Sows were 2.3 (CI_{OR} 1.5, 3.7) times more likely to shed *Salmonella* than grow-finish pigs, and 4.0 (CI_{OR} 2.4, 6.8) times more likely to shed than nursery pigs; grow-finishers were 1.7 (CI_{OR} 1.1, 2.8) times more likely to shed *Salmonella* than nursery pigs. Pooled samples from pens that received pelleted feed were 8.2 (CI_{OR} 3.2, 20.6) times more likely to be *Salmonella*-positive than samples from pens with non-pelleted feed (Table 5.5). Pens allowing for nose-to-nose contact among pigs were 2.2 (CI_{OR} 1.3, 4.0) times more likely to be *Salmonella*-positive than pens without such contact (Table 5.5).

5.3.4 Variance component estimation (pooled fecal samples)

The estimates of variance in the occurrence of *Salmonella* positive pooled fecal samples at the herd and production phase levels were 2.24 (standard error [s.e.], 1.31) and 1.34 (s.e., 0.57), respectively. Using the latent variable method[12] the proportion of variance residing at the herd level [Eq. 5.1] was 33%, while 20% of total variance was due to production phase [Eq. 5.2].

5.3.5 *Salmonella* recovery from pooled vs. individual samples

Overall, *Salmonella* was isolated from 38% (113/295) of pooled grow-finish samples and 25% (73/294) of individual samples. The odds of *Salmonella* recovery from grow-finishers were 2.9 times (CI_{OR} 1.8, 4.5; P<0.001) higher from pooled than individual samples. In sows, 51% (102/200) of pooled samples and 38% (38/99) of individual samples were *Salmonella*-positive; however, no statistical test for differences between sampling strategies was done for this production phase as paired pooled and individual samples were not collected from the same pen or animals.

5.3.6 *Salmonella* serovar and phage type distribution (both pooled and individual samples)

The serovar prevalence for each production phase is shown in Table 5.6. Nineteen distinct serovars were identified. Multiple serovars (2-8 per farm) were detected on all but 1 farm. Fewer serovars were detected in individual samples (7 and 8 typed serovars, for sows and grow-finish, respectively) than in pooled samples (13, 12 and 12 typed serovars, for sows, grow-finish and nursery, respectively). The 5 most common serovars were *S.* Derby (28.5%), *S.* Typhimurium, var. Copenhagen (19.4%), *S.* Putten (11.7%), *S.* Infantis (6.7%), and *S.* Mbandaka (6.2%) (Table 5.4). Phage typing results for all *S.* Typhimurium, *S.* Typhimurium, var. Copenhagen, *S.* Enteritidis and *S.* Heidelberg isolates are presented in Table 5.7. On the 7 farms where these serovars were found, the number of phagetypes isolated per farm ranged from 1 to 6, with multiple phagetypes found on 4 farms. *S.* Typhimurium PT104 was detected on 2 farms, and on both these farms this was the only phagetype present.

The serovar distributions in various production phases were compared for the 5 most prevalent serovars, with the exception of *S.* Mbandaka. Since this serovar was not isolated from the breeding herd, this comparison was limited to nursery pigs and grow-finishers production phases. In an analysis adjusted for sample type (pooled vs. individual), significant differences in serovar distribution were found between production phases; these pair-wise contrasts are presented in Table 5.8.

5.4 Discussion

Existing research on the epidemiology of *Salmonella* in pigs has focused primarily on finishing pigs due to their proximity to the consumer. Still, pigs of other ages can play an important role in the maintenance and dissemination of *Salmonella* on-farm, as well as contribute to food safety issues themselves. In this study we investigated the epidemiology of *Salmonella* throughout all levels of pig production and reported on production phase level factors which could potentially influence the *Salmonella* status of pigs, an important contribution to future surveillance and control efforts for *Salmonella* in western Canada.

Three herds included in this study were initially presumed to be free of *Salmonella*; however, all 10 herds were ultimately found to be *Salmonella*-positive. Although only *S.* Typhimurium and *S.* Choleraesuis commonly cause clinical salmonellosis in pigs, infection by other serovars causes prolonged carrier states and intermittent shedding[13]. Even when *S.* Typhimurium and *S.* Choleraesuis are present within a herd, infection may remain primarily sub-clinical without outbreaks of clinical salmonellosis. In these cases, and in the absence of regular

testing, the presence of *Salmonella* goes unsuspected and undetected. Our observations then emphasize clinical history is not an accurate indicator of herd *Salmonella* status.

The current study parallels previous studies that sows were more at risk for shedding *Salmonella* than both nursery and grow-finish pigs[8, 14-17]. Cull sows are usually shipped to slaughter immediately after weaning, when increased shedding has been observed[16]. Additionally, transport and lairage practices may contribute to increased shedding of *Salmonella* by sows immediately prior to slaughter[18, 19]. For these reasons, potential control efforts should be placed on this population both on-farm and at slaughter to reduce the on-farm *Salmonella* reservoir as well as minimize potential food safety risks.

The use of pelleted feed and nose-to-nose pig contact through pens were two other significant risk factors detected in this study. Other researchers, both in Canada and elsewhere, have also reported strong associations between the use of pelleted feed and farm *Salmonella* status[15, 20-22]. Other research groups reported that the use of acidifying rations reduced the prevalence of *Salmonella* in market-age pigs[23], and that pelleted feed decreased stomach acidity in the pig as compared to coarser feed[24] or increased mucin secretion, contributing to the survival of ingested *Salmonella* and colonization of the pig[25]. Efforts to reduce *Salmonella* at the farm level could incorporate acidification of water or rations or changing feed to coarser-grind rations. Nose-to-nose contact between pigs through pens is a less likely target for intervention, since this is a feature inherent to barn design and unlikely to be easily changed. However, consideration of the possibility of transmission of *Salmonella* and other important pathogens between pens and production units should be taken into consideration when designing and building new barns.

Approximately a third of the estimated variance of *Salmonella* shedding resided at the farm level, suggesting that farm-level factors may exert the greatest influence on the outcome[26]. Others have reported farm type as a significant risk factor for *Salmonella* shedding[27], which further supports the premise that farm-level management factors significantly impact the *Salmonella* status of pigs. Within farms, 20% of the variance of *Salmonella* shedding was attributable to production phase, suggesting that production phase specific factors might be also important and concurs with our finding that production phase is a significant risk factor when included as a fixed effect in the regression model. However, previous studies investigating risk factors for *Salmonella* have focused primarily on finishing pigs and little information regarding risk factors for pigs of other ages is available. Further research into production phase level factors which could potentially influence the *Salmonella* status of pigs is required.

One-time sampling of individual pig feces (as compared to repeated or pooled samples) has been identified, among other reasons, for poor sensitivity of *Salmonella* culture[28]. Similarly, in our study, more positives were found in pooled pen samples than from individual pigs. Furthermore, more positive farms were identified when sampling pigs from all production phases. Consequently, the use of pooled pen samples, from all phases of pig production, is recommended as a more reliable means of accurately of establishing the prevalence of *Salmonella* in pig herds.

The observed distribution in *Salmonella* serovars was similar to other findings within Canada[3, 5, 9, 29] and the United States[30, 31], except for one notable exception. *S.* Putten, a serovar that has not been reported by any of these studies, was the third most common serovar in the

study, and was found only in 3 farms in Saskatchewan; these farms also accounted for over 80% of all untypeable isolates. Taken together, this is suggestive of either possible geographical differences in serovar distribution in pigs in western Canada or other common factors that contributed to the transmission of specific serovars between these herds. Molecular methods, such as those used to document transmission of *S.* Typhimurium DT 104 between geographically related herds in Denmark[32], would be necessary to further investigate this observation and further our understanding of the spread of *Salmonella* within and between herds.

Significant differences were observed in serovar prevalence between production phases. Surveillance efforts which focus solely on finisher pigs, either on-farm or at slaughter, would not have detected the full range of serovars present on these farms. As other researchers have noted, an understanding of serovar type and distribution is important because certain serological tests, such as the Danish-mix ELISA (enzyme-linked immunossorbent assay), detect antibodies against serogroups B, C1 and D1 only[5]. Serological response to serovars such as *S.* Mbandaka, *S.* Anatum, or *S.* Putten, would not have been detected by this ELISA. The changes in serovar distribution as pigs progress through the production cycle presents a challenge to *Salmonella* surveillance and control efforts which utilize serological tools only; cost-effective complementary bacteriologic testing of samples from all levels of pig production is necessary for accurate evaluation of *Salmonella* status in pig herds

In this study we identified only three significant risk factors, possibly because the study did not have sufficient power to detect other significant risk factors due to the small number of studied farms. The main study limitation was the use of purposeful selection of farms, which was

necessary to meet the objectives of a concurrent study. For these reasons no conclusions based on this study should be made regarding *Salmonella* prevalence in western Canadian pig farms in general. This study indicates that the breeding herd plays an important role in the persistence of *Salmonella* infection within pig herds, as suggested by other researchers[8, 16]. Molecular fingerprinting methods are needed in order to confirm clonal spread of *Salmonella* from sows to other production phases within these herds. In summary, this study has contributed to future surveillance and control efforts by providing important insight into the on-farm epidemiology of *Salmonella* in western Canada.

5.5 References

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Table 5.1. Distribution of the categorical variables in each production phase that were considered as possible risk factors for the occurrence of *Salmonella* in 10 farrow-to-finish pig farms from Alberta and Saskatchewan.

		Distribution		
Variable	Levels of response	Sows (%)	Nursery (%)	Grow-finish
Sex	gilt(s)	na	34	38
	barrow(s)	na	34	37
	mixed pen	na	32	24
	sow(s)	100	na	na
Fecal score	runny	0	0	3
	normal	93	99	96
	hard/dry	7	1	1
Fed pelleted feed	yes	30	51	29
	no	70	49	71
Fed wet feed	yes	44	78	83
	no	56	22	17
Fed on floor	yes	20	0	8
	no	80	100	92
Pen cleanliness	clean	56	76	25
	slightly wet/dirty	24	23	49
	moderately wet/dirty	16	0	9
	very wet/dirty	4	0	16
Pen floor type	full slatted	25	94	16
	part slatted	66	6	80
	not slatted	9	0	4
Concrete floor	yes	79	4	91
	no	21	96	9
Concrete walls	yes	9	4	54
	no	91	96	46
Nose-to-nose contact between pens	yes	79	53	80
	no	21	47	20
Production phase	sows	100	0	0
	nursery/weaners	0	100	0
	grow-finishers	0	0	100

Table 5.2. Distribution of the continuous variables in each production phase that were considered as possible risk factors for the occurrence of *Salmonella* in 10 farrow-to-finish pig farms from Alberta and Saskatchewan

Variable	Production Phase	Mean	Median	Min	Max	s.d.	n
Age in weeks[a]	Nursery	6.6	7	3	11	2	236
	Grow-finish	16.6	16	8	27	4.7	255
Number of pigs in pen[b]	Sows	5.3	6	1	10	2.7	39
	Nursery	18.8	18	6	70	9.8	255
	Grow-finish	16.4	14	3	120	12.9	295
Pig density (m^2 per pig)[b]	Sows	2.3	2.25	0.75	4.3	1.1	39
	Nursery	0.25	0.25	0.1	0.53	0.08	255
	Grow-finish	0.85	0.74	0.15	3.12	0.47	295

[a]observations on age were not recorded for breeding females
[b]data from pigs in pens only; does not include observations from sows in gestation/farrowing crates (n=161)

Table 5.3. Proportion of all fecal samples positive for *Salmonella* based on bacterial culture for each phase of pig production in 10 farrow-to-finish herds in Alberta and Saskatchewan

Farm	Total #samples	Overall #positive	Sows (pooled) #positive	Sows (ind) positive	Nursery (pooled) #positive	GF (pooled) #positive	GF (ind) #positive
1	120	77	17/20	10/10	17/30	21/30	12/30
2	96	54	14/20	5/10	1/16	17/25	17/25
3	120	95	17/20	5/10	22/30	28/30	23/30
4	120	2	1/21	0/9	0/30	1/30	0/30
5	120	34	13/20	5/10	2/30	11/30	3/30
6	120	46	12/20	3/10	5/30	15/30	11/30
7	119	18	5/19	2/10	9/30	2/30	0/30
8	104	58	17/20	7/10	13/14	15/30	6/30
9	108	11	5/20	0/10	2/19	3/30	1/29
10	116	12	1/20	1/10	10/26	0/30	0/30
All farms	1143	407/1143	102/200	38/99	81/255	113/295	73/29

Table 5.4. Unconditional associations between predictor variables and the occurrence of *Salmonella* positive pooled fecal samples from pens on 10 farrow-to-finish pig farms from Alberta and Saskatchewan

Variable	Levels of response	OR	95% CI (OR)	P-value
Sex	overall			0.018
	gilt(s)	0.43	0.26 to 0.70	0.001
	barrow(s)	0.58	0.36 to 0.51	0.026
	mixed pen	0.19	0.09 to 0.40	0.001
	sow(s)	Reference		
Fecal score	overall			0.071
	normal	0.23	0.04 to 1.36	0.11
	hard/dry	0.67	0.08 to 5.70	0.72
	runny	Reference		
Fed pelleted feed	yes	2.59	1.21 to 5.53	0.014
	no	Reference		
Fed wet feed	yes	1.77	1.09 to 2.89	0.020
	no	Reference		
Fed on floor	yes	1.77	1.08 to 3.46	0.087
	no	Reference		
Pen cleanliness	overall			0.008
	slightly wet/dirty	1.40	0.94 to 2.10	0.10
	moderately wet/dirty	1.21	0.59 to 2.46	0.60
	very wet/dirty	0.31	0.12 to 1.25	0.016
	clean	Reference		
Pen floor type	overall			0.46
	part slatted	1.92	1.32 to 2.80	0.001
	not slatted	1.16	0.30 to 4.53	0.83
	full slatted	Reference		
Concrete floor	yes	2.16	1.49 to 3.13	0.000
	no	Reference		
Nose-to-nose contact between pens	yes	1.95	1.17 to 3.23	0.009
	no	Reference		
Production phase	overall			0.089
	nursery/weaners	0.48	0.31 to 0.75	0.001
	grow-finishers	0.33	0.21 to 0.53	0.000
	sows	Reference		
Number of pigs in pen		0.95	0.93 to 0.97	0.000
Pig density	Pigs per meter^2	1.95	1.32 to 2.89	0.001

Table 5.5. Final multivariable regression model for associations between predictor variables and pen *Salmonella* status on 10 farrow-to-finish pig farms from Alberta and Saskatchewan

Variable		OR	95% CI (OR)	P-value
Fed pelleted feed	Yes	8.2	3.3 to 20.7	0.000
	No	reference		
Nose-to-nose contact	Yes	2.2	1.3 to 3.9	0.005
	No	reference		
Production phase	Nursery	0.2	0.1 to 0.4	0.000
	Grow-finish	0.4	0.3 to 0.4	0.000
	Sows	reference		
	Grow-finish	1.8	1.1 to 2.8	0.019
	Sows	4.1	2.4 to 6.8	0.000
	Nursery	reference		

Table 5.6. *Salmonella* serovars isolated from 10 farrow-to-finish pig farms in Alberta and Saskatchewan, grouped according to production phase

Serovar	Sows, pooled	Sows, individual	Nursery, pooled	Grow-finish, pooled	Grow-finish, individual	Total
S. Derby	20	12	6	48	33	119
S. Typhimurium var. Copenhagen	23	6	24	19	9	81
S. Putten	12	4	7	14	12	49
S. Infantis	8	3	4	7	6	28
S. Mbandaka	0	0	14	8	4	26
S. Give	8	8	1	1	1	19
S. Anatum	5	2	3	5	2	17
S. Ohio	0	0	3	1	0	4
S. Rubislaw	2	1	0	1	0	4
S. Livingstone var. 14+	1	0	3	0	0	4
S. Typhimurium	0	0	0	0	3	3
S. Worthington	3	0	0	0	0	3
S. Give var. 15+	0	0	1	1	0	2
S. Enteriditis	1	0	0	1	0	2
S. Ohio var. 14+	1	0	1	0	0	2
S. Brandenburg	2	0	0	0	0	2
S. Lexington var. 15+	0	0	0	1	0	1
S. Heidelberg	0	0	1	0	0	1
S. Kentucky	1	0	0	0	0	1
Untypeable	15	2	15	10	8	50

Table 5.7. *Salmonella* phage types isolated from 10 farrow-to-finish pig farms in Alberta and Saskatchewan

Serovar	Phage type	# of isolates	% of isolates
S. Typhimurium var.	UT5	30	34.5%
Copenhagen	21	16	18.4%
	104	13	14.9%
	22	5	5.7%
	208 var	5	5.7%
	135	4	4.6%
	146a var	3	3.4%
	208	1	1.1%
	142 var	1	1.1%
	Untypeable	2	2.3%
	UT3	1	1.1%
S. Typhimurium	27	2	2.3%
	U276	1	1.1%
S. Enteritidis	11b	1	1.1%
	20a	1	1.1%
S. Heidelberg	10	1	1.1%
	Total	87	100%

Table 5.8. Differences in *Salmonella* serovar distribution between production phases on 10 farrow-to-finish pig farms in Alberta and Saskatchewan

Serovar	Contrast	OR	$CI_{ORlower}$	$CI_{ORupper}$
S. Derby	grow-finish vs. nursery	10.2	4.2	24.9
	grow-finish vs. sows	1.5	0.9	2.5
	sows vs. nursery	6.7	2.6	16.9
S. Infantis	sows vs. nursery	3.1	0.9	10.8
S. Putten	sows vs. nursery	3.2	1.2	9
S. Typhimurium var. copenhagen	nursery vs. grow-finish	3.0	1.4	6.4
	sows vs. grow-finish	3.3	1.7	6.4
S. Mbandaka	nursery vs. grow-finish	4.4	1.7	11.3

CHAPTER 6

SUMMARY AND CONCLUSIONS

The relevance of *Salmonella* as a food safety issue has grown rapidly over the last two decades. Many European countries already have on-farm monitoring and control programs in place, and others are taking steps to develop and implement their own programs and remain competitive in the global marketplace. This has largely occurred in response to the European Union's recent Zoonoses Directive 2003/99/EC which mandates the monitoring of *Salmonella*, primarily in poultry and in pigs[1]. In Canada, the CQA® (Canadian Quality Assurance) program for hog producers has developed a set of best practices for minimizing or eliminating physical, chemical and biological hazards that can affect food safety (http://www.cqa-aqc.ca/); however, only the province of Quebec currently has a program in place specifically for the monitoring and control of *Salmonella* in pigs. This voluntary, industry-backed program was launched in 2004 and is based on serological testing of pigs at slaughter (Letellier, A., personal communication). International pressures could potentially create the incentive to develop similar programs in other provinces.

The Canadian swine industry represents only 2% of the internationally produced pork and yet it is the world's third largest exporter with 22% of global pork trade[2]. This makes Canada's industry potentially vulnerable to international food safety trends. Alberta and Saskatchewan together produce about 20% of Canadian pork[2,3]. Availability of accurate and cost-effective diagnostic tests is vital to any pathogen-related monitoring or control program. However, after two decades of extensive research of this topic, and specifically on the accuracy of diagnostic tests for *Salmonella*, the scientific recommendations are still confusing or ambiguous. Therefore,

the main objectives of this thesis were to identify, appraise and summarize existing primary research examining the accuracy of the main diagnostic tests for *Salmonella* in pigs, using a research synthesis approach. This approach included a transparent and replicable systematic review, quantitative meta-analysis and meta-regression. The tests chosen were bacterial culture, RT-PCR and ELISA, which would be primarily considered for potential monitoring or control programs in Canada. Concurrently, these tests were evaluated using both traditional statistical and Bayesian approaches under field conditions by sampling pigs from 10 farrow-to-finish farms in Alberta and Saskatchewan that had been purposively selected based on the *Salmonella*-presumptive status. The *Salmonella* serovar distribution, sampling approaches and risk factors for shedding of *Salmonella* spp. in breeding, nursery and grow-to-finish phases were also evaluated on these farms to add to the existing knowledge of the epidemiology of *Salmonella* in pigs in western Canada.

Systematic reviews are most commonly used to estimate the effect of interventions. In recent years there has been increased momentum to use this methodology to examine the accuracy of diagnostic tests; the diagnostic review presented in this thesis is among the first to be conducted in the areas of food safety and veterinary public health. Systematic reviews of diagnostic and screening test evaluations are carried out for the same reasons as systematic reviews of treatment interventions: to generate summary estimates of test performance based on all available evidence, to evaluate the quality of published studies, and to account for variation in findings between studies[4].

A comprehensive and replicable search of 10 electronic databases and grey literature sources (proceedings of the International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork 2001, 2003, and 2005, web pages of the Inventory of Canadian Agri-Food Research ICAR and the National Pork Board) was conducted to retrieve potentially relevant primary research. The abstract-based citations were screened for relevance and evaluated for methodological soundness and reporting, through two subsequent assessments by two independent reviewers. Data were extracted from all relevant studies that also met four minimum methodological soundness criteria (Chapter 2) and reported sufficient data for potential meta-analyses. Thirty eight references were included in the review, reporting 129 unique test comparisons for bacterial culture (as compared to other culture), 24 individual-level and 7 herd-level test comparisons for ab-ELISA (as compared to culture), 17 test comparisons for PCR (as compared to culture), and 10 test comparisons for ag-ELISA (as compared to culture).

Test accuracy was most often reported sensitivity (Se) and specificity (Sp), or raw data were available for their calculation. A wide range of reported Se and Sp was reported for each test; e.g. for bacterial culture Se estimates ranged from 17% to 100% and to a lesser extent for PCR (in comparison with culture), where Se ranged from 55% to 100%. The majority of test comparisons included bacterial culture as the reference standard although culture protocols differed significantly among studies.

When reported estimates of Se and Sp were examined through meta-analysis, significant heterogeneity was observed for all tests. For this reason, meta-analysis forest plots and reported pooled estimates were presented only for visual evaluation and as a summary of available

information. Diagnostic reviews are valuable for obtaining summary or pooled estimates of test accuracy, but only in the absence of significant heterogeneity between results from different studies. Where significant heterogeneity exists, the use of meta-regression analysis is recommended in order to investigate the factors potentially associated with variability between studies[5]. A meta-regression analysis was consequently used to investigate the extensive variability in test accuracy. The main objectives were to identify the test protocol, methodological soundness and reporting variables that might explain the variation in reported test Se and Sp among studies, and to quantify potential associations between these variables and reported test Se and Sp (Chapter 3).

When culture was compared to other culture protocols, variations in both index test protocols and reference standard protocols contributed significantly to variation in culture Se (Se_c). The type of enrichment used in both index and reference tests, agar type (index), enrichment incubation temperature and study population were also significant predictors of Se_c. Multiple and parallel enrichments and selective agars were associated with markedly increased Se_c, as compared with single enrichment techniques. Incubation of enrichment media at 42C, rather than 37C, also increased Se_c, confirming that higher temperatures are preferable for highly contaminated samples[6-8], which are typically collected at the farm and slaughter levels. Culture conducted on samples collected at slaughter resulted in increased Se_c when compared to on-farm samples, presumably because pigs shed larger numbers of *Salmonella* after transport or in lairage and larger quantity may also result in more viable organisms in the sample resulting in a greater probability of recovering *Salmonella* organisms.

When ab-ELISA was compared to culture, the reported cutoff value of the ab-ELISA was significantly associated with the observed ab-ELISA Se and Sp, and explained 42-79% of the total variance between unique test comparisons. Numerous studies reported varying test accuracy estimates or test agreements at the individual or herd level using various ab-ELISA cutoff values[8-12]; our analysis has shown that the difference in ab-ELISA accuracy is large and predictable when ab-ELISA cutoff is changed. This information may aid in decisions regarding appropriate cut-off values in ab-ELISA-based *Salmonella* surveillance and control programs.

The Se of PCR, as compared to culture, was overall quite good (mean 91%), but varied with different sample matrices. PCR Se was lower for fecal samples than for carcass swabs or lymph tissue. The use of broth-enriched PCR as a screening tool for pig feces may be useful particularly when prevalence is low in targeted populations[13], and results would be available 1 to 2 days sooner than if conventional culture were used. Ag-ELISA is another rapid test which may potentially be useful for detecting *Salmonella* in pigs. However, the lack of sufficient unique test comparisons in this test category precluded the use of more extensive meta-regression.

The tests examined in this review were all evaluated relative to bacterial culture, and the culture protocols which were used as reference standards varied considerably. This has important implications since different culture protocols will have different accuracies, and the accuracy of the index test will therefore vary relative to the reference standard used. The consistent use of a single good reference standard is an important aspect of study quality[14]; without a common reference standard, comparison of results between studies is difficult, if not impossible. This systematic review and meta-analysis has highlighted that the lack of a single, good, universally

applied reference test is a serious problem in studies evaluating tests for detecting *Salmonella* in pigs.

The systematic-review and meta-analysis (Chapter 2) and then subsequent meta-regression (Chapter 3) have confirmed significant heterogeneity among reported unique test comparisons within each of the three tests that were evaluated in the study, thus requiring caution in interpretation of findings. The overall methodological soundness and reporting of primary studies included in this review was poor, with consistent lack of the use or reporting of blinding, reference standards, randomization and selection criteria. It is evident from these observations that significant improvement in the design and reporting of studies in this area is necessary. Consequently, the food safety and veterinary public health research community should formally consider ways for standardizing the conduct and reporting of the studies evaluating diagnostic test accuracy.

The consistent use of a single relevant reference standard is a particularly important feature that should be addressed at the international level[4]. With no gold standard available, tests are evaluated *relative* to the selected reference standard. Consequently, the apparent accuracy of tests may differ depending on which reference test was used for comparison. Other aspects of study methodological soundness and reporting, such as blinding and randomization, could also affect reported estimates of test accuracy[14]. A good example of international initiatives for improving the methodological soundness and reporting of primary research addressing the accuracy of diagnostic tests already exists in the international public health arena and may be considered for formal international adoption within food safety and veterinary public health

fields. Standardization guides, such as the STARD initiative (Standards for Reporting Diagnostic Accuracy)[15] and the QUADAS tool (Quality Assessment of Diagnostic Accuracy Studies)[16], offer very appealing frameworks and concepts. While these tools were developed for application in the human health fields, researchers in the areas of food safety and veterinary public health are strongly encouraged to adopt this methodology as well. Our study has also provided valuable insight into test protocol-related factors cause variation in test Se and Sp. This information should prove useful in future research efforts aimed at identifying a highly accurate and reliable reference standard against which to measure either new or existing tests.

Traditionally, tests are evaluated by comparing the results of the test of interest (index test) against the results of another test which is assumed to be correct (reference or "gold" standard). The problem is that there is no true gold standard for detecting *Salmonella* in pigs. The advantage of the Bayesian approach is that the Se and Sp estimates for two or more tests can be obtained in one model relative to the true, but unknown (latent) disease status[17], thus no gold standard is required. Within this context, the latent class becomes "ever exposed" (culture, RT-PCR or ELISA positive) or "never exposed" (culture, RT-PCR and ELISA negative), and reflects the pigs potential exposure to contamination – past and present – within its environment. Su et al. previously demonstrated the usefulness of the Bayesian approach for herd-level test evaluation using different sampling schemes for *Salmonella* in pigs[18]; however, to our best knowledge this approach to evaluating diagnostic tests for *Salmonella* based on individual pig samples has not been reported.

In Chapter 4, bacterial culture and RT-PCR, and ab-ELISA for *Salmonella* were applied to fecal and blood samples, respectively, collected from nursery and grow-to-finish pigs from 10 farrow-to-finish farms in western Canada. The results were analyzed using a Bayesian and conventional statistical analysis only in grow-to-finish pigs at the individual sample level. A low sero-prevalence (8/247) observed in nursery pigs precluded the analyses within this production stage. Interestingly, apparent culture prevalence in these pigs was fairly high (80/247). At the time of sampling, these pigs were 3-6 weeks old (mean 6.6) and it's possible that most were too young to develop a test-detectable immune response.

In grow-to-finish pigs, the Se and Sp of all tests resulting from the Bayesian analysis were similar to results obtained from traditional estimates of Se and Sp at ELISA cutoff value of OD40%. Culture Se of approximately 72% and 66% were observed for pooled pen samples and individual samples, respectively (Sp was assumed to be 100%). Culture and PCR Se were found to be significantly correlated, illustrating that it is necessary to use statistical models which take this dependency into account. The primary difference between the two approaches was that the Bayesian approach eliminated most of the variability in the estimates of culture and PCR Se and Sp, and in the estimates of population prevalences, associated with the choice of ELISA cut-off value.

The results of our systematic review (Chapter 2) and field 'test evaluation' study (Chapter 4), clearly confirm that the use of culture and serology in parallel is more meaningful at the group level than at the individual level. Still, knowledge of the individual-level test accuracy estimates is relevant because test Se and Sp estimates at the herd-level are dependent on the Se

and Sp at the individual level, the number of pigs tested, the true within-herd prevalence in infected herds, and the number of positive reactors used to classify the herd as positive[19]. The difference between Bayesian and traditional approaches may be of only theoretical interest if both approaches lead to similar findings, as it was observed in this study for ELISA Se and Sp. However, the latent class approach did have significant advantages over traditional methods. First, in the Bayesian model *neither* test is assumed to be perfect while traditional methods require the assumption that the reference test is 100% correct. Secondly, if prior information is available for any of the tests being examined or for prevalence within the sampled population, this information can be incorporated into the model to obtain updated, more precise posterior estimates of test accuracy. Finally, by defining the latent disease status class "ever exposed" vs. "never exposed", all evidence of either previous exposure (serological status) or current infection (culture and PCR status) from pigs can be used to define the risk of recent *Salmonella* exposure to a pig, pen or herd. This has important implications for *Salmonella* monitoring and control programs which focus on identifying high-risk farms defined by within-herd *Salmonella* prevalence, as well implications for other food-borne pathogens which are subject to monitoring and control at the farm level.

The sampling was conducted from May to August 2004. The ab-ELISA kit used in this study (Svanovir, Svanova Biotech, Uppsala, Sweden) was removed from the market in 2007 (A. Jalali, SVANOVA Biotech; personal communication); however, this assay was based on the slight modification of Danish mix-ELISA[20] which uses coating antigens (LPS) of serogroups B, C1 and D (O-antigens 1, 4, 5, 6, 7 and 12). These antigens are used in other assays including the IDEXX Herdchek (IDEXX Laboratories Inc.) and Salmotype (Salmotype Labordiagnostik)

ELISA test kits; therefore, the information generated by this study may be applicable to these other assays as well.

The primary purpose of this thesis was to evaluate the reported accuracy of three selected diagnostic tests for *Salmonella* in pigs using research synthesis methods, and compare test estimates under field conditions using conventional statistics and Bayesian methods. The latter study was also utilized to explore the epidemiology of *Salmonella* infection throughout all phases of pig production in 10 farrow-to-finish western Canadian farms. The breeding herd has been relatively overlooked in studies investigating the epidemiology of *Salmonella* in swine, as the focus of most reported primary research has been on finishing pig populations due to their proximity to the consumer. In Canada, researchers in Quebec examined *Salmonella* epidemiology in all phases of pig production within one large integrated production system[21]. In western Canada, only the grow-to-finish populations have been studied[22-24].

In chapter 5, the distribution of *Salmonella* serovars in the breeding, nursery, and grow-to-finish pigs, and risk factors for shedding are reported for the 10 herds from Alberta and Saskatchewan which were enrolled in the diagnostic accuracy study (Chapter 4). Nineteen distinct serovars were detected and the most common serovars isolated from these herds were *S.* Derby (28.5%), *S.* Typhimurium, var. Copenhagen (19.1%), *S.* Putten (11.8%), *S.* Infantis (6.8%) and *S.* Mbandaka (6.1%). These findings are relatively similar to those reported in other studies in North America[21,22,24-27]. There was some indication of regional differences in serovar distribution, as *S.* Putten was isolated in the 3 herds from Saskatchewan but not in the 7 herds from Alberta. Significant differences in serovar distribution were observed among production

phases, which may indicate change in serovar-specific immunity as pigs move through different production phases. This aspect may also have implications for serology use. *Salmonella* was isolated more frequently from sows than from either nursery or grow-to-finish pigs, a trend reported by other researchers[28-32]. *Salmonella* was also isolated more frequently from pooled pen feces than from individual feces, and included a broader range of serovars. Consequently, the use of pooled pen samples in all phases of pig production is recommended as a more reliable means of accurate evaluation of *Salmonella* shedding in pig herds.

The use of pelleted feed, the opportunity for nose-to-nose contact between pens, and the phase of production were significant risk factors for shedding *Salmonella*. Pelleted feed has been consistently associated with increased *Salmonella* positive status or increased shedding in other studies and could be considered a primary target for on-farm *Salmonella* control[30,33-35]. Studies are also needed to investigate the potential effect of different feed types on the *Salmonella* status of pigs and herds under field conditions in western Canada. Nose-to-nose contact might be a less likely target for intervention, but may be considered when designing and building new barns.

Shedding of *Salmonella* was more prevalent in the breeding herd than in other production phases; any program to monitor or control *Salmonella* on-farm should therefore take this population into consideration. Research is needed to better understand factors which contribute to the persistence of *Salmonella* within the breeding herd and to identify potential interventions for this population. Sow management has the potential to affect the prevalence of *Salmonella* in nursery and grow-to-finish pigs. Future studies should examine the effect of *Salmonella* control in sows on the risk of infection and contamination in other points in the food chain.

Thirty-three percent of the estimated variance of *Salmonella* shedding resided at the farm while 20% resided at the production phase level, suggesting that farm- and production-phase factors other than pelleted feed and nose-to-nose contact (which were pen-level factors in this study) may be associated with the *Salmonella* status of pigs. A small study size (n= 10 herds) has precluded more extensive investigation of risk factors. Previous studies investigating risk factors for *Salmonella* have focused primarily on finishing pigs and little information regarding risk factors for pigs of other ages is available. Further research is needed to separate potentially specific production phase level factors for shedding *Salmonella*. This study was limited by the purposeful selection of farms, which was necessary to meet the objectives of Study 2 (Chapter 4). While this does not bias the interpretation of the risk factor analysis, no conclusions based on this study should be made regarding *Salmonella* prevalence in western Canadian swine farms in general.

Even after almost two decades of extensive research efforts the existing global primary research is not sufficient to provide definitive answers regarding the accuracy of specific diagnostic tests for *Salmonella* in pigs. Much of the information included in the systematic review was published after 2000; therefore, some may consider it "too soon" for a systematic review to be useful or appropriate. Undoubtedly, the low number of studies included for most test comparison categories confirms that further work in this area is required. However, if research continues in the current manner, with poor study quality and reporting and without standardization of reference tests, it is unlikely that the global pool of knowledge will substantially improve. Rather than "too soon", perhaps it is "just in time". Identification of the most common problems and reasons for variability between studies now may stimulate initiatives

to standardize the conduct and reporting of diagnostic test evaluations in the fields of food safety and veterinary public health sooner than might otherwise happen, and significantly improve research in both the short and long terms.

The systematic review described in this thesis addressed the question "What is the accuracy of selected diagnostic tests used for detecting *Salmonella* in swine?" Due to significant heterogeneity in all test categories, and insufficient data for some, this question could not be answered. That does not mean, however, that this was a futile exercise. On the contrary, valuable information on reasons for heterogeneity and identification of fundamental quality issues in the conduct and reporting of research in this area was generated, demonstrating the usefulness of research synthesis methodology for evaluating diagnostic tests in the areas of food safety and veterinary public health. The challenge to the conduct of systematic reviews in these areas is the lack of standard reference tests and the poor study design and/or reporting of relevant research. A long-term practical solution would be the adoption of standards such as the STARD and QUADAS tools by both researchers and journal editors. In the immediate future, the problem could potentially be addressed by attempting to contact primary authors of relevant research for clarification, though this would be prohibitive if a large number of studies were involved. It may be a useful exercise to repeat the current systematic review using this strategy to determine whether the results would be significantly improved.

There remains no clear answer to the question "what is the best test to use to monitor *Salmonella* in Canadian pigs?" If culture is chosen, we must then ask "what is the best culture protocol to use?" From the results of this review, there is no doubt that the reported accuracy of

culture varies widely. The evidence is not sufficiently strong to recommend any one culture protocol, although the results of the meta-regression suggest using higher enrichment incubation temperatures and avoiding the use of selenite enrichment media. It is obvious that the most sensitive culture protocol is one that uses multiple enrichment media and selective agars; however, this is unlikely to be considered the "best" test given the additional costs associated with this approach. If the objective of a monitoring and control program is to identify herds with highest on-farm prevalence, relative to other farms using the same test, then a culture protocol of lower sensitivity is likely sufficient. Each "lower sensitivity" culture protocol must still be evaluated against a highly accurate reference standard in order to obtain the best possible estimate of the actual sensitivity, if estimates of true on-farm prevalences are required or if results are to be directly comparable results from other regions, programs or laboratories. A final problem with the use of culture for monitoring on-farm *Salmonella* in pigs revolves around the logistics of on-farm sample collection, which involves considerable time, costs and manpower. Due to rapid infection acquired during transport and lairage[36-38] culture of samples obtained at slaughter will not accurately reflect on-farm *Salmonella* prevalence. For these reasons, neither bacterial culture nor PCR analysis of samples collected at slaughter would be the test of choice for any potential farm-level monitoring program for finishing pigs in western Canada.

The most promising choice for monitoring the *Salmonella* status of finishing pigs remains the use of antibody-capture ELISA. Collection of sera or muscle for "meat juice" at the abattoir is convenient and amendable to automation, as is the assay itself. Unlike culture, there are no additional costs associated with positive tests; therefore the overall cost of monitoring will not be affected by the prevalence of *Salmonella* in different pig populations. The serological status of

the pig is unaffected by transport and lairage, and reflects on-farm exposure. The meta-regression generated important information on the predicted change in ELISA sensitivity and specificity associated with changing ELISA cutoff values for defining *Salmonella*-positive status; this may aid policy makers in selecting the optimal cut-off value for potential monitoring and control programs. This study did not examine which ELISA would be most suitable for use in western Canada, and therefore, the choice of ELISA remains entirely subject to practical and technical considerations.

The primary disadvantage of ELISA may be that most commercially available assays are based on lipopolysaccharide O-antigens 1, 4, 5, 6, 7 and 12 of *S.* Typhimurium and *S.* Choleraesuis, which should detect *Salmonella* serogroups B, C1 and D. Theoretically, other serogroups would not be detected. This is of concern as serovars from other serogroups are commonly isolated from Canadian pigs, in numbers which may vary between regions. For example, in Study 2 (Chapter 4) approximately 20% of all typed isolates were from serogroups E1, F or G2, while a study in Ontario found almost 48% of isolates to be from serogroups E1, G2, K or L[10]. It is necessary to determine which serovars are prevalent in the target population to in order to determine if a given ELISA may be useful. It may even be necessary to develop a new or modified ELISA to specifically target the most prevalent serovars if they are substantially different from serogroups B, C1 and D. A second disadvantage to ELISAs is that they are not useful for detecting *Salmonella* exposure in very young pigs, and may be less useful in older, breeding females due to cross-reactivity with antibodies to other enterobacteriaceae. The ELISA would therefore not be suitable for on-farm collection and monitoring programs which include all age groups.

Like culture, the use of an ELISA with less than perfect – or even unknown – accuracy would be acceptable if the objective is to identify herds with the most sero-positive animals, relative to other farms. Also like culture, the ELISA must be evaluated against a highly accurate reference standard in order to obtain the best possible estimate of true accuracy, if estimates of true on-farm prevalences are required or if results are to be directly comparable to results from other regions, programs or laboratories. In a population of animals with unknown disease status, there is questionable validity in evaluating the accuracy of ELISA – which requires a detectable immune response that increases dependent on the passage of time and exposure to a sufficient infective dose – relative to culture, which detects the *Salmonella* organism which may be decreased or eliminated from the pig as a result of the immune response. It is perhaps surprising that individual pigs are found to be both sero-positive and culture-positive as often as they are. Challenge studies evaluate the accuracy of the ELISA in animals of known disease status, and though accurate estimates can be obtained for the experimental population, this approach will overestimate the accuracy of the test in the field situation. Furthermore, the accuracy of one ELISA evaluated relative to another ELISA of unknown accuracy is uninterpretable. At this time, there appears to be no truly good method of evaluating the accuracy of an ELISA to detect the *Salmonella* status of pigs under field conditions. Consequently, the correlation between the ELISA and on-farm bacterial status may be more useful than evaluation of the accuracy of the ELISA relative to imperfect and questionable reference standards.

There are mixed views within the research and industry communities as to whether a monitoring and control program is necessary in western Canada; opinions differ about whether

such a program would ever be implemented. At the time of writing this thesis, the pork industry in Canada is plagued by major problems, such as the recent H1N1 "swine flu" pandemic which has caused some disruption in international pork trade, and the "COOL" (Country Of Origin Labeling) requirements recently legislated in the United States which may impact the export of live Canadian hogs to that country. It is understandable that the issue of *Salmonella* control in pigs in Canada is low on the radar at this time. However, consider the following: the European Union (EU), as of 2008, has legislated the monitoring and control of *Salmonella* in pigs in member countries; within Canada, only Quebec has a monitoring and control program in place. Earlier this year, Quebec pork processor Les Aliments Lucyporc, Yamachiche, Quebec was the first Canadian integrated pork packer to earn approval to export pork to the EU (http:// www.cattlenetwork.com/Content.asp?contentid=301717). Though information is not publicly available on the rationale behind this decision, it is probably not coincidental that the first EU approved pork exporter in Canada is located in the only province following the EU lead on the issue of *Salmonella* in pigs. As the role of *Salmonella* in food safety is receiving priority attention by our international trading partners, it is essential that within Canada researchers and the pork industry remain aware of this issue.

Information on the national prevalence and distribution of *Salmonella* serovars and the standardization and evaluation of available diagnostic tools might be too meager to consider introducing a national monitoring control program at the present time. Investigations into the on-farm epidemiology of *Salmonella* in Canadian pigs have largely been limited to Alberta, Ontario, Quebec and more recently Saskatchewan[22-25,39]. Manitoba produces the largest number of hogs of any of the western provinces, yet to the best of our knowledge there are no published reports

describing the *Salmonella* status of Manitoba swine herds; research into the prevalence and distribution of *Salmonella* in this pig population is needed. Information regarding the performance of select diagnostic tests in the western Canada population is sparse, and to date has involved only small numbers of herds or pigs. More extensive study involving larger numbers of herds representing all major pig populations in western Canada is required. Cost-benefits of tests selected for potential use will need to be weighed. All tests targeted for use in a national monitoring or control program should be subjected to inter-laboratory validation involving all participating testing centers to determine the repeatability of results between laboratories, regions and pig populations.

The studies described within this thesis have provided valuable information about the performance of selected diagnostic tests for *Salmonella* in pigs, sources of variability in estimates of test accuracy, and insight into the epidemiology of *Salmonella* throughout all phases of pig production western Canada. Development of either a perfect test for *Salmonella* in pigs or a simple solution to dealing with *Salmonella* infection in pigs or pork products is unlikely in the foreseeable future. However, each study and each piece of new information increases our ability to monitor and control this pathogen. Although currently there are no programs in western Canada to monitor or control *Salmonella* in pigs, international and public pressures could potentially challenge the industry to consider the introduction of programs similar to the current industry-backed program in the province of Quebec. Working proactively to identify optimum testing strategies and interventions ensures that researchers, policy makers, pig producers, and processors are prepared for this challenge.

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APPENDIX A
SYSTEMATIC REVIEW TOOLS

A.1 Relevance Screening Level I

1. Does this abstract describe EITHER the evaluation of a diagnostic test(s) [serology/ELISA, bacterial culture or PCR] OR the use of 2 or more of these tests applied simultaneously to the same sample population, for detecting *Salmonella* infection in swine feces, blood, lymph nodes, carcass swabs or pork "meat juice"?

[ ] YES **(if yes, check all that apply below)**

- [ ] The abstract is evaluating one or more diagnostic tests used for detecting *Salmonella* infection in swine feces, blood, lymph nodes, carcass swabs or pork "meat juice". **(include)**
- [ ] The abstract describes the use of 2 or more tests, used simultaneously on the same sample population, to detect *Salmonella* infection in swine feces, blood, lymph nodes, carcass swabs or pork "meat juice". **(include)**
- [ ] The abstract is limited to the evaluation of the methodology of a diagnostic test(s) (serology/ELISA, bacterial culture or PCR) used for detecting *Salmonella* infection in swine feces, blood, lymph nodes, carcass swabs or pork "meat juice" **(exclude)**
- [ ] The abstract mentions predictability (such as, but not limited to, Se and Sp, predictive values, correlation, likelihood ratios, percent agreement) of a diagnostic test for *Salmonella* infection in swine feces, blood, lymph nodes, carcass swabs or pork "meat juice". **(include)**
- [ ] The above statements may apply to this citation; however there is not enough information in the abstract or the abstract is missing. **(include)**
- [ ] None of the above statements apply to this citation. **(exclude)**

[ ] NO **(exclude)**

A.2 Relevance Screening Level II

1. Is the language of this article English?

   [ ] YES **(include)**
   [ ] NO **(exclude)**

2. Is the article describing **(check all that apply):**

   [ ] The comparison of two or more tests for the detection of *Salmonella* in swine, with both tests being limited to bacterial culture, serology (ELISA), or PCR? **(include)**
   [ ] The use of two or more tests, limited to bacterial culture, serology (ELISA), or PCR, used simultaneously on the same sample population, for the detection of *Salmonella* in swine? **(include)**
   [ ] The abstract is limited to the evaluation of the methodology of diagnostic test(s) [bacterial culture, serology (ELISA), or PCR] for the detection of *Salmonella* in swine. **(exclude)**
   [ ] The evaluation or use of a SINGLE test via "challenge" trials/tests on animals of known *Salmonella* status, and the test being limited to bacterial culture, serology (ELISA), or PCR, for detecting *Salmonella* infection in swine? **(exclude)**
   [ ] None of the above apply. **(exclude)**

3. Please indicate what type of article the abstract represents:

   [ ] Primary study **(include)**
   [ ] Conference proceeding **(include)**
   [ ] Thesis **(include)**
   [ ] Report **(include)**
   [ ] Review article **(exclude)**

4. Comments:

A.3 Quality Assessment

1. Were the estimates of test performance reported, or is a sufficient amount of raw data presented for post-hoc analysis?

   [ ] YES **(include)**
   [ ] NO **(exclude)**

2. If 2 or more tests are being used/compared, is the time period between tests short enough to be reasonably sure that the subject's *Salmonella* status did not change between the two tests? OR For challenge trials: Was the time from challenge administration to measurement of outcome sufficient to have the outcome of interest?

   [ ] YES **(include)**
   [ ] NO **(exclude)**
   [ ] Not reported **(include)**
   [ ] A test or tests were used to compare/evaluate a previously established herd-level *Salmonella* status. **(exclude)**

3. Were samples stored appropriately AND processed/tested within a reasonable period of time after collection?
   [ ] YES **(include)**
   [ ] NO **(exclude)**
   [ ] Not reported **(include)**

4. Was the test protocol(s) described in sufficient detail to permit replication of the test?
   [ ] YES **(include)**
   [ ] NO **(exclude)**

A.4 Secondary Quality Assessment and Data Extraction Level I (general)

1. Reference Type
   [ ] Journal article
   [ ] Proceedings
   [ ] Other (specify)

2. Funding Source(s):

3. In what country(s) did the participants (researchers/laboratories) originate?

4. What is the study design?
   [ ] Case control
   [ ] Prevalence/cross sectional
   [ ] Cohort
   [ ] Randomized control trial
   [ ] Challenge trial
   [ ] Laboratory-methodology
   [ ] Other (specify)

5. In what country(s) did the samples originate?

6. When did sample collection take place? (yyyy or yyyy-yyyy)
   [ ] Enter timeframe here _____
   [ ] Not stated

7. What was the time-frame between sample collection and processing? (i.e. none, 3 days, 2 months, 1 year)
   [ ] Enter timeframe here _____
   [ ] Not stated

8. Were the same test(s) applied to ALL samples or a random selection of the samples?
   [ ] YES
   [ ] NO

9. Were the same tests applied to all subjects and all samples, regardless of the result of one or more tests?
   [ ] YES
   [ ] NO
   [ ] Does not apply (1 test only)

10. Were the tests independent of each other (one test did not form part of another test being used)?
    [ ] YES
    [ ] NO
    [ ] Does not apply (1 test only)

11. Was the test protocol(s) described in sufficient detail to permit replication of the test?
    [ ] YES
    [ ] NO (if NO, briefly describe missing information)

12. Were test results interpreted without knowledge of the results of the other test(s)? (e.g. blinding)
    [ ] YES
    [ ] NO
    [ ] Does not apply (1 test only)

13. Were uninterpretable/intermediate test results reported?
    [ ] YES
    [ ] NO

14. What type of samples were collected? Please specify source (check all that apply)
    [ ] individual feces, antemortem (specify voided, rectal, etc)
    [ ] individual feces, post-mortem (specify section of gut sampled)
    [ ] pooled feces, antemortem (specify pen, voided, rectal, etc)
    [ ] pooled feces, post-mortem (specify section of gut sampled)
    [ ] rectal swab (indicate on farm, lairage/holding pen, at slaughter)
    [ ] individual blood
    [ ] pooled blood
    [ ] individual meat-juice (indicate location sampled or NA)
    [ ] pooled meat-juice (indicate location sampled or NA)
    [ ] lymph nodes (indicate anatomical location and if individual or pooled)
    [ ] carcass swab (indicate individual or pooled)
    [ ] other (describe)

15. Please indicate the population represented in this paper. (check all that apply)
    [ ] Farm Level (specify the number of farms)
    [ ] Research environment (describe)
    [ ] Does not apply - laboratory/methodology only
    [ ] Not reported
    [ ] Other (specify)

16. Were the farms/groups/pigs selected a "random sample" or a "convenience sample"? (answer question for highest level i.e. if farms were being studied, answer for "farms", not "pigs")
    [ ] Random sample
    [ ] Convenience sample

16. (cont'd)

[ ] Not reported
[ ] Does not apply

17. Was the sample population clinically ill or healthy/asymptomatic?

[ ] Clinical Salmonellosis
[ ] Subclinical Salmonellosis
[ ] Does not apply, as this article describes laboratory-methodology only
[ ] Not reported

18. What was the age group/production phase of the sample population? (check all that apply)

[ ] nursing piglets (indicate research or on-farm)
[ ] weaned pigs (indicate research or on-farm)
[ ] grow-finish pigs (on-farm)
[ ] finisher pigs (abattior)
[ ] sows (on-farm)
[ ] sows (abattior)
[ ] other (specify)
[ ] does not apply - laboratory-methodology
[ ] source of samples not reported

19. Were criteria for selecting the sample population clearly describe

[ ] YES
[ ] NO
[ ] Does not apply - laboratory/methodology only

20. List the selection criteria.

21. List the exclusion criteria.

22. Were withdrawals or losses (subjects AND samples) from the study, if any, explained?

[ ] YES
[ ] NO
[ ] Does not apply - laboratory/methodology only

23. How many different culture protocols are reported? (check all that apply)

[ ] NONE
[ ] FEWER than TWO
[ ] FEWER than THREE
[ ] FEWER than FOUR
[ ] FEWER than FIVE
[ ] FEWER than SIX

24. How many different serum ELISA protocols are reported? (check all that apply)
    [ ] NONE
    [ ] FEWER than TWO
    [ ] FEWER than THREE
    [ ] FEWER than FOUR
    [ ] FEWER than FIVE
    [ ] FEWER than SIX

25. How many different PCR protocols are reported? (check all that apply)
    [ ] NONE
    [ ] FEWER than TWO
    [ ] FEWER than THREE
    [ ] FEWER than FOUR

26. What was the purpose of the study? (check all that apply)
    [ ] test comparison or evaluation
    [ ] prevalence assessment
    [ ] pathogenesis study
    [ ] vaccine trial
    [ ] other intervention trial (speficy)

27. What measure can be extracted from this reference? (check all that apply)
    [ ] Se (reported)
    [ ] Sp (reported)
    [ ] kappa (reported)
    [ ] percent agreement (reported)
    [ ] 2x2 data (2 test comparison)
    [ ] correlation between tests
    [ ] the only info available is %positive by each test
    [ ] other reported(specify)

28. What type of test comparisons are made/can be made? (check all that apply); specify by(#) how many of each test are used i.e. culture vs culture (3), or Culture (1) vs ELISA (1) :
    [ ] Culture vs. Culture ( )
    [ ] Culture ( ) vs. ELISA ( )
    [ ] Culture ( ) vs. PCR ( )
    [ ] ELISA vs. ELISA ( )
    [ ] ELISA ( ) vs. PCR ( )
    [ ] PCR vs. PCR ( )
    [ ] Single test only (specify)

A.5 Data Extraction Level II (test comparison – protocol and outcomes)

The following variables describe the data which are to be extracted from each unique test comparison:

VARIABLE	DESCRIPTION
refID	Article unique identifier
Population	Describe the population of pigs sampled (e.g. nursery, grow-finish, on-farm, abattoir, etc.)
Sample Type1	Type of sample matrix tested by Test 1
Sample Type2	Type of sample matrix tested by Test 2
Test1 Sample Size	Weight/volume of sample used in Test 1
Test2 Sample Size	Weight/volume of sample used in Test 2
Reference Standard	Describe how the reference standard was defined (e.g. positive by Test 2 only; positive by Test 1 or Test 2; etc)
N test 1	Number of samples tested by Test 1
N test 2	Number of samples tested by Test 2
Level	Sampling level (individual, pen/group, herd)
Test1_Type	Category of test, Test 1 (culture, ab-ELISA, ag-ELISA, PCR)
Test1	Within each article, assign unique identifier to each test within a category in order of appearance in article (e.g. Culture1, Culture2, PCR1, PCR2, etc.). Then, record the identifier for the index test here)
Test1_ID	Combined unique identifier (Test1_ID) with refID to create unique test protocol identifier (e.g. refID 221, Culture1 becomes 221Culture1)
Test2_Type	Category of test, Test 2 (culture, ab-ELISA, ag-ELISA, PCR)
Test2	Within each article, assign unique identifier to each test within a category in order of appearance in article (e.g. Culture1, Culture2, PCR1, PCR2, etc.). Then, record the identifier for the reference test here)
Test2ID	Combined unique identifier (Test2_ID) with refID to create unique test protocol identifier (e.g. refID 221, Culture1 becomes 221C1)
ELISA OD%	For each ELISA protocol reported in this record, record the reported optical density cutoff value
SP neg cutoff	If ELISA cutoff value is reported as an S/P ratio, record the negative cutoff value here
SP pos cutoff	If ELISA cutoff value is reported as an S/P ratio, record the positive cutoff value here
cell a	For 2x2 cross-tabulated test results, record the value of cell "a" here
cell b	For 2x2 cross-tabulated test results, record the value of cell "b" here
cell c	For 2x2 cross-tabulated test results, record the value of cell "c" here

cell d	For 2x2 cross-tabulated test results, record the value of cell "d" here
Kappa	Reported kappa value
Kappa s.e.	Reported kappa value standard error, standard deviation or confidence interval
Per_agree	Reported percent agreement
Per_agree s.e.	Reported percent agreement standard error, standard deviation or confidence interval
Correlation	Reported correlation
Correlation p-value	Reported correlation associated P-value
T1 Se	Reported sensitivity for Test1
T1 Se CI lower	Reported sensitivity for Test1, lower confidence limit
T1 Se CI upper	Reported sensitivity for Test1, upper confidence limit
T1 Sp	Reported specificity for Test1
T1 Sp CI lower	Reported specificity for Test1, lower confidence limit
T1 Sp CI upper	Reported specificity for Test1, upper confidence limit
T2 Se	Reported sensitivity for Test2
T2 Se CI lower	Reported sensitivity for Test2, lower confidence limit
T2 Se CI upper	Reported sensitivity for Test2, upper confidence limit
T2 Sp	Reported specificity for Test2
T2 Sp CI lower	Reported specificity for Test2, lower confidence limit
T2 Sp CI upper	Reported specificity for Test2, upper confidence limit
Test1 % positive	Proportion of samples positive by Test1
Test2 % positive	Proportion of samples positive by Test2
Test1 inconclusive	Number of inconclusive test results reported for Test1
Test2 inconclusive	Number of inconclusive test results reported for Test2
Serovars detected	List serovars detected, number of different serovars detected, or "not reported"
NOTES	Record any pertinent information not captured by above fields

A.6 Culture protocol details

1. How many different culture protocols are reported? (check all that apply)
   [ ] NONE
   [ ] FEWER than TWO
   [ ] FEWER than THREE
   [ ] FEWER than FOUR
   [ ] FEWER than FIVE
   [ ] FEWER than SIX

2. Does this article only report the protocol as "published/reported" elsewhere?
   [ ] NO
   [ ] YES (please record citation of referenced article)

(Note: questions 3-16 will be repeated for each unique culture protocol within this reference)

3. Sample cultured:
   [ ] Feces (describe source)
   [ ] Lymph nodes (describe source)
   [ ] Carcass swab
   [ ] Other (indicate type, source)

4. Size of initial sample: (e.g. 1 g, 10 ml, etc)

5. Sample size cultured:

6. Pre-enrichment broth: (check all that apply)
   [ ] Buffered peptone water (BPW)
   [ ] Other (describe)
   [ ] Not applicable

7. Volume of pre-enrichment broth (e.g. 10 ml; if more than one pre-enrichment, list volume by broth)

8. Incubation temperature: (if more than one broth, list temperature by broth)

9. Incubation time (e.g. 20-24 hrs): (if more than one broth, list time by broth)

10. What selective enrichment broth(s) were used?
    [ ] Tetrathionate
    [ ] Selenite
    [ ] Rappaport Vassiliadis
    [ ] Other (indicate type, additives if any)

11. What was the enrichment incubation temperature? (if more than 1, list temperature by broth)

12. What was the incubation time? (if more than one, list time by broth)

13. What selective agars were used? (note any modifications reported)
    [ ] XLT4
    [ ] Rambach (RAM
    [ ] Modified semi-solid RV (MSRV)
    [ ] Brilliant Green (BG)
    [ ] *Salmonella*-Shigella (SS)
    [ ] Chromogenic
    [ ] MacConkey
    [ ] XLD
    [ ] DIASALM
    [ ] Other

14. For each agar listed above, outline (in a comma-delimitated format) the agar, type of inoculant (broth), incubation temp, incubation time (or time plates read) at time 1, time 2(if applicable). (e.g. XLT4, TT, 35C, 24h, 48h):

15. Outline any additional steps not captured by the above questions:

16. Notes: (for any pertinent information not captured by above questions):

A.7 ELISA protocol details

1. How many different ELISA protocols are reported in this reference? (check all that apply)
    [ ] NONE
    [ ] FEWER than TWO
    [ ] FEWER than THREE
    [ ] FEWER than FOUR
    [ ] FEWER than FIVE
    [ ] FEWER than SIX

(Note: questions 2-8 will be repeated for each unique ELISA protocol within this reference)

2. Check the appropriate ELISA type below:
    [ ] HerdChek (Idexx)
    [ ] Salmotype
    [ ] Svanovir
    [ ] VetSign
    [ ] Other commercial ag-ELISA (specify)
    [ ] In-house ag-ELISA (provide laboratory name or description)
    [ ] VIDAS-SLM antigen capture
    [ ] Other antigen capture (specify)

3. Does this article only report the protocol as "published/reported" elsewhere?
    [ ] NO
    [ ] YES (please record citation of referenced article)

4. Is the protocol reported only as "according to manufacturer's instructions"?
    [ ] NO
    [ ] YES

5. As far as you can determine, are there any deviations from "manufacturer's directions?" (does not apply to in-house ELISA's)
    [ ] No, or not stated
    [ ] Yes (briefly describe deviations)

6. From what *Salmonella* serotypes were antigens for this ELISA obtained? (check all that apply)
    [ ] *S.* Choleraesuis
    [ ] *S.* Typhimurium
    [ ] Other (list)
    [ ] Serotype source was not reported

7. Where the specific antigens incorporated in the ELISA reported? (i.e. 1, 4, 7, etc)
   [ ] NO
   [ ] YES (list antigens in ascending order, comma delimited)

8. Was the wavelength used to measure the antibody levels reported?
   [ ] NO
   [ ] YES (record wavelength here)

A.8 PCR protocol details

1. How many different PCR protocols are reported in this reference? (check all that apply)
   [ ] NONE
   [ ] FEWER than TWO
   [ ] FEWER than THREE
   [ ] FEWER than FOUR
   [ ] FEWER than FIVE

(Note: questions 2-16 will be repeated for each unique PCR protocol within this reference)

2. Indicate PCR "Type" (e.g. "PCR", "multiplex PCR", rt-PCR, etc)

3. Describe sample/tissue type:

4. Were samples frozen at any time?
   [ ] NO
   [ ] YES

5. Was PCR done direct on sample or from pre-enriched or cultured sample?)
   [ ] Pre-enriched/enriched
   [ ] Direct on sample

6. Pre-enrichment broth used?
   [ ] NO
   [ ] YES (if yes, list broth type, temp, time)

7. Enrichment broth used?
   [ ] NO
   [ ] YES (if yes, list broth type, temp, time)

8. Selective agar used?
   [ ] NO
   [ ] YES (if yes, list agar type, temp, time)

9. Was *Salmonella* confirmed via isolation/serotyping?
   [ ] NO
   [ ] YES

10. What was the volume/weight of sample used:

11. Describe the extraction method (e.g. phenol/chloroform, diatomaceous earth, etc)

12. Primer Pair information: (check and describe all that apply)
    - [ ] Name/ID
    - [ ] Primer designer
    - [ ] Gene source
    - [ ] #Base pairs
    - [ ] Size of product
    - [ ] Kit name
    - [ ] Not reported

13. Volume of extract used:

14. Positive control information: (check and describe all that apply)
    - [ ] Strain source
    - [ ] ID designation
    - [ ] Not reported

15. Negative control: (describe)

16. Comments:

APPENDIX B: LIST OF RELEVANT ARTICLES EXCLUDED AT QUALITY ASSESSMENT

Reference:	**Reason for Exclusion:**
Achterberg R, Maneschijn-Bonsing J, Bloemraad R, et al. Detecting *Salmonella* antibodies in pork. New Food 2006;8:56-58.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Anderson RC, Genovese KJ, Harvey RB, et al. Assessment of the long-term shedding pattern of *Salmonella* serovar Choleraesuis following experimental infection of neonatal piglets. J Vet Diagn Invest 2000;12:257-260.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Arnold T, Scholz HC, Marg H, et al. Impact of invA-PCR and culture detection methods on occurrence, and survival of *Salmonella* in the flesh, internal organs and lymphoid tissues of experimentally infected pigs. J Vet Med B Infect Dis Vet Public Health 2004;51:459-463.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Asai T, Fujii S, Osumi T, et al. Isolation and serological survey of *Salmonella* in pigs in Japan. J Vet Med Sci 2002;64:1011-1015.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Bager F, Baggesen DL, Nielsen B. Control of *Salmonella* in the Danish national pig herd. Proc of the 8th International Congress on Animal Hygiene 1994.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Baggesen DL. Failure to prove the effect of feeding on experimental *Salmonella* Typhimurium infections in pigs. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Crete, Greece) 2003.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Baggesen DL, Wingstrand A, Carstensen B, et al. Effects of the antimicrobial growth promoter tylosin on subclinical infection of pigs with *Salmonella enterica* serotype Typhimurium. Am J Vet Res 1999;60:1201-1206.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Baum DH. Vaccine and epidemiologic studies of *Salmonella* infections in swine. Iowa State University. United States -- Iowa: Iowa State University, 1997.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Baum DH, Ward S, Baum CL, et al. Statistical process control methods used to evaluate the serologic responses of pigs infected with three *Salmonella* serovars. J Swine Health Prod 2005;13:304–313.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Beloeil PA, Chauvin C, Proux K, et al. Impact of the *Salmonella* status of market-age pigs and the pre-slaughter process on *Salmonella* caecal contamination at slaughter. Vet Res 2004;35:513-530.	No estimates of test performance extractable, nor sufficient data for post hoc calculation

Reference:	Reason for Exclusion:
Beloeil PA, Chauvin C, Proux K, et al. Longitudinal serological responses to *Salmonella enterica* of growing pigs in a subclinically infected herd. J Swine Health Prod 2003;60:207-226.	No estimates of test performance extractable, nor sufficient data for post hoc calculation (results are from different sample types combined)
Blaha T, Ehlers J, Methner U, et al. Proficiency test of four *Salmonella* antibody ELISA tests for their harmonization. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Crete, Greece) 2003.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Boughton C. Infection of pigs following exposure to contaminated pen floors. Proc of the 6th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork (Rohnert Park, USA) 2005.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Camitz A, Holmquist G, Ballagi A, et al. HerdChek *Salmonella* antibody ELISA for the serological monitoring of *Salmonella* infection in swine. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	Test protocol(s) not described in sufficient detail
Charles SD, Abraham AS, Trigo ET, et al. Reduced shedding and clinical signs of *Salmonella* Typhimurium in nursery pigs vaccinated with a *Salmonella* Choleraesuis vaccine. J Swine Health Prod 2000;8:107-112.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Christensen J, Baggesen DL, Soerensen V, et al. *Salmonella* level of Danish swine herds based on serological examination of meat-juice samples and *Salmonella* occurrence measured by bacteriological follow-up. Prev Vet Med 1999;40:277-292.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Cook ACJ. Measuring the impact of *Salmonella* control in finishing pigs - lessons from a pilot study. Pig Journal 2004;53:157-163.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Cote S, Letellier A, Lessard L, et al. Distribution of *Salmonella* in tissues following natural and experimental infection in pigs. Can J Vet Res 2004;68:241-248.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Dahl J. Monitoring changes in the association between *Salmonella*-serology and microbiology over time. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Dahl J, Wingstrand A, Nielsen B, et al. Elimination of *Salmonella* Typhimurium infection by the strategic movement of pigs. Vet Rec 1997;140:679-681.	No estimates of test performance extractable, nor sufficient data for post hoc calculation

Reference:	Reason for Exclusion:
Davies PR, Morrow WEM, Jones FT, et al. Risk of shedding *Salmonella* organisms by market-age hogs in a barn with open-flush gutters. J Am Vet Med Assoc 1997;210:386-389.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Davies RH, Bedford S, Shankster S. Enhanced culture techniques for the detection of *Salmonella*. Vet Rec 2001;148:539-540.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Ekeroth L, Alban L, Feld N. Single versus double testing of meat-juice samples for *Salmonella* antibodies, in the Danish pig-herd surveillance programme. Prev Vet Med 2003;60:155-165.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Enøe C, Andersen S, Wachmann H, et al. Estimation of sensitivity and specificity of an indirect enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against *Salmonella enterica* in meat juice and of microbiological examination of caecal content and mesenteric caecal lymph nodes for *S. enterica*. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	Test protocol(s) not described in sufficient detail
Enøe C, Boes J, Dahl J, et al. Sensitivity of cultivation of *Salmonella enterica* in pooled samples of pig faeces. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Crete, Greece) 2003.	Test protocol(s) not described in sufficient detail
Erdman MM, Harris IT, Harris DL. Isolation of *Salmonella* using pooled pen feces from 37 U.S. swine farms. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Erdman MM, Harris IT, Torremorell M, et al. Occurrence of *Salmonella* serotype Typhimurium DT104 on a commercial swine farm before, during, and after depopulation and repopulation. J Am Vet Med Assoc 2005;227:460-466.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Feder I. Conventional and molecular diagnosis for the detection of *Salmonella* Choleraesuis in porcine feces. Kansas State University. United States -- Kansas: Kansas State University, 1999.	No estimates of test performance extractable, nor sufficient data for post hoc calculation (data from artificially contaminated feces only)
Feld NC, Ekeroth L, Møgelmose V, et al. Correlation between color of meat juice samples and *Salmonella* antibody levels in the Danish mix-ELISA. Proc of the 6th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork (Rohnert Park, USA) 2005.	No estimates of test performance extractable, nor sufficient data for post hoc calculation

Reference:	Reason for Exclusion:
Gibson K, Ritter L, Blaha T, et al. Monitoring the dynamics of *Salmonella* prevalence in commercial swine herds. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	Test protocol(s) not described in sufficient detail
Grafanakis E, Leontides L, Genigeorgis C. Seroprevalence and antibiotic sensitivity of serotypes of *Salmonella enterica* in greek pig herds. Vet Rec 2001;148:407-411.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Gray JT, Fedorka-Cray PJ. Development of an ELISA for the detection of swine exposed to *Salmonella* spp. Abstracts of the General Meeting of the American Society for Microbiology 1996;96.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Gray JT, Fedorka-Cray PJ, Stabel TJ, et al. Influence of inoculation route on the carrier state of *Salmonella* Choleraesuis in swine. Vet Microb 1995;47:43-59.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Gray JT, Fedorka-Cray PJ, Stabel TJ, et al. Natural transmission of *Salmonella* Choleraesuis in swine. Appl Environ Microbiol 1996;62:141-146.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Hamilton D, Bobbitt J, Lester S, et al. Effect of pre-slaughter handling and serology on *Salmonella* in pigs. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Crete, Greece) 2003.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Hanes DE, Koch WH, Miliotis MD, et al. DNA probe for detecting *Salmonella* Enteritidis in food. Mol Cell Probes 1995;9:9-18.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Harkins KR, Harrigan K, Dickson S. A rapid presence absence test for *Salmonella* from hog carcass sponges using fluorescent probes and cytometric technology. Abstracts of the General Meeting of the American Society for Microbiology 2003;103:P-067.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Hurd HS, McKean JD, Griffith RD, et al. Estimation of the *Salmonella enterica* prevalence in finishing swine. Epidemiol Infect 2004;132:127-135.	Test protocol(s) not described in sufficient detail
Hurd HS, McKean JD, Wesley IV, et al. The effect of lairage on *Salmonella* isolation from market swine. J food prot 2001;64:939-944.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Jauho ES, Boas U, Wiuff C, et al. New technology for regiospecific covalent coupling of polysaccharide antigens in ELISA for serological detection. J Immunol Methods 2000;242:133-143.	No estimates of test performance extractable, nor sufficient data for post hoc calculation

Reference:	Reason for Exclusion:
Jayarao BM, Biro G, Kovacs S, et al. Prevalence of *Salmonella* serotypes in pigs and evaluation of a rapid presumptive test for detection of *Salmonella* in pig feces. Acta Veterinaria Hungarica 1989;37:39-44.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Jensen AN, Dalsgaard A, Stockmarr A, et al. Survival and transmission of *Salmonella enterica* serovar Typhimurium in an outdoor organic pig farming environment. Appl Environ Microbiol 2006;72:1833-1842.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Jensen AN, Hoorfar J. Optimal purification and sensitive quantification of DNA from fecal samples. J Rapid Methods Autom Microbiol 2002;10:231-244.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Kich JD, Cardoso M, Coldebella A, et al. Development of an ELISA test for *Salmonella* serological monitoring in Brazil. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	Test protocol(s) not described in sufficient detail
Kozlowski MB, Leonard FC, Egan J, et al. On-farm control measures for the reduction of salmonellosis in pigs. Proc of the 6th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork (Rohnert Park, USA) 2005.	Test protocol(s) not described in sufficient detail
Kramer TT, Rhiner JA. Detection and distribution of *Salmonella* Choleraesuis in Iowa swine. Proc of the 96th Meeting of the US Animal Health Association 1992.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Kranker S, Alban L, Boes J, et al. Longitudinal study of *Salmonella enterica* serotype Typhimurium infection in three Danish farrow-to-finish swine herds. J Clin Microbiol 2003;41:2282-2288.	Test protocol(s) not described in sufficient detail
Kristensen S. Effect of benzoic acid in the feed on *Salmonella* Typhimurium in weaned pigs. Proc of the 6th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork (Rohnert Park, USA) 2005.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Lawhorn B, Dubose BS, Thompson J, et al. Group relationship of *Salmonella* ELISA antibody status of grower-finisher hogs to fecal shedding detectable by culture. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Lehmann J, Weber, Höschler, et al. The combination of two *Salmonella* - antigen test systems for reliable diagnostic of salmonellosis in stockbreeding pigs.Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	No estimates of test performance extractable, nor sufficient data for post hoc calculation

Reference:	Reason for Exclusion:
Letellier A. Population of a farrowing unit by *Salmonella* negative animals. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Crete, Greece) 2003.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Letellier A, Cote S, Surprenant C, et al. Use of serology to evaluate the impact of clinical salmonellosis in swine on the herd status and on the contamination of pig carcasses from affected herds. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Leyk W. *Salmonella* serology – which samples should be used: comparison of meat juice and serum samples of the same pigs. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Crete, Greece) 2003.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Maes D, Gibson K, Trigo E, et al. Evaluation of cross-protection afforded by a *Salmonella* Choleraesuis vaccine against *Salmonella* infections in pigs under field conditions. Berl Munch Tierarztl Wochenschr 2001;114:339-341.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Mejia W, Zapata D, Mateu E, et al. Lack of specificity of a combination of Rappaport-Vassiliadis broth and XLT4 agar for the isolation of *Salmonella*e from pig faeces. Vet Rec 2005;156:150-151.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Methner U, Rosner H, Müller G, et al. Studies on the influence of ochratoxin A administration on *Salmonella* Typhimurium infection in pigs. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Mul MF, van der Gaag MA. Control of *Salmonella* at pig finishing farms with a farm decision tree. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Crete, Greece) 2003.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Narayanaswamy S, Krishnappa G. Comparative evaluation of different serological tests for diagnosis of salmonellosis in pigs. Mysore J Ag Sci 1997;31:94-97.	No estimates of test performance extractable, nor sufficient data for post hoc calculation

Reference:	Reason for Exclusion:
Nielsen B, Baggesen D, Bager F, et al. The serological response to *Salmonella* serovars Typhimurium and Infantis in experimentally infected pigs. The time course followed with an indirect anti-LPS ELISA and bacteriological examinations. Vet Microb 1995;47:205-218.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Nietfeld JC, Feder I, Kramer TT, et al. Preventing *Salmonella* infection in pigs with offsite weaning. J Swine Health Prod 1998;6:27-32.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Nietfeld JC, Kelly B, Dritz SS, et al. Comparison of conventional and delayed secondary enrichment for isolation of *Salmonella* spp. from swine samples. J vet diagn invest 1998;10:285-287.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Nollet N, Houf K, Dewulf J, et al. Distribution of *Salmonella* strains in farrow-to-finish pig herds: a longitudinal study. J Food Prot 2005;68:2012-2021.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
O'Connor JL, Snow LC, Cook AJ. A trial investigating the effect of organic acids in weaner pigs' diets on *Salmonella* prevalence in finisher pigs. Res Vet Sci 2005;78:S1–S46.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Olsen JE, Aabo S, Nielsen EO, et al. Isolation of a *Salmonella*-specific DNA hybridization probe. APMIS 1991;99:114-120.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Pangloli P, Dje Y, Oliver SP, et al. Evaluation of methods for recovery of *Salmonella* from dairy cattle, poultry, and swine farms. J Food Prot 2003;66:1987-1995.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Proux K, Cariolet R, Fravalo P, et al. Contamination of pigs by nose-to-nose contact or airborne transmission of *Salmonella* Typhimurium. Vet Res 2001;32:591-600.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Quessy S, Guevremont E, Beauchamp G, et al. Risk factors associated with presence of *Salmonella* in pigs in Canada. Proc of the 6th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork (Rohnert Park, USA) 2005.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Reissbrodt R, Gaull F, Fehlhaber K. Evaluation of a new chromogenic *Salmonella* plating medium. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Roesler U, Altrock Av, Heller P, et al. Effects of fluorequinolone treatment acidified feed, and improved hygiene measures on the occurrence of *Salmonella* Typhimurium DT104 in an integrated pig breeding herd. J Vet Med B Infect Dis Vet Public Health 2005;52:69-74.	No estimates of test performance extractable, nor sufficient data for post hoc calculation

Reference:	Reason for Exclusion:
Roesler U, Heller P, Waldmann KH, et al. Immunization of sows in an integrated pig-breeding herd using a homologous inactivated *Salmonella* vaccine decreases the prevalence of *Salmonella* Typhimurium infection in the offspring. J Vet Med B Infect Dis Vet Public Health 2006;53:224-228.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Rowe T, Leonard F, Kelly G, et al. *Salmonella* serotypes present on a sample of Irish pig farms. Vet Rec 2003;153:453-456.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Scholz HC, Arnold T, Marg H, et al. Improvement of an invA-based PCR for the specific detection of *Salmonella* Typhimurium in organs of pigs. Berl Munch Tierarztl Wochenschr 2001;114:401-403.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Schwarz P, Bessa MC, Kich JD, et al. The correlation between serology and isolation of *Salmonella* in pigs at slaughter in southern Brazil. Proc of the 6th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork (Rohnert Park, USA) 2005.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Skovgaard N, Christensen SG, Gulistani AW. *Salmonella*s in Danish pigs: a comparison of three isolation methods. J Hyg 1985;95:69-75.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Sorensen LL, Alban L, Nielsen B, et al. The correlation between *Salmonella* serology and isolation of *Salmonella* in Danish pigs at slaughter. Vet Microb 2004;101:131-141.	No estimates of test performance extractable, nor sufficient data for post hoc calculation (results of ag-ELISA and culture pooled)
Spiehs MJ. Evaluation of dietary manipulations to improve growth performance and reduce *Salmonella* Typhimurium prevalence and shedding in finishing swine. University of Minnesota. United States -- Minnesota: University of Minnesota, 2004	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Springer S, Lindner T, Steinbach G, et al. Investigation of the efficacy of a genetically-stabile live *Salmonella* Typhimurium vaccine for use in swine. Berl Munch Tierarztl Wochenschr 2001;114:342-345	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Srinand S, Robinson RA, Collins JE, et al. Serologic studies of experimentally-induced *Salmonella* Choleraesuis var Kunzendorf infection in pigs.Am J Vet Res 1995;56:1163-1168.	Test protocol(s) not described in sufficient detail
Stankevicius A, Wasyl D, Jablonski A, et al. One-tube nested PCR for the detection of *Salmonella* sp. in swine faeces. Bull Vet Inst Pulawy 2006;50:35-39.	Test protocol(s) not described in sufficient detail
Stege H, Christensen J, Nielsen JP, et al. Prevalence of subclinical *Salmonella enterica* infection in Danish finishing pig herds. Prev Vet Med 2000;44:175-188.	No estimates of test performance extractable, nor sufficient data for post hoc calculation

Reference:	Reason for Exclusion:
Stege H, Feld NC, Baggesen DL, et al. Subclinical *Salmonella* infection in Danish finishing pig herds: association between serological and bacteriological testing. Epidemiologie et Sante Animale 1997:07.13.01-07.13.03.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Stege H, Jensen TK, Moller K, et al. Prevalence of intestinal pathogens in Danish finishing pig herds. Prev Vet Med 2000;26:279-292.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Steinbach G, Blaha T, Methner U. Estimating the prevalence of *Salmonella* spp. in swine herds: Influence of sensitivity and specificity of *Salmonella* detection. J Vet Med B Infect Dis Vet Public Health 2002;49:438-444.	Test protocol(s) not described in sufficient detail
Stone GG, Oberst RD, Hays MP, et al. Detection of *Salmonella* serovars from clinical-samples by enrichment broth cultivation PCR procedure. J Clin Microbiol 1994;32:1742-1749.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Stone GG, Oberst RD, Hays MP, et al. Combined PCR-oligonucleotide ligation assay for rapid detection of *Salmonella* serovars. J Clin Microbiol 1995;33:2888-2893.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Suh DK, Song JC. Simultaneous detection of Lawsonia intracellularis, Brachyspira hyodysenteriae and *Salmonella* spp. in swine intestinal specimens by multiplex polymerase chain reaction. J Vet Sci 2005;6:231-237.	Test protocol(s) not described in sufficient detail
Swanenburg M, Urlings HAP, Snijders JMA, et al. *Salmonella* in slaughter pigs: prevalence, serotypes and critical control points during slaughter in two slaughterhouses. Int J Food Microbiol 2001;70:243-254.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Szaszák A, Blaha TG, Deen J, et al. Evaluation of the suitability of a commercially available ELISA test as a nonitoring tool for estimating the *Salmonella* prevalence of commercial swine herds. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Leipzig, Germany) 2001.	Test protocol(s) not described in sufficient detail
Tapchaisri P, Wangroongsarb P, Panbangred W, et al. Detection of *Salmonella* contamination in food samples by dot-ELISA, DNA amplification and bacterial culture. Asian Pac J Allergy Immunol 1999;17:41-51.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Tsai-Hsin C, Jen-Chieh P, Wen-Zhe H, et al. Development of PCR primers for the detection of *Salmonella enterica* serovar Choleraesuis based on the fliC gene. J Food Prot 2005;68:1575-1580.	No estimates of test performance extractable, nor sufficient data for post hoc calculation

Reference:	Reason for Exclusion:
van der Wolf PJ, Wong DMALF, Wolbers WB, et al. A longitudinal study of *Salmonella enterica* infections in high- and low-seroprevalence finishing swine herds in the Netherlands.Vet Q 2001;23:116-121.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
van Winsen RL, van Nes A, Keuzenkamp D, et al. Monitoring of transmission of *Salmonella enterica* serovars in pigs using bacteriological and serological detection methods. Vet Microbiol 2001;80:267-274.	No estimates of test performance extractable, nor sufficient data for post hoc calculation (results different tests pooled)
Vassiliadis P, Mavrommati C, Efstratiou M, et al. A note on the stability of Rappaport-Vassiliadis enrichment medium. J Appl Bacteriol 1985;59:143-145.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Wilkins, Waldner, Rajić , et al. Estimation of Sensitivity and Specificity of Culture and Danish-Mix ELISA for detection of *Salmonella* in swine using Bayesian methods.Proc of the 6th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork (Rohnert Park, USA) 2005.	Test protocol(s) not described in sufficient detail
Williams DR, Hunter D, Binder J, et al. Observations on the occurrence of *Salmonella* Choleraesuis and other *Salmonella*s in 2 herds of feeder pigs. J Hyg 1981;86:369-377.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Xirouchaki E, Vassiliadis P, Trichopoulos D, et al. A note on the performance of Rappaport's medium, compared with Rappaport-Vassiliadis broth, in the isolation of *Salmonella*s from meat products, after pre-enrichment. J Appl Bacteriol 1982;52:125-127.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Elder RO, Duhamel GE, Mathiesen MR, et al. Multiplex polymerase chain reaction for simultaneous detection of Lawsonia intracellularis, Serpulina hyodysenteriae, and *Salmonella*e in porcine intestinal specimens. J Vet Diagn Invest 1997;9:281-286.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Lehmann J, Roesler U, Lindner T, et al. Discrimination of vaccinated and infected pigs by *Salmonella*-specific IGa antibodies. Proc of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Foodborne Pathogens in Pork (Crete, Greece) 2003.	No estimates of test performance extractable, nor sufficient data for post hoc calculation
Reynisson E, Josefsen MH, Krause M, et al. Evaluation of probe chemistries and platforms to improve the detection limit of real-time PCR. J Microbiol Methods 2005;66:206-216	No estimates of test performance extractable, nor sufficient data for post hoc calculation

APPENDIX C: ON-FARM SAMPLE AND DATA COLLECTION TOOLS

C.1 Farm details and herd management information

1. General Farm Information

legal required
Legal Land Location Q____Sect_____Twp _____Rng_____ Mer_____
GPS northing_____ Westing_____

A. Herd type: ☐farrow-finish ☐other (specify)_____
B. Operation type: ☐commercial ☐breeding stock ☐multiplier
C. Number of animals finished/year: _____
D. ☐ **CQA Registered** ☐ **CQA Validated**

E. Get age and weight if possible, one or the other is required. Or can give number of days in each phase but make sure that is clearly indicated i.e. 3 weeks in nursery vs. age at leaving nursery

Production Phase	Age out	Weight out
Farrowing (weaning age)		
Nursery		
Pre-grow		
Grow		
Finish		

F. Pig Flow (NA applies if animals originate within herd i.e. raise own gilts, own boars, own finishers, no outside animals brought in)

Breeding females entering unit originate from a maximum of:
☐ One source ☐ Two sources
☐ Three sources ☐ Four or more sources ☐ Not applicable

Breeding males entering unit originate from a maximum of:
☐ One source ☐ Two sources
☐ Three sources ☐ Four or more sources ☐ Not applicable

Piglets entering nurseries originate from a maximum of (farrowing units):
☐ One source ☐ Two sources
☐ Three sources ☐ Four or more sources ☐ Not applicable

Feeder pigs entering finishing units originate from a maximum of (nurseries):
☐ One source ☐ Two sources
☐ Three sources ☐ Four or more sources ☐ Not applicable

Is batch farrowing (all-in all-out) used? (circle one) YES NO N/A
Is batch production used in nursery? YES NO N/A
Is batch production used in grow-finish? YES NO N/A
(Note: ANY pigs left in the room before the next batch is not all-in all –out)
F-F: are all phases of production housed on: ☐one site ☐two sites ☐3 sites
If one site, are all phases of production housed in: ☐one barn ☐2 barns ☐3 or more

G. Biosecurity (check all that apply)

☐ Shower in ☐ Shower out ☐ Wash hands prior to entry
☐ Change clothes to farm-provided apparel ☐ Wash footwear prior to entry
☐ Change footwear to farm-provided footwear
☐ Other (describe)_____

Down time required between farm visits:_____ Strictly enforced? ☐ Yes ☐ No
Are there quarantine facilities for incoming animals? ☐ Yes ☐ No
Are quarantine facilities routinely used? ☐ Yes ☐ No ☐ N/A
Average number of days incoming animals are quarantined _____
Is the same truck used to transport animals and feedstuff? ☐ Yes ☐ No
Is there a toilet in your production facilities? ☐ Yes ☐ No
Is there a sink for washing your hands? ☐ Yes ☐ No
Are cats/dogs allowed into the barn on a regular basis? ☐ Yes ☐ No
Do birds have access to barn (i.e. often seen indoors)? ☐ Yes ☐ No
Have rodents or rodent pellets been observed in the barn? ☐ Yes ☐ No

H. Treatment records

Are treatments recorded for:

☐Breeding animals ☐Weaned pigs
☐Suckling pigs ☐Grow-finish
☐Feed additives

2. Breeding/Gestation/Farrowing

A. Housing/Hygiene

Are sows/gilts: ☐AI'd ☐hand-mated ☐pen-mated (check all that apply)
If pen-mated, how long (on average) are animals housed in pens?_____

Gestation: Are sows/gilts housed: (check all that apply)
☐in pens if yes, duration_____ number per pen_____
floor type **C/FS/PS** size of pen _____
☐in stalls if yes, floor type **C/FS/PS**
(C=solid concrete , FS = full slat, PS = part slat, any variation make a note in the margin)

Total area (square meters or feet) of the breeding/gestation/farrow production area _____

Is litter bedding used? ☐ Yes ☐ No
If yes, which type? _____
If concrete floor is used, is manure removed from pens at least once a week? ☐ Yes ☐ No
How frequently is the production area washed AND disinfected? _____times/year
Which type of disinfectant is used?_____
If batch production is used:
Are rooms completely emptied between batches: ☐ Yes ☐ No
Are pens cleaned to a manure-free stage between batches? ☐ Yes ☐ No
Are cleaned pens disinfected between batches? ☐ Yes ☐ No
Average number of time production area is kept empty between batches_____

B. Health

In the breeding population (sows and gilts): Has there been a change in health problems in the last 6 months? ☐ Yes ☐ No
If yes, has it been ☐**increased** ☐**decreased**
Did you have to treat your pigs for diarrhea during the last 6 months? ☐ Yes ☐ No
If yes, did you treat ☐individuals ☐groups can select both if it applies
Did you have to treat your pigs for respiratory problems during the last 6 months? ☐ Yes ☐ No
If yes, did you treat ☐individuals ☐groups can select both if it applies
Is there a pen where sick pigs are isolated? ☐ Yes ☐ No
Are hands usually (at least 9/10 times) washed or disposable gloves used after treating sick animals? ☐ Yes ☐ No

C. Feed –

Breeding/gestation

Is feedstuff served pelleted? ☐ Yes ☐ No
Do you use mash feed? ☐ Yes ☐ No
How is feedstuff served? Check all that apply ☐ Dry ☐ Wet ☐ Fermented
Where is feedstuff prepared? ☐ on farm ☐ commercial feedmill
Has the feed type changed in the last 6 months? ☐ Yes ☐ No
Are antibiotics being added to the feed? ☐ Yes ☐ No
If yes, which ones?_____
List any other additives in the feed or water (i.e. acids, whey, other)_____
Briefly describe feeding practices in breeding/gestation/farrowing (brief description of feed type and duration just a few words ie feed wet to gestating, dry to breeding etc, or whatever is appropriate)_____
_____
_____

Feed – Farrowing
Is feedstuff served pelleted? ☐ Yes ☐ No

Do you use mash feed? ☐ Yes ☐ No
How is feedstuff served? ☐ Dry ☐ Wet ☐ Fermented
Where is feedstuff prepared? ☐ on farm ☐ commercial feedmill
Has the feed type changed in the last 6 months? ☐ Yes ☐ No
Are antibiotics being added to the feed? ☐ Yes ☐ No
If yes, which ones?_____
List any other additives in the feed or water (i.e. acids, whey, other)_____
Briefly describe feeding practices in breeding/gestation/farrowing (brief description of feed type and duration)_____
_____

3. Nursery

A. Housing/Hygiene (note: data on floor type etc is to be captured on a per-pen basis on separate form)

At what age are piglets weaned and moved to the nursery area? _____
Total area (square meters or feet) of the production area _____
How frequently is the production area washed AND disinfected? _____times/year
Which type of disinfectant is used?_____
Is litter bedding used? **Yes No**
If yes, what type?_____
If batch production is used:
Are rooms completely emptied between batches: ☐ Yes ☐ No
Are pens cleaned to a manure-free stage between batches? ☐ Yes ☐ No
Are cleaned pens disinfected between batches? ☐ Yes ☐ No
Average number of days production area is kept empty between batches_____

B. Health

In the nursery population:
Has there been a change in health problems in the last 6 months? ☐ Yes ☐ No
If yes, has it been ☐**increased** ☐**decreased**
Did you have to treat your pigs for diarrhea during the last 6 months? ☐ Yes ☐ No
If yes, did you treat ☐individuals ☐groups
Did you have to treat your pigs for respiratory problems during the last 6 months? ☐ Yes ☐ No
If yes, did you treat ☐individuals ☐groups
Is there a pen where sick pigs are isolated? ☐ Yes ☐ No
Are hands usually washed after treating sick animals? ☐ Yes ☐ No
Are runt pigs held back and mixed with other ages? ☐ Yes ☐ No

C. Feed

Is feedstuff served pelleted? ☐ Yes ☐ No
Do you use mash feed? ☐ Yes ☐ No
How is feedstuff served? Check all that apply ☐ Dry ☐ Wet ☐ Fermented
Where is feedstuff prepared? ☐ on farm ☐ commercial feedmill
Has the feed type changed in the last 6 months? ☐ Yes ☐ No
Are antibiotics being added to the feed? ☐ Yes ☐ No
If yes, which ones?_____
List any other additives in the feed or water (i.e. acids, whey, other)_____
Briefly describe feeding practices in the nursery (brief description of feed type and duration) ie feed wet mash for 1 week, dry pellets for 1 week,
etc)_____
_____

4. Grow Area (if just one G-F area, skip this section and go to 5 – Finisher)

A. Housing/Hygiene (note: data on floor type etc is to be captured on a per-pen basis on separate form)

Total area (square meters or feet) of the production area _____
How frequently is the production area washed AND disinfected? _____times/year
Which type of disinfectant is used?_____
Is litter bedding used? ☐**Yes** ☐**N0**
If yes, what type?_____
If batch production is used:
Are rooms completely emptied between batches: ☐ Yes ☐ No
Are pens cleaned to a manure-free stage between batches? ☐ Yes ☐ No
Are cleaned pens disinfected between batches? ☐ Yes ☐ No
Average number of days production area is kept empty between batches_____

B. Health

In the grow population:
Has there been a change in health problems in the last 6 months? ☐ Yes ☐ No
If yes, has it been ☐**increased** ☐**decreased**
Did you have to treat your pigs for diarrhea during the last 6 months? ☐ Yes ☐ No
If yes, did you treat ☐individuals ☐groups
Did you have to treat your pigs for respiratory problems during the last 6 months? ☐ Yes ☐ No
If yes, did you treat ☐individuals ☐groups
Is there a pen where sick pigs are isolated? ☐ Yes ☐ No
Are hands usually washed after treating sick animals? ☐ Yes ☐ No

Are runt pigs held back and mixed with other ages? ☐ Yes ☐ No

C. Feed

Is feedstuff served pelleted? ☐ Yes ☐ No
Do you use mash feed? ☐ Yes ☐ No
How is feedstuff served? ☐ Dry ☐ Wet ☐ Fermented
Where is feedstuff prepared? ☐ on farm ☐ commercial feed mill
Has the feed type changed in the last 6 months? ☐ Yes ☐ No
Are antibiotics being added to the feed? ☐ Yes ☐ No
If yes, which ones?_____ What amount?_____
List any other additives in the feed or water (i.e. acids, whey, other)_____
Briefly describe feeding practices in the grow area (brief description of feed types and duration)
_____
_____

5. Finishing

A. Housing/Hygiene (note: data on floor type etc is to be captured on a per-pen basis on separate form)

Total area (square meters or feet) of the production area _____
How frequently is the production area washed AND disinfected? _____times/year
Which type of disinfectant is used?_____
Is litter bedding used? **Yes No**
If yes, what type?_____
If batch production is used:
Are rooms completely emptied between batches: ☐ Yes ☐ No
Are pens cleaned to a manure-free stage between batches? ☐ Yes ☐ No
Are cleaned pens disinfected between batches? ☐ Yes ☐ No
Average number of days production area is kept empty between batches_____

B. Health

In the finishing population:
Has there been a change in health problems in the last 6 months? ☐ Yes ☐ No
If yes, has it been ☐**increased** ☐**decreased**
Did you have to treat your pigs for diarrhea during the last 6 months? ☐ Yes ☐ No
If yes, did you treat ☐individuals ☐groups
Did you have to treat your pigs for respiratory problems during the last 6 months? ☐ Yes ☐ No
If yes, did you treat ☐individuals ☐groups
Is there a pen where sick pigs are isolated? ☐ Yes ☐ No
Are hands usually washed after treating sick animals? ☐ Yes ☐ No

Are runt pigs held back and mixed with other ages? ☐ Yes ☐ No

C. Feed

Is feedstuff served pelleted?	☐ Yes	☐ No	
Do you use mash feed?	☐ Yes	☐ No	
How is feedstuff served?	☐ Dry	☐ Wet	☐ Fermented
Where is feedstuff prepared?	☐ on farm	☐ commercial feedmill	
Has the feed type changed in the last 6 months?	☐ Yes	☐ No	
Are antibiotics being added to the feed?	☐ Yes	☐ No	
If yes, which ones?_____			
Do you use Tylosin as a growth promoter?	☐ Yes	☐ No	

List any other additives in the feed or water (i.e. acids, whey, other)_____

Briefly describe feeding practices in the finishing area (brief description of feed type and duration)

_____

_____

Shipping information

To what slaughterhouse do you ship to?_____

What day of the week do you normally ship?_____

What are your next 3 scheduled shipping dates?_____

Approximately how many hogs are you shipping each time?_____

Approximately what time do your hogs arrive at the plant?_____

C.2 On-farm sample collection form

Production phase:						**Date:**			**Herd ID**							
Sample #	Pen ID	♂ Female Barrow Mix	Breed	Age (weeks)	# in pen	Duration in pen	Pen Score 0=clean 1=1/4 2=half 3=all	Pen Dimension	Fecal Fresh	Fecal 0=runny 1=normal 2=dry	Current Tx	Floor: Full, partial, solid	Concrete or Other	Nose to Nose	Cement Walls	Bedding & type
41460		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	
41461		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	
41462		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	
41463		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	
41464		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	
41465		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	
41466		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	
41467		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	
41468		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	
41469		♂ F B M	R W				0 1 2 3		Y N	0 1 2	Y N U	F P S	C O	Y N	Y N	