Quantification of Salmonella from porcine samples to identify potential critical control points in pre-harvest pork production

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UMI
Quantification of *Salmonella* from porcine samples to identify potential critical control points in pre-harvest pork production

by

Jared Keith Gailey

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Veterinary Microbiology

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Iowa State University
Ames, Iowa
2004
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For the Major Program
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GENERAL INTRODUCTION

Background
Salmonella is one of the most common foodborne pathogens in the United States. Each year it is responsible for approximately 1.4 million illnesses, 15,000 hospitalizations, and 500 deaths. Additionally, it has been estimated that the annual economic cost due to Salmonella infections is $2.4 billion.

Salmonellosis is frequently associated with poultry products, especially raw eggs. However, other common sources of Salmonella include pork, beef, other meat products, vegetables exposed to fecal material, pets, and pet foods. In recent years, a great deal of research has been conducted on the contributions of pork to human salmonellosis. Various portions of the pork production process have been scrutinized in an attempt to locate critical control points at which Salmonella can be contained, especially in the pre-harvest portion of the process. Suggested critical control points discussed in recent research include holding, transport, and the identification and segregation of high prevalence herds both in slaughter and transport.

Need for quantitative data
When conducting a hazard analysis to determine critical control points, two types of information about a specific pathogen are needed. First, the prevalence of the pathogen, or how often it occurs, must be identified. Next, the quantity of the pathogen, or how much of the pathogen exists in a positive sample, must be determined. This allows specific targeting at areas of the process where the pathogen occurs most often and at the highest load. Unfortunately, little quantitative data about Salmonella in pork currently exists and most potential critical control points have been identified using only prevalence data.
Dissertation Organization

This dissertation summarizes the doctoral efforts of Jared K. Gailey. The first section is literature review containing the information necessary to understand the subsequent summaries of my research. It reviews the cumulative knowledge of many respected researchers in the field of swine and *Salmonella* interactions. This review also illustrates the need for the research presented in this dissertation.

The next three sections are summaries of my doctoral research, arranged in chronological order. These sections will each be submitted to the *Journal of Food Protection* for publication. The first paper is a validation of a new enumeration technique for the quantification of *Salmonella*. This technique was found to be equivalent to the existing method, but requires much less time and media and therefore improves our ability to collect enumeration data. The second paper is a summary of enumeration data collected using the new method. Using this method, we were able to quantify the *Salmonella* loads in 114 samples from three Midwest abattoirs. Using this quantification data, an expected distribution of *Salmonella* loads in infected animals could be determined. With this distribution the likelihood of illnesses caused by pork can be predicted. The third paper uses this distribution, the prevalence of *Salmonella* in intestinal contents and lymph nodes, and a composite dose response curve in a model. This model evaluates the effects of hypothetical interventions applied in the last weeks of finishing as well as in the transport/holding portion of pork production. The effects measured include prevalence, average *Salmonella* carcass load, and potentially infectious doses on the carcass or in retail cuts. This model allows us to predict appropriate locations for critical control points in the pre-harvest portion of pork production. It was found that the final weeks of finishing and transport/holding were both appropriate critical control points.

The last two sections of this dissertation are appendices. Appendix A summarizes other research in which I participated in during my doctoral education and is intended to demonstrate the scope of my experience. During the course of
my doctoral education, I was able to participate in many other research opportunities that provided me opportunities to expand my views, understanding, and abilities. Appendix B is the source code for the model in my third research paper. This is included to allow replication of the model in the future, should other researchers desire to do so.

This dissertation is a summary of my doctoral experience. I hope that the research and insights provided herein will assist other investigators as they explore the relationships between pork, Salmonella and food safety.
LITERATURE REVIEW

Introduction

*Salmonella* is a member of the family Enterobacteriaceae. Members of this family are characterized as small gram-negative rods, generally motile by peritrichous flagella. Members of this family are aerobic or facultatively anaerobic and are commonly found in enteric samples. A common distinguishing family characteristic is the ability to produce acid through the fermentation of glucose (1).

Salmonellae are commonly divided into two groups, typhoidal and non-typhoidal. Typhoidal salmonellae (*Salmonella* Typhi, S. Paratyphi A, S. Paratyphi B, and S. Paratyphi C) is the cause of typhoid fever which has a case fatality rate of 10-20%, without antibiotic treatment and is responsible for 600,000 deaths annually worldwide. Since nearly all cases of typhoid fever occur in third world countries, the remainder of this review will focus on non-typhoidal salmonellae, one of the primary causes of foodborne illness in the United States (2).

Non-typhoidal Salmonellae are a diverse group of over 2400 serotypes (3). Some serotypes are commonly associated with a specific host. These include *Salmonella* Abortusovis in sheep, S. Gallinarium in birds, S. Choleraesuis in swine and S. arizonae in cold-blooded animals (4). The five most common serotypes causing human disease in 2002 were Typhimurium, Enteritidis, Newport, Heidelberg, and Javiana (5). The infective dose of non-typhoidal *Salmonella* is generally believed to be greater than $10^5$ cells, but many outbreaks have been caused by estimated doses of $10^1 - 10^3$ cells (6-9).

Salmonellae can grow between 7 and 48°C, with an optimal growth temperature of 37°C. Growth can also occur between pH 4-8 and above water activities of 0.93. Although they are readily killed by heat and acid, salmonellae can survive both freezing and drying. These characteristics, coupled with its ubiquitous
presence in animals and the environment, make *salmonellae* one of the most important foodborne agents in the United States (10).

**History of Salmonella**

The first record of the *Salmonella* species was by Eberth who noted its presence in spleen sections taken from a victim of typhoid fever. In 1884 Gaffky successfully cultivated what he termed “typhoid bacillus”. In 1886 D.E. Salmon and T. Smith first isolated, what would be later named in honor of Salmon, *Salmonella* Choleraesuis. As scientists continued to make discoveries, additional *Salmonella* serovars were recognized. By the 1920’s, approximately 20 serovars had been identified. White initiated a serovar classification scheme based on the O (somatic) and H (flagellar) antigens in 1926. In 1941 Kauffman extended the original method, and this eventually led to the present *Salmonella* serotyping method, the Kauffman-White scheme. Until recently, each serovar was written as a separate *Salmonella* species even though it was understood that each was really a specific serotype. Recent changes in nomenclature dictate that nearly all serovars of food safety importance belong to the species *Salmonella* enterica subspecies enterica. For example: *Salmonella enterica* subsp. enterica serovar Typhimurium (11). According to the nomenclature currently used by the Centers for Disease Control (CDC) it would be written *Salmonella* ser. Typhimurium the first time it is written and *Salmonella* Typhimurium thereafter (12).

**Salmonellosis**

**Symptoms**

*Salmonella* infection is usually accompanied by symptoms typical of many enteric diseases. These include inflammation of the intestines, headache, abdominal pain, diarrhea (occasionally bloody), nausea and occasionally vomiting.
Fever is almost always present and dehydration may be a concern in very young or elderly patients. Occasionally, the infection may evolve from enteric disease to a more serious localized infection of tissue or blood. The time to onset of symptoms ranges from 6-48 hours and symptoms generally continue for 1-2 days. In rare instances, arthritic sequelae may follow 3-4 weeks after infection. Deaths are rare and occur mainly in very young, elderly, or immuno-compromised populations. Sick individuals generally shed *Salmonella* in the feces for up to two weeks, but approximately 1% of adults and 5% of children can shed asymptptomatically for over a year. As symptoms generally resolve without the use of antibiotics, supportive treatment is recommended, although in life threatening cases the use of antibiotics may be appropriate (2,13).

**Sources of disease**

Common sources of salmonellae include poultry, pork, beef, other meat products, as well as eggs, vegetables exposed to fecal material, pets, and pet foods. *Salmonella* can generally be controlled by following basic sanitation guidelines. Especially important precautions include hand washing (after handling uncooked meats, diapers, or pets), keeping cooked and uncooked foods separated, thoroughly cooking animal products, as well as the proper treatment of leftovers (2,14).

**Incidence**

Mead et al (15) estimated that *Salmonella* causes over 1.4 million illnesses, 15,000 hospitalizations, and 500 deaths annually. Although fatalities due to *Salmonella* are very rare, *Salmonella* is responsible for 30% of all foodborne related deaths, mainly as a function of the vast number of illnesses that occur. A study that tracked the vehicles of foodborne illnesses from 1973-1987 indicated that *Salmonella* infections come from a wide range of sources (Table 1) (16).
Cost

While most cases of salmonellosis have few lasting consequences, salmonellosis is one of the most important foodborne illnesses from an economic standpoint. When calculating the economic impact of illnesses, items such as hospital bills, doctor visits, missed work, and loss of family income due to death must be considered. In 1985 Archer and Kvenberg (17) estimated that each hospitalized case of salmonellosis cost $2,025 and non-hospitalized cases an average of $675. In 1989, Todd (18) estimated that the average cost for each case of salmonellosis is $1,350. In 2002, the Economic Research Service branch of the USDA estimated that the annual economic cost due to Salmonella infections was $2.4 billion with $100 million of that being direct medical costs (19).

Pathogenesis

Upon ingestion, Salmonella rapidly travels to the intestines. Once in the intestine, laboratory models show that Salmonella can be detected inside the epithelial cells lining the gut within 20 min and in macrophages after one hour. Although some studies posit that the intestinal symptoms associated with salmonellosis are caused by a toxin, most researchers believe that symptoms are caused by the destruction of surface epithelial cells. This destruction, which takes place in less than 12 hours in laboratory models, prevents fluid uptake by the intestine resulting in exudative diarrhea (20,21).

Uptake of Salmonella is facilitated by a class of cells known as M cells. These cells line the outside of a lymphoid structure, the Peyer’s patch, which is especially prominent in the ileum. Salmonella appears to have a special affinity for these cells and is rapidly engulfed into a vacuole by them. Salmonella also appears to be able to invade other non-phagocytic cells through a process known as membrane ruffling (22). Interestingly, Fedorka-Cray et al (23) found that intranasally
inoculated esophagotamized weanling pigs that many enteric samples, including lymph nodes, became infected even though the removal of the esophagus should have prevented infection. This indicates that lymph system may be a route of *Salmonella* infection.

Once *Salmonella* is taken up into the vesicles, no replication takes place for at least three hours. In order for the pathogen to replicate in the vacuole it must prevent the lysosome from destroying it. One mechanism may be acidification of the vacuole which neutralizes the toxic effect of the lysosome once it joins the vacuole. Another possibility is that *Salmonella* is able to prevents the lysosome from binding with the vacuole (24).

In the mouse model, once the *Salmonella* reaches the Peyer’s patch, it is disseminated to the liver and spleen via the reticulo-endothelial system. One day after experimental infection, $10^1$-$10^2$ cells are found in both the liver and the spleen. After 5-7 days levels of $10^7$-$10^8$ CFU are found, which corresponds to a doubling time of 200 minutes. An infection of this level rapidly kills the mice, but most human illness is largely limited to an infection of the intestinal epithelium (25).

**Detection of *Salmonella***

**General principles**

*Salmonella* isolation is neither an easy nor standardized procedure. Generally, isolation protocols use a nonselective pre-enrichment to revive injured cells, a selective enrichment to encourage the growth of *Salmonella*, selective plating media to isolate *Salmonella*, and biochemical tests or serological assays to confirm the presence of *Salmonella*. However, a recent survey by Waltman and Mallinson (26) of 74 laboratories isolating *Salmonella* from poultry found that 17 different combinations of enrichment media were used. Incubation time and temperature varied greatly, and 14 different post-enrichment plating media were
used. This lack of a uniform method suggests that there is no "perfect" isolation procedure. This is not especially surprising when one considers the large number of Salmonella serotypes and the wide variety of hosts that can carry this organism. Fricker (27) noted that procedures that are appropriate for one sample may not be appropriate for others. Hence, it is prudent to evaluate the isolation procedures in relation to the specific sample of interest.

Key ingredients in Salmonella isolation

In the isolation of Salmonella, many selective inhibitors have been used to aid in the selection of Salmonella. These selective inhibitors include bile salts, desoxycholate, thiosulfate, brilliant green, malachite green, crystal violet, tetrathionate, magnesium chloride, an incubation temperature of 42°C, and motility. Additionally, antibiotic compounds including novobiocin, sulphadiazine, sulphacetamide, sulphamethazine, and mandelic acid have been used to restrict the growth of competing organisms. However, a study by Arroyo and Arroyo (28) found that although most of these compounds effectively inhibit gram positive and many gram negative organisms, they tend to have little selective power against other members of the family Enterobacteriaceae. As a result, small numbers of Salmonella may be difficult to detect in the presence of large numbers of competing enteric organisms.

While inhibitory compounds are designed to allow Salmonella to preferentially multiply, most agars use chemical reactions to differentiate Salmonella from other organisms. The reaction of iron salts and hydrogen sulfide is a commonly used to differentiate Salmonella based on the characteristic black colony morphology. In some media, the failure to ferment lactose is used for differentiation. In the presence of phenol red, colonies failing to ferment lactose maintain a red color while colonies that produce acid from lactose turn yellow (i.e. E. coli). Recently several
agars using chromogenic compounds have been developed that differentiate *Salmonella* based on colony color.

**Media used in the isolation of *Salmonella***

A plethora of broths and agars have been developed for the isolation and detection of *Salmonella*. Rather than discussing each of these, this review will simply focus on the media most commonly used in the isolation of *Salmonella* from swine.

**Buffered peptone water**

Buffered peptone water (BPW) is designed for growth and recovery of injured *Salmonella* cells. Growth in BPW ensures that damaged *Salmonella* are not destroyed when they are placed in a selective medium. The buffering capacity of this medium ensures that the pH during recovery is relatively high as low pH may be detrimental to damaged cells (29).

**GN Hajna broth**

This broth was designed for the culturing of gram-negative organisms, particularly *Salmonella* and *Shigella* from clinical and non-clinical samples. It is generally used as a nonselective enrichment broth to allow for the repair of injured cells prior to enrichment (30).

**Tetrathionate broth**

Tetrathionate broth is used as a selective enrichment for the isolation of *Salmonella* species. It is particularly effective when only small numbers of *Salmonella* are present in samples containing large numbers of competing enteric flora. Selectivity is achieved with thiosulfate and tetrathionate, as well as bile salts (31).

**Rappaport-Vassiliadis R10 broth**
This medium is generally used following pre-enrichment of the sample. It is used for many different sample types, particularly those containing heavy loads of competing bacteria. Selectivity is achieved with magnesium chloride (raises the osmotic pressure), malachite green and a low pH (5.1). The recommended incubation temperature is 42°C (32).

**Modified semi-solid Rappaport-Vassiliadis medium**

This medium is a modification of the above mentioned RV medium specifically designed to detect motile *Salmonella* from feces and food products. In addition to magnesium chloride, malachite green and a low pH, selectivity is enhanced through the use of Novobiocin and 2.7g of agar per liter. This media has been used as a rapid means for isolating motile *Salmonella* from food products (33).

**Xylose lysine tergitei 4 agar (XLT)**

This agar is specially designed to prevent the overgrowth of competing enteric bacteria. Selectivity is achieved through the addition of Tergitol 4 supplement while differentiation is achieved through fermentation of xylose, lactose, sucrose, the decarboxylation of lysine and particularly the production of hydrogen sulfide which gives colonies a black center (34).

**Modified brilliant green agar**

Modified brilliant green agar is typically used in the isolation *Salmonella* from water, sewage and foods. It is especially useful for culturing *Salmonella* that produce little or no hydrogen sulfide. Selectivity is achieved using brilliant green and the antibiotic sulphamandelate. Differentiation is achieved through the failure to ferment lactose and sucrose which produces a red colony (35).

**Rambach agar**

This agar is used for the differentiation of members of the family *Enterobacteriaceae*. Desoxycholate inhibits gram positive organisms. Differentiation is achieved through acid production from propylene glycol and the utilization of lactose identified by β-galactosidase. *Salmonella* colonies produce a
characteristic pink/red color that allows for differentiation from other members of the family *Enterobacteriaceae* (36).

**Isolation methods**

Currently, the majority of published studies of *Salmonella* in swine use one of two protocols (37). The first protocol is similar to the International Standard Organization protocol currently used in the EU to test for *Salmonella* (38). It consists of a nonselective pre-enrichment in buffered peptone water (BPW) overnight at 37°C, a 1:100 transfer to Rappaport-Vassiliadis broth (RV broth) at 42°C for 24 h, streaking onto XLT and modified brilliant green agar plates overnight at 37°C, followed by biochemical confirmation (39). The second protocol entails using a nonselective pre-enrichment in GN Hajna broth (GN) for 24 h as well as selective enrichment in tetrathionate broth (TT) 48 h at 37°C. This is followed by 1:100 dilution into RV broth for 24 h at 37°C, streaking onto brilliant green agar plates with sulfadiazine (BGS) overnight at 37°C, and biochemical confirmation (40).

Currently our research lab at the National Animal Disease Center uses a protocol that incorporates portions from both of these methods. In our method, half of the sample is placed in BPW for pre-enrichment and the other half in TT selective enrichment. This is followed by 1:100 dilution into RV broth +40µg/ml novobiocin for 24 h at 42°C. This is followed by a 1:100 dilution into RV broth for 24 h at 42°C. This RV is then struck onto XLT agar and modified brilliant green agar and incubated for 24 h at 37°C. Presumptive positive colonies are plated on Raumbach agar for 24 h at 37°C. A positive colony is then picked, slanted onto tryptic soy agar, and sent to NVSL for serotyping.
Significant *Salmonella* outbreaks

Great understanding of bacterial pathogens can be gained by studying outbreaks associated with them. A comprehensive review of all outbreaks attributable to *Salmonella* is well beyond the scope of this paper. Rather, a few significant and representative outbreaks will be discussed.

Perhaps the first recorded outbreak of foodborne salmonellosis occurred in 1888 when Gaertner isolated *Salmonella* Enteritidis from the organs of a man who had eaten meat from an emergency slaughtered cow. The man, one of 58 individuals who became ill, died within 36 hours of eating 1.5 lbs of the affected meat (41).

Of particular concern are outbreaks involving susceptible or high profile populations. A good example of this was an outbreak involving infant formula that occurred between November and December 1985 in the United Kingdom. A small whole in the spray drier used to process the formula was found to contain a reservoir of *Salmonella* Ealing. Several months worth of product were contaminated at low levels by this source. Seventy-six people became ill with this uncommon serotype; of which 48 were infants, 14 were siblings or parents, 2 were adults who had consumed other dried milk products produced at the same plant, and 12 adult cases appeared to have no link. Seven of the infants were admitted to the hospital and one died as a result of the illness (42). This case illustrates that a small lapse in plant sanitation or maintenance can have serious consequences in vulnerable populations.

The general public tends to associate *Salmonella* with the consumption of raw eggs, perhaps with good reason. One of the largest outbreaks to ever occur in the United States was associated with raw eggs. In September of 1994, health officials in Minnesota noticed an unusual increase in the number of *Salmonella* Enteritidis isolations. A case-control study linked this increase to the consumption of Schwan's ice cream and this was later validated when *Salmonella* Enteritidis was found in
unopened ice cream containers. Apparently, the shipping company that was hauling pasteurized ice cream pre-mix to the production facility was using the same tankers to haul raw eggs from egg breaking plants. Failure to adequately sanitize before hauling the pre-mix resulted in the pasteurized product becoming contaminated. A total of 150 cases were identified in Minnesota alone, but as *Salmonella* is considerably underreported, the estimated number of individuals affected was much higher. Given that Schwan’s ice cream is sold nation wide, extrapolation of the Minnesota outbreak data resulted in an estimate of 224,000 illnesses nationwide (43). This case clearly illustrates the impact that cross-contamination of pasteurized or cooked products with sources of *Salmonella* can have.

A 1994 outbreak in Wisconsin illustrates the importance of sanitation in meat production as well as proper preparation of the product by the consumer. During the Christmas/New Year period of 1994 health care workers identified 158 cases of *Salmonella* Typhimurium infection. Nearly all of the affected individuals had eaten raw hamburger, (a common practice in some areas of Wisconsin) obtained from a single abattoir, at household and social gatherings. It appears that a grinder used to prepare the ground beef was not properly cleaned and sanitized. The grinder served as a reservoir for the *Salmonella* and this, coupled with the failure of the consumer to properly prepare the product, resulted in the outbreak (44).

Perhaps the most difficult *Salmonella* outbreaks to prevent are those involving foods that are normally eaten raw, such as fruits and vegetables. An excellent demonstration of this is a 1995 multinational outbreak of *Salmonella* Newport associated with alfalfa sprouts. This outbreak was caused by single contaminated lot of alfalfa seeds produce by a Dutch seed broker. This lot was distributed to growers through international channels and caused outbreaks in Denmark, Canada, Georgia, Oklahoma, Oregon, Pennsylvania, Vermont, Virginia, and West Virginia. While the multinational nature of this outbreak makes it difficult to estimate the total number of illness causes by this contaminated lot, 133 cases were isolated from
Oregon and Canada (45). Since sprouts undergo no bactericidal treatment, the outbreak would have been difficult to prevent, but the advisability of eating alfalfa sprouts can certainly be questioned. This outbreak also demonstrates the compounding effect international trade can have on foodborne outbreaks.

Pork and pork by-products have also been implicated in many *Salmonella* outbreaks. An outbreak of particular interests occurred in Canada. In the summer of 1999, public health workers in Alberta noticed a dramatic rise in the occurrence of *Salmonella* Infantis. Investigation determined that the most probable source was dog treats made from pig ears. In fact, 48/94 pig ears sampled for Salmonella from retail outlets were positive. Apparently victims were becoming infected either directly from the treats themselves (i.e. not washing hands after handling them) or were infected after their dogs developed infections (46). Although this is not a typical foodborne illness, it does illustrate that control of *Salmonella* at the abattoir or farm could positively impact other vectors of *Salmonella*.

For the pork industry, perhaps the most significant outbreak occurred in Denmark during the summer of 1993. In the early 1990’s, Danish research indicated that up to 50% of all *Salmonella* cases were attributable to pork and pork products (47). In the midst of this heightened awareness, an outbreak involving over 500 victims occurred in the Copenhagen area. The culprit was determined to be a single slaughterhouse that was receiving infected pigs from a limited number of relatively large farms (48). This increased awareness, coupled with a significant outbreak, prompted the Danish Ministry of Agriculture to implement a nationwide *Salmonella* control program in the swine industry. The program included control of *Salmonella* in feed, breeding, multiplying and finishing herds as well as control measures at the abattoir (49). The implementation of this program increased international interest in controlling *Salmonella* in pork and fueled the desire for increased research of potential intervention strategies.
Salmonella in pork production

When devising intervention strategies, it is important to consider the whole production process. Traditionally the focus of food safety began at the abattoir, with little attention given to the origin of the animal, the route by which they arrived at the abattoir, or its distribution after fabrication. In the past decade, attention has shifted to the whole process. In order to develop effective intervention strategies, many researchers have evaluated possible intervention points where Salmonella can be controlled. The pre-harvest portion of the process presents one of the best opportunities to control Salmonella in swine, as Salmonella that is prevented from entering the plant cannot be spread to carcasses or retail cuts.

A 1964 study by Hansen et al (50) found that swine slaughtered within 3 hours had a 10% (6/60) prevalence of Salmonella in the colon, while those held for 3 days at the abattoir had a 35% (25/72) prevalence. Additionally, a greater diversity of serotypes was isolated from the group held for 3 days. The authors felt that the holding pen was an important source of Salmonella contamination, and the implementation of changes in holding practices could improve food safety.

In 1967 Williams and Newell (51) found that although only 1/491 rectal swabs were positive at the farm, up to 72% of the animals were positive when sampled 1.5 hours after arriving at the abattoir. In a 1968 follow-up study, the same authors (52) found that although none of 276 rectal swabs at the farm were positive for Salmonella, 49/176 rectal swabs were positive after transport and 1.5 hours of holding. In both studies, some of the serotypes found after slaughter matched those isolated from the holding pens prior to the arrival of the pigs. Serotypes were also found which matched the isolates found in feed used on the farm. The authors felt that although some of the animals may have become infected during holding, most of the increase was a result of stress-induced excretion of Salmonella that was present, but not being shed, at the farm. Based on these studies, the authors felt
the most effective intervention points would be controlling *Salmonella* in the feed as well as reducing stress during transport.

In an effort to strengthen the theory that transport-stress is an important factor in the increased detection of *Salmonella*, Williams and Newell (53) tested 20 animals prior to 3.75 hours of simulated transport. While none of the animals were positive prior to transport, six of the animals were positive at the end of the simulated transport.

From this period of research, two separate opinions developed. The first is that *Salmonella* excretion levels at the farm are low because *Salmonella* infection levels are low and that the increase seen at shipping is due to mingling of animals and exposure to a contaminated environment during transport and holding. The second opinion was that infection occurs frequently at the farm from feed and other sources, but it is difficult to measure using rectal swabs and that the stress from shipping and holding results in an increase in detectable shedding (51).

Judging by the lack of papers published on this subject during the 70's and 80's, the debate and interest in this area of study apparently subsided. However, the Danish outbreak and the desire to find pre-slaughter interventions has reignited interest in stress and the holding pen as potential sources of *Salmonella* in slaughter pigs. A recent survey (54) of European and American experts indicates that the two opinions described by Williams and Newell still exist. European experts tended to believe that prevention of infection at the farm would control the number of animals shedding *Salmonella*, while American experts felt that holding and transport were important causes of contamination regardless of initial on-farm levels of *Salmonella*.

Following the increased concern about in *Salmonella* in swine, the Pre-harvest Food Safety and Enteric Diseases unit of the USDA Agricultural Research Service's National Animal Disease Center developed a research program to explore pre-harvest interventions for *Salmonella* in swine. The first study conducted, an evaluation of transport stress, found that animals receiving the more stressful
transport treatment had a slightly lower *Salmonella* prevalence upon slaughter than the control group. However, when on-farm and post-slaughter *Salmonella* prevalences in animals from the same farm were compared, a significant increase in *Salmonella* isolation rates after slaughter was noted. Serotyping results indicated a greater diversity existed among the post-slaughter positives than the on-farm isolates. In addition to greater diversity, the predominant post-slaughter serotype changed weekly, while the farm serotype remained constant. Because the swine were transported in disinfected trailers, these results suggest that a non-farm source of *Salmonella* may have been responsible for the increased isolation rates, as opposed to shedding reoccurrence (55).

However, this study compared different samples at the farm (feces) and post-slaughter (colon contents and visceral tissues) therefore, sampling differences may partially explain the increase in isolation. In a follow-up study, identical samples from nearly 600 market weight swine were compared (superficial inguinal and ileocecal lymph nodes, cecal contents, feces, and tissue for serology). In this study, pen mates were necropsied at the farms of origin and the abattoir. This study also demonstrated a significant difference in *Salmonella* isolation rates between the farm (5%) and the abattoir (39.7%) (following 2-3 hours of holding prior to slaughter). The study also demonstrated an increase in the variety of *Salmonella* serotypes recovered from animals necropsied at the abattoir (56).

Given this study, it became necessary to evaluate the physiological possibility of swine becoming infected in as little as two hours. It was found that when swine were exposed to fecal-slurry containing approximately $10^3$ CFU/g *Salmonella*, 3/6 pigs exposed for 30 minutes, 12/16 exposed for two hours, and 8/8 exposed for six hours became infected (57).

Taking these observations into account, swine exposed to the holding pen appear to a major source of *Salmonella* in a packing plant environment. Swanenburg et al (58) stated that the impact of transport and holding tend to be
underestimated while the contributions from the farm are overestimated. Several authors have indicated that intestinal carriage of *Salmonella* is a significant risk factor for the contamination of the carcass and further processed pork products (59,60). Morgan et al (61) stated that “the source of carcass contamination was primarily intestinal *Salmonella* infections and the extent of carcass contamination was determined by the number of *Salmonellas* entering the abattoir in the intestine of slaughtered pigs”.

**The need for enumeration**

Despite the literature available that implicates the holding pens as a significant food safety hazard, the potential danger posed by the holding pen cannot be adequately evaluated because the samples in the previously mentioned studies tested only for the presence/absence of *Salmonella*. In fact, despite the large amount of research done on *Salmonella*, very little quantitative data exists. This is an important gap in knowledge for as Paracelsus, the father of toxicology said, "Dosis facit venenum." ("The dose makes the poison.") (62). One would suppose that viscera containing $10^1$ CFU/g *Salmonella* would not pose the same threat of illness as viscera containing $10^6$ CFU/g. Therefore, information about the load of *Salmonella* being brought into the plant by swine is vital to the understanding of the hazards posed by holding pens and other pre-harvest portions of pork production. In fact, concern over the inability to appropriately judge risk based on available data was voiced during the Oct. 9-10, 2001 Swine Work Group meeting of the National Pork Board (63). It is also of note that the International Commission on Microbiological Specifications for Foods (ICMSF) Working Group on Microbial Risk Assessment indicated that:

In the area of exposure assessment there are extensive data on the microbial ecology of particular raw materials and foods, but these data are often incomplete or inadequately quantitative...The numbers and
probability of pathogens contaminating food at different stages of the food chain are poorly documented and certainly not quantitative (64).

Additionally, as the focus of research shifts from identifying the primary sources of pre-slaughter contamination to removing these sources, an understanding of population dynamics and ecology of *Salmonella* becomes increasingly important. Particularly, factors such as the quantity of organisms, their distribution throughout the pen, seasonal trends, and reactions to stressors such as cleaning, drying, and sanitizers become vital pieces of the puzzle necessary to effectively eliminate the pathogen. Unfortunately, the current methods available to study these dynamics are limited.

**Enumeration techniques**

There are currently very few techniques available for the quantification of *Salmonella*. Direct plating, in which a 0.1ml portion of the sample is spread over the surface of a selective plating medium and colonies of the proper morphology are enumerated, has been used in research when a *Salmonella* with a known antibiotic resistance is studied. Unfortunately, plate counts are relatively ineffective in testing field samples as *Salmonella* colonies are obscured by the overgrowth of other enteric bacteria.

Another approach, used by Wood and Rose (65), is the most probable number technique (MPN). In this technique, samples are serially diluted to the presumed *Salmonella* extinction level, and then 3-10 replicate samples from each dilution are placed in one or more nonselective enrichment media (Figure 1). Following an appropriate incubation period each of these replicate samples are transferred to one or more selective enrichment media. After incubation, each sample is plated onto one or more selective plating media. Morphologically suspect colonies are then biochemically evaluated, and based on this evaluation, each of the replicate samples are classified as positive or negative. The pattern of *Salmonella*
positive/negative tubes is then compared to a statistically derived table for an approximation of the number of *Salmonella* present in the original sample. In effect, each quantitative sample becomes 15 to 50 qualitative samples. The MPN method has many drawbacks including the amount of labor, the laboratory space required, the large amounts of media required, the difficulty of handling large numbers of samples, and overall cost.

As cumbersome as the method is, MPN is the standard method for the enumeration of *Salmonella* (66). Due to the time consuming nature of the method, few researchers, and even fewer industry microbiologists, are willing to use the MPN method. As a result, large amounts of qualitative data exist, but quantitative data is quite limited.

It is evident that if the need for quantitative data is to be filled, a less cumbersome, costly, and labor-intensive method for enumeration must be developed. Several attempts at modifying this technique have been made, with limited success. Whittimore (67) developed a single 10-fold dilution series technique designed for the enumeration of relatively small quantities of *Salmonella* (<10³) in the absence of competitive flora. This technique would not be suitable for samples with large variations in the quantity of *Salmonella*, and large amounts of background flora can also interfere with the test. Pumfrey and Nelson (68) developed a MPN method using a DNA probe. This method reduced labor at the plating media step but the retained the labor-intensive pre-enrichment and enrichment phases. Humbert et al (69) developed a miniaturized MPN procedure that utilized a 96 well format which significantly reduced the labor involved with transferring of samples from the nonselective to the selective enrichment. Regrettably, the differentiation between positive and negative replicates was still quite labor intensive as presumptive positives were identified with enrichment serology and then confirmed on Rambach agar.
In order for a test to become widely used, it must have certain attributes. Specifically, it must require a limited number of transfers, be contained in a simple and efficient format, use only a limited amount of lab space and labor, be easily and quickly read, and not be cost limiting.

**Usefulness of enumeration data**

Once a simplified method for the quantification of *Salmonella* is developed, then extensive enumeration data can be collected more easily. As previously mentioned, there is little quantification data for *Salmonella* in pork. However, the USDA baseline survey of *Salmonella* on pork carcasses did quantify *Salmonella* loads. In 169 positive carcasses, 54% were below the detectable range (<0.03 MPN/cm\(^2\)), 31% were between 0.03-0.30 MPN/cm\(^2\), 10% were between 0.31-3.0 MPN/cm\(^2\), and only 5% were between 3.1-30.0 MPN/cm\(^2\) (70). Work by Laubach et al (71) found that quantities of *Salmonella* in head meat were generally less than 10\(^2\) CFU, but levels higher than 10\(^3\) CFU were occasionally seen.

The potential of quantitative data is illustrated by the work of Fegan et al (72). The prevalence of *E. coli* O157 in cattle is well characterized, but little work has been done on the amount of this organism present in the feces of infected animals. Recent research by Fegan et al documented the enumeration *E. coli* O157 from feces at a cattle abattoir. They found that nearly 75% of the positive samples had less than 10\(^2\) CFU/g and that *E. coli* O157 made up a very small percentage of the total *E. coli* population. This study provided valuable information on the ecology of *E. coli* O157 and will aid those wishing to conduct a quantitative risk assessment.

Perhaps one area in which quantitative data for *Salmonella* in pork does exist is studies on the persistence of infection. While most of this data comes from animals experimental inoculated with large doses of *Salmonella*, the data is useful in understanding magnitude of organisms shed throughout the course of infection.
Several studies all report similar trends (63, 73-75). While the peak shedding levels varied from $10^6$ to $10^3$, within two to four weeks of infection, typical numbers of *Salmonella* recovered were less than $10^2$. This indicates that the animals that are shedding the greatest amounts *Salmonella* are most likely the animals most recently infected. This is an important observation about *Salmonella* ecology as it indicates that recently infected animals may pose the greatest risk.

**Use of models in identification of control points**

Once enumeration data is collected, this data can be inserted into various models to aid in understanding the dynamics of the pork production and pinpointing effective intervention points. Currently, several available models are handicapped by the lack of quantitative data. A model by Berends (76) predicted that 70% of all carcass contamination results from the animal entering the plant in a contaminated condition. While the most important causes of contamination were polishing machines and inept evisceration procedures, singeing and scalding were most likely to reduce *Salmonella* levels. Pearce (77) et al also identified singeing and scalding as important *Salmonella* critical control points. Additionally, polishing and dehairing were identified as steps likely to increase contamination.

Expanding models beyond the abattoir to the entire pork production system, van der Gaag et al (78) found the most important stages of production to be the finishing stage and the abattoir. Another paper by this group (79) found that the most cost effective interventions were at lairage prior to slaughter, followed by interventions in slaughter process and in the finishing stage of growth.

**Predicting illness**

One drawback of models without a quantitative component is the inability to predict potential illnesses prevented or caused by a specific process. Without this
capability, it is difficult to determine points in the production process that reduce human illness and not just Salmonella prevalence. Alban et al (80) attempted a quantitative risk assessment of human salmonellosis due to Salmonella Typhimurium DT104 in dry cured sausages. The authors admit that quantitative data for sausage meat does not exist, but they utilized data from other cuts to estimate the potential human illnesses. The model predicted low incidence of contamination and associated human illness.

In the Alban paper, there was considerable discussion of the ability of small levels of Salmonella to cause illness. Blaser and Newman (8) discuss six outbreaks caused by levels lower than $10^3$ organisms. In some of the outbreaks, the infective dose may have been as little as one organism. Much of the infectious dose information available for Salmonella is based on a group of studies (81, 82) from the 1950's in which volunteers were given known amounts of Salmonella. Several dose response models (83-85) have been generated using these data. Perhaps one of the best dose-response models for estimating illnesses in a model was generated by Latimer et al (86). The advantage of this model is that it provides estimates of illness probabilities for low doses of Salmonella, as well as providing illness probabilities for Salmonella of various pathogenicity levels. Given the wide variety of Salmonella, a model using an illness probability range rather than a single estimate is more likely to be representative of real world parameters in which the pathogenicity of Salmonella varies widely.

**Conclusion**

This review of current literature has covered the importance of Salmonella as a foodborne pathogen and some prominent detection methods and media. Additionally, it has identified the need for enumeration of Salmonella in research. Given the few available quantitative methods, development of a new method would aid in the collection of quantitative data. Once a method is validated and
quantitative data is collected, then the information can be included in models of the pork production system. The addition of quantification data to new or existing models will allow the estimation of illnesses prevented or caused by specific portions of the farm to fork continuum. These models will be very useful in the analysis of hazards and the creation of critical control points to combat these hazards. The improved understanding of *Salmonella* ecology and the pork production chain will enhance the safety of pork products.

**References**


personal communication: Dr. Elizabeth Wagstrom-National Pork Board, AVP Science and Technology


32


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<tr>
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</tr>
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<td><strong>Total</strong></td>
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Table 1. A summary of the food types associated with foodborne Salmonellosis in the U.S. from 1973-1987
Number of *Salmonella* per 5 tubes

1
10
100
1,000
10,000

.2ml into each tube

10/ml
100/ml
1,000/ml
10,000/ml

BPS

2ml

18ml

100,000/ml

100,000 Salmonella/2g

2g of feces

1,000,000 Salmonella/2g

Figure 1. A schematic representation of the Most Probable Number method of enumeration
Validation of a New Most Probable Number Method for *Salmonella*

A paper to be submitted to the Journal of Food Protection

Jared K. Gailey, H. Scott Hurd, and James S. Dickson

**ABSTRACT**

Currently there is a need for detailed enumeration data in the study of *Salmonella*. Enumeration of *Salmonella* is generally accomplished using the most probable number (MPN) technique. While generally accepted, the labor and media intensive nature of this technique discourages enumeration and limits the number of samples that can be enumerated. In an effort to reduce the labor associated with enumeration and to increase the number of samples that can be enumerated, a modified most probable number technique, termed the “RX tube”, was designed and is evaluated in this paper. This technique includes commonly used media (modified semi-solid Rappaport-Vassiliadis agar, XLT4 agar, and tetraphionate broth) in a tube format. The RX tube was capable of detecting approximately 1 *Salmonella* cell for 18/21 strains. Using artificially contaminated fecal samples of a known *Salmonella* concentration there was a correlation of 0.97 between tryptic soy agar plate counts (TSA) and MPN, 0.95 between RX and TSA, and 0.95 between RX and MPN. When naturally contaminated samples were used, a correlation of 0.77 between RX and MPN results was found. Additionally, RX and MPN were found to be equivalent within a 0.5 log tolerance. Finally, the RX tube used approximately one-fourth of the labor required by MPN. The RX method appears to be equivalent with the MPN method and requires significantly less labor, making enumeration of *Salmonella* more feasible.
INTRODUCTION

When attempting to define the risk posed by any infectious disease, two important pieces of information are needed: the prevalence and the quantity of the agent. For some organisms, such as the pathogenic *E. coli*’s, multiple methods exist to determine both prevalence and quantity. In *Salmonella* research, a wide array of qualitative methods exist (18,19), but the only generally accepted method for quantification is the most probable number (MPN) method (17). Plate count methods used in the enumeration of many other infectious organisms are generally unreliable for *Salmonella*, as current media tends to allow the growth of many competing organisms, thereby obscuring *Salmonella* colonies. In the enumeration of *Salmonella* in swine samples, the method described by Wood and Rose (20), which follows the standard MPN procedure (17) is often used.

In the literature describing the epidemiology of *Salmonella* in swine, the prevalence of infected animals and of *Salmonella* in the environment is often reported (3,9,10,11,14). In these studies, only the numbers of animals/samples that are positive or negative were considered. Commenting on this, the International Commission on Microbiological Specifications for Foods (ICMSF) Working Group on Microbial Risk Assessment indicated that “In the area of exposure assessment there are extensive data on the microbial ecology of particular raw materials and foods, but these data are often incomplete or inadequately quantitative...The numbers and probability of pathogens contaminating food at different stages of the food chain are poorly documented and certainly not quantitative.” (13).

Clearly, there is a dearth of quantitative information in the study of *Salmonella* in the food supply. This is most likely attributable to the large amounts of labor and media involved in the MPN method. If less cumbersome methods for enumeration could be developed, perhaps more scientists would be willing to combine quantitative *Salmonella* research with their qualitative work.
Developing a new quantification method for an organism with as many diverse serotypes as Salmonella is a daunting task. Finding a single characteristic shared by all serotypes is nearly impossible. However, a reasonable place to start is with motility. Although several poultry isolates, including Salmonella. Gallinarum and S. Pullorum, are non-motile, the vast majority of Salmonella are motile (16). Motility is the basis for AOAC Official Method 993.07 (1), motility enrichment on modified semi-solid Rappaport-Vassiliadis (MSRV) medium. Motility enrichment on MSRV has been shown to be a rapid and sensitive method for detecting Salmonella from heavily contaminated samples following either pre-enrichment or selective enrichment (2, 4, 5, 6). However, the plates are relatively fragile and inoculating them can be time consuming when doing large numbers of samples.

Another common attribute used to differentiate Salmonella from other enteric bacteria is the production of H₂S. Again, while the majority of Salmonella produce H₂S, there are some naturally occurring strains that either produce no H₂S or only produce it weakly. Xylose lysine tergitol 4 (XLT) medium, which has become a popular medium in recent years, bases identification on the production of H₂S. It is similar to xylose lysine desoxycholate (XLD ) medium (a standard AOAC medium(1)) except that it contains tergitol 4, to inhibit Proteus and Pseudomonas, and additional peptone to enhance the H₂S reaction (19). A comparison of five plating media for Salmonella found that after selective enrichment in tetrathionate (TT) broth, MSRV and XLT had the best sensitivities and specificities (8).

Based on these facts, selection based on motility and H₂S production is likely to detect the majority of Salmonella isolates. By combining these commonly used isolation media in a test tube format, we have created a test that has the potential to make the enumeration of Salmonella less difficult.

The object of this paper is to describe and compare the ability of these common media in a new format, the RX tube, to enumerate Salmonella from naturally and artificially contaminated samples.
MATERIALS AND METHODS

RX tube preparation. The media used in this test included TT, MSRV, and XLT. TT provided for the selective enrichment of the initial sample, MSRV selected for *Salmonella* on the basis of motility, and H₂S produced by *Salmonella* utilizing XLT served as a visual indicator. To prepare the RX tube, all media were prepared according to manufacturers instructions (Difco, Sparks, MD). One ml of XLT, cooled to 50°C, was placed in the bottom of a 16X150mm tube (figure 1), allowed to solidify, and then 4 ml of 50°C MSRV was added to the tube. After the MSRV cooled, 0.5ml of autoclaved mineral oil was layered over the MSRV. The tube was stored no more than 2 weeks at 2-8°C. Immediately prior to using the tube, 4.5 ml of TT was added to the tube, taking care not to disturb the MSRV layer. Any tubes in which the TT penetrated more than 10mm below the MSRV surface were discarded to prevent non-motile organisms from accessing the XLT. The tubes were inoculated with 0.5 ml of the test sample and incubated at 42°C for 96±12 hours.

RX tube interpretation. Tubes which remained green, as well as tubes with yellowing less than 20mm below the MSRV surface, were considered negative. Tubes with a distinct yellowing that extended more than 20mm were considered presumptive positives. Any tube with visual H₂S production (blackening) more than 40mm below the MSRV surface was considered positive (figure 2). All presumptive positives were confirmed by decanting the TT, inserting a loop at least 20mm into the MSRV and streaking the loop onto Rambach agar (24hrs at 37°C).

Validation of individual RX tubes. Prior to validating the RX tube as an enumeration tool, the detection capabilities of single tubes were tested. The detection threshold of the tube was tested using 21 different field isolates from swine, representing the most commonly isolated swine serotypes in our laboratory
culture collection (*Salmonella* ser. Typhimurium var. copenhagen (n=4), Derby (n=4), Heidelberg (n=2), and Agona, Anatum, Bovis morbificans, Brandenburg, Hadar, Infantis, Manhattan, Newport, Reading, Senftenberg, Saint Paul (n=1)). Of these isolates, three were not expected to produce positive results based on in-lab evaluation of motility and \( \text{H}_2 \text{S} \) production: a Typhimurium copenhagen (with poor motility in motility GI medium, no motility on a MSRV plate and low \( \text{H}_2 \text{S} \) production), a Heidelberg (no and low motility in motility GI medium and MSRV), and the Bovis morbificans (high motility on motility GI medium and high \( \text{H}_2 \text{S} \) production, but very limited motility on MSRV).

The isolates were prepared by adding 0.5 ml of the stock culture to 4.5 ml of tryptic soy broth (TSB) and incubated for 18±2 hours at 37°C. These cultures were then serially diluted to approximately 10 cells/ml and 0.1 ml for each isolate was inoculated into each of 15 RX tubes. From the TSB cultures, ten replicates of 0.1ml were also direct plated onto tryptic soy agar (TSA). The TT in each tube was also inoculated with \( 10^5 \) CFU/ml of non-characterized competitive flora isolated from the cecum of a *Salmonella* negative sow using buffered peptone water (BPW). After incubating, each of the 15 tubes were classified as positive, negative or presumptive positive.

The sensitivity and specificity of the RX tube was determined using 96 randomly chosen RX positive tubes and 99 RX negative tubes (n=195 tubes) inoculated with field samples. The presence/absence of *Salmonella* was confirmed by streaking each tube onto Rambach agar (RA), and comparing the true presence/absence of *Salmonella* with the result of the RX tube.

**Validation of RX as an enumeration tool.** Throughout this portion of the evaluation, 5 tube replications with a total of five 1:10 serial dilutions (a total of 25 RX tubes per test), based on standard MPN format (17), were used. All comparisons were to the MPN method using the principles described by Wood and
Rose (20). Specifically, borosilicate 16X150mm test tubes (n=25) containing 4.5ml BPW were inoculated with 0.5ml of the sample. The tubes were covered and incubated at 37°C for 24 hours. Following incubation, 0.1ml of the BPW was transferred to 10ml of RV and incubated at 42°C for 24 hours. A 3mm loopful was then streaked onto XLT agar and incubated at 37°C for 24 hours. One suspect colony from XLT was chosen and streaked to RA (37°C for 24 hours) for confirmation. The quantity of *Salmonella* present in the original sample for both the MPN method and the RX tube were computed using standard MPN tables.

To evaluate the RX tube as an enumeration tool, trials were first carried out using seeded samples with known amounts of *Salmonella*. The amount of *Salmonella* was verified with duplicate plate counts using TSA plates. A total of 65 comparisons were conducted using 5 different serotypes (Derby, Anatum, Typhimurium var. copenhagen, Heidelberg, and Newport). For each serotype, three replicates each were done for 10^0 and 10^1, five replicates were done for 10^2 and two replicates were done for 10^3 CFU/ml of *Salmonella*.

Following the enumeration of seeded samples, 33 naturally contaminated samples, obtained for Midwestern abattoirs, were evaluated. These samples included swine feces (n=11), tonsils (n=10), cecal contents (n=7), and ileocecal lymph nodes (n=5). All samples were prepared for testing using the methods previously described by Hurd et al (12). Each of these samples was analyzed using both the RX tube and the MPN method as outlined above.

**Statistical Analysis.** Data were entered into Excel (Microsoft, Redmond, WA) and statistical analysis was performed using JMP version 4 (SAS institute, Cary, NC). Correlation between MPN and RX was determined using matched pairs analysis. Sensitivity and specificity of the individual tubes was determined using standard formulas (15).
In this study, the null hypothesis was not that the methods were equivalent, but rather that the methods were different. In this situation, traditional statistical tests such as matched-pairs analysis may not be the most appropriate test. Therefore an equivalence test using two one-sided t tests was also used to compare the RX tube to MPN results. Equivalence tests are often used for testing generic drugs where the objective is not to prove that the generic drug is better than the brand-name drug, but that they are equal. Briefly stated, an equivalence test consists of two null hypotheses; the difference between the two tests (D) is less than or equal to an arbitrarily chosen lower bound of acceptability (D ≤ B_l) and the difference between the two tests is greater than or equal to an arbitrarily chosen upper bound (D ≥ B_u). The arbitrary bounds are chosen based on a range of variation that is considered acceptable in a particular situation. If both H_0: D ≤ B_l and D ≥ B_u are rejected, then the difference is equivalent to zero, based on the upper and lower bound (7). Woodward (21) pointed out that for a five tube MPN, the 95% confidence limits range from approximately 24% to 324% of the MPN result. On a log scale, this translates to a confidence interval of (+) 0.50 logs and (-) 0.62 logs. For the purposes of this paper, ± 0.50 logs was chosen as the upper and lower bound for the equivalence test. For descriptive purposes, ±0.25 logs was also evaluated.

**RESULTS**

**Individual tube results.** For the 21 strains tested, only the three strains that were motility- or H₂S-impaired failed to be detected at approximately 1 cell of *Salmonella* levels. Based on TSA plate counts, the number of *Salmonella* cells actually placed in each tube ranged from 0 to 5 with an average of 1.7. The number of RX tubes (n=15) detecting *Salmonella* for remaining strains ranged from 9 to 15 tubes. The negative tubes in this range can be accounted for by the random
variability inherent in diluting to a single cell. For the strains with normal motility and 
H$_2$S production, the detection limit is close to one cell, even in the presence of
competing flora.

For the 195 tubes confirmed on Rambach agar, 93 of the 96 positive tubes
contained *Salmonella* while 93 of the 99 negative RX tubes did not contain
*Salmonella*. Thus an individual RX tube has a sensitivity of 96.9% and a specificity of
93.9%.

**RX tube as an enumeration tool results.** The results of the 65
laboratory prepared samples were similar, whether they were obtained using TSA
plate counts, RX tubes or MPN. Using matched-pairs analysis, there was a
correlation of 0.97 between TSA and MPN, 0.95 between RX and TSA, and 0.95
between RX and MPN. Using the equivalence test, all three tests were equivalent
within the ± 0.5 log confidence interval inherent in the MPN and RX test procedure
(p<0.01). The MPN and RX tests were also equivalent (p<0.01) within a ±0.25 log
certainty interval. Both MPN and RX tended to give higher results than TSA.

Naturally contaminated samples tested (n=33) ranged from positive, but
below the detectable limit (i.e. the 10 gram sample was positive for *Salmonella*, but
the small portion of this sample enumerated was negative), to 4.7 logs by both MPN
and RX with a mean of 1.36 for MPN and 1.33 for RX, while no TSA data was
collected as these were naturally contaminated samples. Matched-pairs analysis
showed a correlation of 0.77 between RX and MPN results. The equivalence test
indicated the RX and MPN were equivalent within the ± 0.5 log confidence interval
(p<0.01).

**Cost and time for both enumeration methods.** Table 1
summarizes the cost and time involved for a single enumeration test. There was
little difference in media preparation time and total test time. Media cost for the RX
test was roughly half of the cost for the MPN test. Most importantly, since the RX
test required no transfers, the MPN test required nearly four times as much technician time as the RX test.

**DISCUSSION**

The “RX tube”, or modified semi-solid RV and XLT tube, was developed in order to expedite research in the quantitative epidemiology of *Salmonella*. The RX tube was equivalent to the traditional MPN test for both the lab and naturally contaminated samples tested in this validation. More importantly, RX tube also required less media expense and technician time. Additionally, the RX tube can detect low levels (~1 cell) of *Salmonella*, even in the presence of competing flora. Finally, since labor is only needed to inoculate and read the tubes, testing large numbers of samples is easier than with the standard MPN methodology.

While the RX test is an improvement over the traditional MPN, room for further modification and improvement still exists. This method was validated using common swine serotypes and normal swine flora. Under these circumstances, the apparent sensitivity, specificity, and single-cell detection capabilities appear to be adequate. In other testing applications, the test may need to be modified to better detect the expected serotypes. The 96-hour incubation time could potentially be a problem if incubator space were sparse. If the method could be modified to shorten the incubation time, but maintain the detection abilities, this could improve the method. Finally, since most of the samples tested had large amounts of competitive flora, this study was unable to determine how well the test might work in relatively sterile products such as processed foods.

This validation has demonstrated that the RX tube is an equivalent replacement for the standard MPN methodology. Having an enumeration method that is less media- and labor-intensive should make it more feasible for researchers to quantify *Salmonella*. Hopefully, this increased research capability will provide the
quantitative results that are urgently needed to understand the epidemiology of *Salmonella* in the food supply.

**REFERENCES**


Add 0.5ml of sample

- Creates an anaerobic environment
- Enrichment
  - Separates Salmonella on the basis of motility
  - Production of $H_2S$ indicates the presence of Salmonella

Mineral oil 0.5ml
Tetrathionate broth 4.5ml
Modified Semi-solid RV media 4ml
XLT 4 agar 1ml

Figure 1. A schematic diagram of the RX tube
Figure 2. A photo showing positive and negative reactions for the RX tube
Table 1. A comparison of the RX and MPN method for one sample consisting of 5 dilutions with 5 replicate tubes (n=25 tubes) assuming ten positive tubes

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Quantification of *Salmonella enterica* in Market Swine at the Abattoir

A paper to be submitted to the Journal of Food Protection

J.K. Gailey, H.S. Hurd, J.S. Dickson, A.M. O'Connor, and J.D. McKean

**ABSTRACT**

The prevalence of *Salmonella* in samples collected at the swine abattoir is well documented. However, little quantitative data exists for the pork production process. The purpose of this paper is to obtain quantitative data for three porcine samples commonly collected at the abattoir. For this study 630 samples (cecal contents, feces and ileocecal lymph nodes) were collected from 210 animals at three Midwest abattoirs. Of the samples tested 114 (18%) were positive for *Salmonella*. Of these samples, 34 were below the detectable quantification limit (i.e. they were positive by culture but negative by quantification). In the 57 quantifiable cecal content samples, an average of 1.8 log CFU/g was detected. For the 8 quantifiable fecal samples, the average was 1.9 log CFU/g while in the 15 quantifiable ileocecal lymph node samples, the average was 2.9 log CFU/g. All three samples had a hyperbolic distribution with the largest number of samples having lower amounts of *Salmonella*. It was interesting to note that ileocecal lymph nodes averaged one log higher than the other samples. These results will be useful for understanding *Salmonella* ecology and conducting hazard analyses to identify potential critical control points in pork production.

**INTRODUCTION**

Maintaining a safe and sanitary pork production facility requires constant vigilance. Not only are there issues with employees contaminating the product, but, the very animals that are slaughtered are constantly transporting pathogens into the
facility. Recent research found that 40% of the animals entering a production facility were harboring *Salmonella* in their intestinal tract (13). In a plant that handles 8,000 animals a day, this translates into 3,200 opportunities for contamination.

Swine appear to be the major source of *Salmonella* in the abattoir environment. Several authors have indicated that intestinal carriage of *Salmonella* is a significant risk factor for the contamination of the carcass and further processed pork products (6,23). Morgan et al. stated that “the source of carcass contamination was primarily intestinal *Salmonella* infections and the extent of carcass contamination was determined by the number of *Salmonellas* entering the abattoir in the intestine of slaughtered pigs”(17). Berends et al calculated that 70% of all contamination is directly introduced into the abattoir by the animals themselves (4).

Despite the assertions that infected swine are the primary source of *Salmonella* in the slaughter plant, the potential danger posed by these animals is difficult to completely evaluate. While most of the studies of *Salmonella* in swine report observed prevalences (5, 7,10, 20), there is a noticeable lack of enumeration data in the literature. As Paracelsus, the father of toxicology stated, “Dose makes the poison”(18). Without quantification data (i.e. the dose), it is difficult to determine the severity of the *Salmonella* contamination, but one would suppose that viscera containing $10^1$ CFU/g *Salmonella* would not pose the same threat of contamination, and subsequent human illness, as viscera containing $10^5$ CFU/g.

Enumeration of *Salmonella* is a particularly difficult and time consuming undertaking and is often omitted from research. However, knowing the quantity of a pathogen, in addition to the prevalence, paints a much clearer picture of the ecology of that organism. For example, the prevalence of E. coli O157 in cattle is well characterized, but little work has been done on the amount of this organism present in the feces of infected animals. Recent research by Fegan et al (8) documented the enumeration E. coli O157 from feces at a cattle abattoir. They found that nearly 75% of the positive samples had less than $10^2$ CFU/g and the E. coli O157 made up
a very small percentage of the total E. coli population. This study provided valuable
information on the ecology of E. coli O157 and will aid those wishing to conduct a
quantitative risk assessment.

Quantitative risk assessments are systematic models that use current
scientific knowledge to estimate the risk of human illness associated with certain
activities or food products. In 1997, Lammerding and Paoli (14) emphasized the
need for quantitative risk assessments in the food safety arena. While a quantitative
risk assessment is a scientifically sound approach to evaluating the risk of human
illness, performing a quantitative risk assessment for Salmonella is hindered by the
current dearth of quantitative data. In order for quantitative risk assessment to
proceed, it is imperative that quantitative data be generated in addition to qualitative
data.

The purpose of this descriptive study was to provide enumeration data for
several commonly collected post-mortem porcine samples. This quantitative
description of Salmonella load entering the packing plant (and potentially the food
chain) via the intestinal tract of the animals will be valuable for future risk
assessments of Salmonella in pork products.

MATERIALS AND METHODS

For this experiment, samples were collected from three separate Midwest
swine abattoirs. Visceral samples were collected, in conjunction with other studies
conducted by our lab, from 90 animals at abattoir A, while 60 samples each were
collected at both abattoirs B and C. From each animal, cecal contents (10ml), feces
(10g) and the ileocecal lymph node (average weight 4.6g, range 1.3-13.8 g) was
collected. All samples were returned to the lab within 4 hours for processing. The
exact weight of each of the samples was recorded to facilitate determining the
CFU/g. Feces and cecal samples were added to 90ml of buffered peptone water
(BPW) and homogenized using a stomacher (Seward, London, UK) at 260 RPM for
one minute. Ileocecal lymph nodes were dipped in ethanol, flamed and individually macerated in a sterile bag with a rubber mallet. Phosphate buffered saline (PBS) (15 ml) was added to the bag and each lymph node sample was homogenized using a stomacher at 260 RPM for one minute. Five 0.5ml replicates from these homogenates were used as the original dilution for MPN enumeration using the RX tube method previously described by Gailey et al (9). All enumerations consisted of 5 tube replicates with 5 dilutions (n=25 tubes total). Enumeration results were obtained using standard MPN tables (21).

Following inoculation of the RX tubes, 10ml of the PBS from the ileocecal lymph nodes was also added to 90ml of BPW. In addition to quantification, the BPW from the three samples was directly cultured by incubating at 37°C for 24 hours. Each was then subcultured 1:100 in (RVN) Rappaport-Vassiliadis broth + novobiocin (20mg/L), incubated for 24 h at 42°C and then transferred 1:100 to Rappaport-Vassiliadis (RV) broth and incubated 24 h at 42°C. RV samples were screened using the Assurance gold EIA Salmonella ELISA kit (BIOCONTROL, Bellevue, WA). ELISA positive samples were then streaked from RV onto xylose lysine tergitol 4 (XLT) and modified brilliant green agar (MBG) and incubated for 24 h at 37°C. Suspect colonies on XLT and MBG were confirmed using Rambach agar (24 h at 37°C). As direct culture used a much greater sample volume than the enumeration, the positive/negative status of each sample was based on the culture results.

RESULTS

Of the 630 samples tested (n=210 for each sample type), 114 (18%) were positive for Salmonella. Of these positive samples, 73 were cecal contents, 18 were feces, and 23 were ileocecal lymph node samples. At the abattoir level, 74 of the positive samples were from abattoir A, 10 were from abattoir B, and 30 were from abattoir C.
Of the 114 positive samples, 34 (16 cecal contents, 10 feces, and 8 ileocecal lymph node samples) were below the detectable limit (i.e. they were positive by 10g culture, but negative by quantification). For the cecal contents, the detectable limit averaged 3.6 CFU/g (range 3.1-4.7 CFU/g). In the feces, the average detectable limit was 5.7 CFU/g (range 2.8-11.2 CFU/g), while the average detectable limit for ileocecal lymph nodes was 2.6 CFU/g (range 1.9-4.9 CFU/g).

In the 57 quantifiable cecal content samples, an average of 1.8 log CFU/g was detected (range 0.49-5.0 log CFU/g). For the 8 quantifiable feces samples, the average was 1.9 log CFU/g (range 0.5-4.5 log CFU/g) while in the 15 quantifiable ileocecal lymph node samples, the average was 2.9 log CFU/g (range 1.1-5.2 log CFU/g). All three samples had a hyperbolic distribution with the largest number of samples having lower amounts of *Salmonella* (figure 1). For cecal contents, feces, and ileocecal lymph node samples respectively, 72.6%, 83.3%, and 52.2% of the samples had less then $10^2$ CFU/g of *Salmonella*.

**DISCUSSION**

Very little quantitative data for *Salmonella* in the abattoir has been published to date. This descriptive study provides quantitative baselines for *Salmonella* in some commonly collected porcine abattoir samples. The hyperbolic distribution of the data towards lower quantities of *Salmonella* is of interest. Previous work by Hurd et al (12) found that experimentally infected market age swine shed 2.6-4.3 log CFU/g of feces one week after infection. Work by Wood and Rose (24), on 6 week old experimentally infected pigs, found that 3 days after inoculation, cecal contents averaged 5.6 log CFU/g, colon contents averaged 5.5 log CFU/g, and ileocecal lymph nodes averaged 4.4 log CFU/g. After 4 weeks, the cecal and colon contents were at 2.5 and 2.7 log CFU/g respectively and after 8 weeks the ileocecal lymph node averaged -0.2 log CFU/g. Given this data and assuming that infection occurs
randomly any time prior slaughter, one would expect a normal distribution curve centered around 2-4 log CFU/g for positive animals.

Interestingly, the USDA baseline survey of *Salmonella* on pork carcasses also found that the quantities of *Salmonella* were skewed toward the lower range. In 169 positive carcasses, 54% were below the detectable range (<0.03 MPN/cm²), 31% were between 0.03-0.30 MPN/cm², 10% were between 0.31-3.0 MPN/cm², and only 5% were between 3.1-30.0 MPN/cm² (22).

The hyperbolic distribution of the data could be explained in several ways. One possible explanation is that many of the infections acquired more than 8 weeks prior to slaughter persist at a sub-clinical level, but are detected at slaughter (3), and skew the distribution toward lower levels. Another possible explanation is supported by the recent body of work that suggests that transport and holding at the abattoir are a major source of *Salmonella* in swine (11, 15, 16, 19). Using this hypothesis, the lower levels of *Salmonella* represent organisms recently (3-6 hrs) acquired during transport and holding that have not had sufficient time to establish an acute infection. Using this same theory, the higher levels of *Salmonella* would represent recent acute *Salmonella* infection obtained in the last few weeks of finishing.

When comparing the three sample types, it is interesting to note that cecal and fecal contents had nearly the same amount of *Salmonella* on average (1.8 and 1.9 log CFU/g respectively), while the lymph nodes averaged a log higher (2.9 log CFU/g). The data from Wood and Rose (24) would suggest that the cecal and fecal levels should be higher than the lymph node. It may be possible that the ileocecal lymph node is more representative of on-farm infection, while the cecal contents, and possibly feces, are more representative of infection recently acquired (3-12 hours) during transport and holding. If this observation is supported by future research, interventions at the farm and plant level could possibly be evaluated through these samples.
Currently, perhaps due to the difficulty of enumeration, any detectable *Salmonella* is considered a public health risk. The lowest infectious human dose of *Salmonella* is generally considered to be more than $10^2$-$10^3$ CFU (2). If therefore, any animal below the $10^2$ CFU level were classified as a minimal health risk, 78% of cecal content positive animals and 89% of feces positive animals pose little potential health risk. However, nearly 50% of ileocecal lymph node positive animals have levels that could be considered a significant health risk. It is possible that by monitoring animals for positive ileocecal lymph nodes at the abattoir, the farms that represent the most public health risk could be identified, representing a means to segregate high and low risk farms.

Although this study included samples from only three different Midwest packing plants and 630 samples, it provides a good example of the insights that can be achieved through enumeration of *Salmonella* samples. Perhaps most importantly, we now have a glimpse of which animals may pose the greatest health risk, as well as possible ways to identify these animals. Additionally, we now have information about the range of *Salmonella* loads that can be expected at the abattoir as well as the quantitative distribution of *Salmonella* positive animals. This type of information is very valuable for the hazard analysis portion of a HACCP plan (1) and modeling of this data could help determine the best places for CCPs in the pre-harvest portion of swine production. Clearly, enumeration data can provide useful insights into food safety and *Salmonella* ecology that cannot be seen through prevalence data alone.

**REFERENCES**


Figure 1. The distribution of Salmonella loads for three different sample types collected at three Midwest abattoirs
Use of Quantitative *Salmonella* Data to Identify Potential Critical Control Points in the Pre-harvest Portion of the Farm to Fork Continuum

A paper to be submitted to the Journal of Food Protection

J.K. Gailey, H.S. Hurd, and J.S. Dickson

**ABSTRACT**

Due to the lack of *Salmonella* quantitative data, most models of the pork production system have considered only prevalence data. Recently acquired quantitative data from the abattoir provides an opportunity to create a Monte Carlo simulation model which utilized these data to explore and determine the relative importance of potential critical control points in the pre-harvest portion pork production. This model examined two hypothetical interventions: an intervention that eliminates low loads of *Salmonella* contamination (hypothetically acquired during transport and holding) and an intervention that eliminates animals with high loads of *Salmonella* (hypothetically acquired during the final weeks of finishing). The model considered *Salmonella* carcass contamination from intestinal contents, contamination from head-associated lymph nodes, and contamination from systemic lymph nodes. The model explored the effect of the two interventions on *Salmonella* prevalence, *Salmonella* loads, and human potential infectious doses. The ability to predict potential infections was an important improvement in this model that was made possible through the use of quantitative data. Interventions at the holding pen reduced carcass prevalence by four-fold and lymph node prevalence by half. On-farm and holding interventions reduced potential illnesses attributable to carcass contamination by half, but only an on-farm intervention reduced illnesses attributable to lymph nodes. The model found that both the final weeks of finishing and transport/holding have potential as critical control points. Additionally, this model
demonstrates the usefulness of quantitative data and demonstrates the need for this type of data for other portions of the pork production process.

**INTRODUCTION**

Recent focus on the safety of the food supply has largely focused on two areas. The first initiative, HACCP, involves the identification of critical control points (CCPs) in a production process. Identification of CCPs effectively allows pathogen control resources to be focused on the portions of the food chain where they will be most effective. The second concept is viewing the production of pork as a farm to fork continuum rather than simply a meat processor function. While the meat processing industry has largely embraced HACCP, food safety controls have lagged behind in the pre-harvest portion of the continuum. This lagging is most likely a result of the relative lack of research in the pre-harvest portion of meat production. Without research, identification of effective interventions is difficult and blindly implemented control strategies may be costly and have no effect or may even have unexpected negative consequences.

In the production of pork, there has been an increased interest in the pre-harvest portion of the process. Research suggests potential CCPs may include holding, transport (13), as well as identification and segregation of high prevalence herds both in slaughter and transport (2). The identification of these potential CCPs has largely been based on research that measured the prevalence (i.e. percentage of positive animals/samples) of *Salmonella* positive swine(8, 14, 21, 29). In *Salmonella* research, the hazard analysis that is vital to the creation of a HACCP plan is hindered by the virtual absence of quantitative (i.e. the load of *Salmonella* in a positive sample) data. The understanding of any microbial system requires knowing both how often the organism occurs and how many of the organisms are present. The lack of quantitative data results in an unclear picture of the *Salmonella* ecology as it relates to pork production.
Obtaining quantitative *Salmonella* research data can be difficult and costly and as a result, most research has been focused on prevalence rather than quantification. Recognizing the need for quantitative data, we gathered enumeration data for three commonly collected swine samples (feces, ileocecal lymph nodes, and cecal contents) at three Midwest abattoirs (6).

The purpose of this paper is to outline a model that uses this quantitative data and illustrates how these data can be used to provide a clearer picture of potential critical control points in the pork production system.

**MATERIALS AND METHODS**

**Model construction.** This model consists of an excel spreadsheet used in conjunction with @ Risk 4.0 (Palisade Inc., Newfield NY). In this model (figure 1), only the evisceration and fabrication portion of the slaughter process are considered as this model is aimed at determining the effect of pre-harvest interventions on freshly processed carcasses and retail cuts. Each animal that is slaughtered represents three opportunities for contamination. For each animal there is a probability for external carcass contamination from the intestinal tract during evisceration. The second opportunity for contamination, head-associated lymph nodes, includes the mandibular, parotid, and retopharyngeal lymph nodes. Head-associated nodes were considered separately from systemic lymph nodes as they are more likely to be included in ground product. The third opportunity for contamination, systemic lymph nodes, includes medial iliac, lateral iliac, iliofemoral, subiliac, ventral thoracic, and superficial inguinal lymph nodes. These lymph nodes are more likely to be found in whole-muscle cuts than ground product. Following the approach of Alban et al (1), we assumed that all contamination events consist of 1 gram of material. At this point in the model, the number of carcasses/retail cuts
contaminated with intestinal contents/lymph nodes was calculated based on the number of animals slaughtered and the probability for contamination.

For whole carcasses contaminated with intestinal contents, or retail cuts containing lymph nodes, there was a probability of finding *Salmonella* in the contents or lymph nodes. The whole carcass, head-associated lymph nodes and systemic lymph nodes each had a separate probability of contaminating material containing *Salmonella*. At this point in the model the number of carcasses/retail cuts with *Salmonella* was calculated given the probability of the intestinal contents/lymph nodes containing *Salmonella*. From this number, the prevalence of *Salmonella* positive pork was determined.

The load of *Salmonella* in the contaminating material (intestinal contents or lymph nodes) ranged from $10^0$ to $10^6$ CFU. The load of *Salmonella* in intestinal contents and lymph nodes had a set distribution. Using this distribution, the load of *Salmonella* contaminating the pork was calculated.

Once the load of *Salmonella* on the pork was determined, the frequency of potential infectious doses (PID i.e. the likelihood that consumption of the contaminating material would cause illness without further interventions) occurring was determined. This was done based on the probability of a known *Salmonella* load causing illness.

This model was iterated 5000 times to produce a distribution of possible results.

**Model Inputs**

**Number of animals.** The model contains several inputs (Table 1). The number of pigs in the model was set at 8000 to reflect a typical 8 hour production day with a 1000 head/hr line speed.

**Probability of contamination.** The probability of contamination for each animal during the evisceration step was based on industry data and a triangle
distribution with a minimum probability of 0.01, a mode of 0.03, and a maximum of 0.06 was used in the model.

Given the high density of lymph nodes (4) in the head region combined with the likelihood of head meat becoming ground product, it is the authors' opinion that most head meat will contain lymph nodes and accordingly, a triangle distribution for the probability of head meat containing lymph nodes of 0.8, 0.9, and 1.0 was used. From a anatomical standpoint, systemic lymph nodes are spread throughout the animal with a relatively low density (4), and it was the authors' opinion that the probability of a retail cut containing a lymph node was relatively low and a triangle distribution of 0.0, 0.1, and 0.2 was assigned.

**Probability of contaminating material containing *Salmonella*:** The probability of contaminating material (i.e. intestinal contents, lymph nodes) containing *Salmonella* was determined from existing literature. The probability of intestinal contents containing *Salmonella* is based on a meta-analysis of field experiments conducted by our lab (11, 13, 14, 15, 25, 26). An analysis of these different studies (n=2113 samples) resulted in a triangular distribution with a minimum probability of 0.02, a mode of 0.37 and a maximum of 0.81.

Little data about the prevalence of *Salmonella* in head-associated lymph nodes currently exists on which to base the model. Hurd et al (12) found that 4% of naïve animals exposed to a *Salmonella* contaminated environment had positive mandibular lymph nodes. A Danish study (9) found only 2% of animals had positive mandibular lymph nodes. However, a study of deboned swine head meat found an average 40% of whole tissue samples were positive for *Salmonella* (17). Based on these data, the probability of head-associated lymph nodes containing *Salmonella* was given a triangle distribution of 0.02, 0.2, and 0.4.

It was also difficult to find *Salmonella* prevalence estimates for systemic lymph nodes. In experimental studies, Wood et al (31, 32) found that 4-12% of
systemic lymph nodes were positive in intentionally infected animals. Hurd et al (12) found that 20% of intentionally infected animals had Salmonella positive superficial inguinal lymph nodes. In a descriptive study of cull sows, Larsen et al (14) found that 0-8% (average 2%) of ventral thoracic and subiliac lymph nodes were positive for Salmonella. Base on these data, the probability of systemic lymph nodes containing Salmonella was a triangle distribution set at 0.0, 0.04, and 0.20.

Distribution of Salmonella loads. The distribution of Salmonella loads (MPN/g) among positive animals was based on quantitative data collected at three Midwest abattoirs (6) (Figure 2) for feces, cecal contents, and ileocecal lymph nodes. In the model, each positive carcass/lymph node was randomly assigned a Salmonella load based these distributions. The model for both head-associated and systemic lymph nodes uses the load distribution found for ileocecal lymph nodes. This is based on the assumption that Salmonella growth curves are similar in any infected lymph node regardless of where that lymph node is located. The distribution of Salmonella loads for contaminated carcasses was based on the combination of cecal contents and feces.

Application of hypothetical interventions by manipulating Salmonella distributions: The hypothetical interventions considered using this model were achieved by altering the “distribution of Salmonella loads” input variable. Based on the literature, it appears that the primary factor influencing the quantity of Salmonella shed by swine is the time since the animal was infected. Wood and Rose (33) as well as Berends et al (2) found that in experimentally infected weaned piglets, the animals shedding the highest quantities of Salmonella were those animals most recently infected (less than two weeks). By four weeks post-infection, levels were generally less than $10^2$ CFU, a level that is less likely to cause illness. In the first week following infection Marg et al (18) were able to enumerate high levels of
Salmonella from the feces of nursery pigs given a dose of $10^{11}$ CFU, but little if any Salmonella could be enumerated for the second and third week. Given these findings, one would expect the animals with the highest level of Salmonella at the abattoir to have been recently infected during the finishing stage.

Conversely, O’Connor et al (22) found that in one Midwest abattoir’s holding pens, the average amount of Salmonella ranged from 0.8 to 2.7 log CFU/ml of pen effluent. In the Netherlands, Swanenburg et al (28) found that Enterobacteriaceae levels in holding areas averaged approximately 3.5 log CFU/cm². According to Gorvel and Meresse (7), once Salmonella invades the host cells, there is a three hour lag period prior to replication. Given the short abattoir holding period (average of 2-4 hours), the hostile environment of the stomach, and the lag period prior to replication as an active infection, one would expect Salmonella levels acquired during transport and holding to be relatively low.

From this information, two hypothetical interventions were evaluated using the model. The first intervention was designed to simulate the prevention of infections during holding and transport prior to slaughter. This intervention considered the effect of eliminating all animals with low levels (<$10^2$ CFU) of Salmonella (i.e. any animal with <$10^2$ CFU become classified as negative). The second intervention was designed to simulate the prevention of Salmonella infections during the last few weeks of finishing. This intervention considered the effect of eliminating all animals with high levels (>$/10^2$ CFU) of Salmonella. The baseline to which the hypothetical interventions were compared included all animals regardless of Salmonella load. Altering the distribution of Salmonella loads affected both the number of positive carcasses/retail cuts (i.e. any carcass that was positive with an excluded level of Salmonella becomes classified as a negative animal) and the loads found on contaminated pork.

Probability of infection. Given a Salmonella load, probability of that dose resulting in infection can be estimated. Several papers explore the relationship
between dose of *Salmonella* and human illness (10, 23, 30). Most of these papers rely on data from human feeding trials conducted in the 1950's (19, 20). Latimer et al (16) classified the strains used in these trials as either low or moderately pathogenic organisms. They also recognized that some outbreaks seemed to require smaller infectious doses than the feeding trial data would indicate. They recommended using data obtained from *Shigella dysenteriae* feeding trials as a proxy for these highly pathogenic organisms. Given the outbreaks documented by Blaser and Newman (3) in which lower than expected infectious doses (10\(^{-1}\)-10\(^{2}\) organisms) caused illness, this substitution appears to be justified. Using both the *Salmonella* and *Shigella* feeding trials, Latimer et al (16) created a composite dose-response model. The probability of human infection for various doses used in this model was derived from this composite dose-response model.

**Model Outputs.** The model has three main outputs. The first output in the model was the average prevalence of *Salmonella* attributable to each of the three types of contamination (intestinal contamination, head-associated lymph nodes, and systemic lymph nodes). The second output was the average *Salmonella* load found on positive carcasses or in positive retail cuts. The third output was the average frequency of human potential infectious doses on carcasses or in retail cuts.

**Sensitivity Analysis.** To test how the model performed when the input variables were adjusted, a sensitivity analysis was conducted. All of the input variables in this model were distributions (probability of contamination, *Salmonella* prevalence in contaminating material, and probability of being an infectious dose). In order to determine the effect of changing these distributions on the results of the model, the input variables were both reduced by half as well as doubled. This yielded a total of 27 different scenarios. For these scenarios, a minimum, maximum, mean, median, and a 95% confidence interval was determined. Additionally,
ANOVA analysis for *Salmonella* prevalence and potential infectious doses was conducted to determine if the two treatments were statistically different from the baseline and each other.

**RESULTS**

**Prevalence.** The model results are shown in Table 2. The baseline prevalence of *Salmonella* contamination resulting from intestinal spills averaged 1.3% (95% confidence interval of 0.4% to 2.6%, maximum 4.4%), with intervention one (eliminating low loads) the prevalence was 0.3% (0.1% to 0.7%, 1.1%), while with intervention two (eliminating high loads) the prevalence was 1.0% (0.3% to 1.9%, 3.3%). For head associated lymph nodes, the prevalence of *Salmonella* was 18.6% (7.1% to 30.6%, 36.8%), 8.9% (3.4% to 3.7%, 14.6%), and 9.7% (3.7% to 16.0%, 19.2%) for the baseline, intervention 1 and intervention 2 respectively. For systemic lymph nodes, the prevalence was 0.8% (0.1% to 1.9%, 3.7%), 0.4% (0.1% to 0.9%, 1.8%) and 0.4% (0.1% to 1.0%, 1.9%) respectively.

**Salmonella load.** Of the positive carcasses contaminated with intestinal contents, the average load for the baseline was 1.9 log cfu of *Salmonella*. With the application of intervention 1 the average load increased to 3.6 log cfu while intervention 2 decreased the average level to 1.1 log cfu. For animals with contaminated lymph nodes, both head and systemic, the loads were 2.5, 3.9, and 0.9 logs respectively.

**Potential infectious doses.** All reported potential infectious doses (PID) were the number of potential infections per 8000 animals processed. The baseline potential infectious doses attributable to contamination with intestinal contents were 7.8 (95% confidence interval of 2.1 to 5.9, with a maximum of 28.6). Application of intervention 1 resulted in a 40% reduction to 4.7 PID (1.2 to 9.6, 19.1), while
intervention 2 resulted in a 60% reduction to 3.2 PID (0.8 to 6.7, 15.1). Contaminated head-associated lymph nodes resulted in an average of 160.9 PID (59.3 to 275.5, 418.2), with a reduction of 18% to 131.2 PID (47.4 to 229.4, 344.1) for intervention 1 and an 82% reduction to 29.7 PID (10.0 to 54.4, 78.5) for intervention 2. Contaminated systemic lymph nodes resulted in an average of 6.9 PID (1.1 to 16.9, 39.9). Intervention 1 resulted in a 19% reduction to 5.6 PID (0.9 to 14.1, 34.9), while intervention 2 resulted in an 81% reduction to 1.3 PID (0.2 to 3.3, 7.3). The distribution of these results are shown in figure 3.

**Sensitivity analysis.** Results from the sensitivity analysis are reported in Table 3. For intestinal contamination, intervention 1 and 2 both significantly reduced the number of potential infectious doses, however, there was no statistical difference between the two interventions. Intervention 1 also significantly reduced the prevalence of the *Salmonella* due to intestinal contamination, while intervention 2 did not. For both head-associated and systemic lymph nodes, intervention 1 had no statistical impact on the number of PID, while intervention 2 statistically reduced the PID. Additionally, both interventions 1 and 2 produced a significant but equal reduction in the prevalence of *Salmonella* attributable to both types of lymph nodes.

**DISCUSSION**

This model provided some interesting insights into possible areas where pre-harvest CCPs could be effective in reducing potential infectious doses as well as carcass prevalence. Rather than focusing on the specific numbers produced by this model, the most emphasis should be placed on the illustrated reduction trends. For *Salmonella* spread by intestinal contamination, an intervention applied at the holding pen will have a significant impact on carcass prevalence, while both finishing and holding pen interventions should result in a reduction of PID. For lymph node associated *Salmonella*, interventions at both finishing and holding pen significantly
reduce carcass prevalence, but only finishing interventions appear to reduce potential infections. Potential holding pen interventions include sanitation of the pen (26), limiting exposure to the pen, and the reduction of moisture. Potential finishing interventions (especially in the last few weeks of production) include vaccination, feed treatments, water acidification, and probiotics (5).

The results of any model are based on the input variables used in the model. Since several of the input variables used in this model were uncertain, the sensitivity analysis was useful in dealing with this uncertainty. It is unlikely that the true value of any of the input variables would be less than half or greater than twice the value used. Naturally, as the input variables changed so did the magnitude of the results, but the effect of the interventions were maintained across all possible combinations. Based on this, it is likely that the insights provided by the model are generally applicable.

For abattoirs that have difficulty meeting the FSIS Salmonella standard, reducing the amount of Salmonella in the holding pen is a possible CCP that could help the plant become compliant. It may also be an effective way to reduce potential infective doses resulting from intestinal contamination. However, a single intervention focused only on holding and transport may be overlooking an important CCP. An effective intervention implemented at the end of finishing has the potential to reduce the Salmonella prevalence in lymph nodes as well as significantly reduce PID for both intestinal and lymph node contamination. Therefore, both a finishing and holding level CCP are justified, as they both have the potential to reduce the number of infective doses.

This reduction of potential infective doses fits neatly into the HACCP approach to food safety in which multiple hurdles are used to reduce the probability of food borne illness. Having both prevalence and quantification data aids in the initial hazard analysis that is the beginning step of HACCP. Using this improved hazard analysis, several CCPs likely to improve food safety were identified.
An especially important improvement resulting from the addition of quantitative data to a model is the ability to predict potential infective doses. This capability is especially important in judging the effectiveness of potential interventions. An intervention that reduces the prevalence of the organism, but does not significantly reduce the PID may be an inappropriate intervention. On the other hand, an intervention that has little effect on prevalence but reduces the PID may be worthwhile. The findings of this model agree with the assessments of other researchers. Rostagno et al (24) indicated that the holding pen should be considered as a potential CCP. Swanenburg et al (27) observed that the last portion of the pig's life may be important in the reduction of *Salmonella*. As additional quantitative data is generated, the concepts from this model can be applied throughout the farm to fork continuum. Through these models, the most protective places for CCPs will become clearer and many foodborne illnesses can be averted.

This modeling exercise has shown the value of quantitative data when combined with existing prevalence data. Without both types of data, the complex picture of *Salmonella* ecology is incomplete. Hopefully, this model will inspire future researchers to determine quantity as well as prevalence in future works to further clarify our understanding of *Salmonella* ecology and improve the safety of pork.

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Salmonella enterica infections in market swine with and without transport and

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of short-term lairage on the prevalence of Salmonella enterica in cull sows. J Food
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<table>
<thead>
<tr>
<th>Variable</th>
<th>Distribution</th>
<th>Values</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of contamination opportunities</td>
<td>Constant</td>
<td>8000</td>
<td>Typical production day</td>
</tr>
<tr>
<td>Number of iterations</td>
<td>Constant</td>
<td>5000</td>
<td>NA</td>
</tr>
<tr>
<td>Probability of intestinal contamination</td>
<td>Triangular</td>
<td>Min:0.01 Mode:0.03 Max:0.06</td>
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<td>Probability of intestinal contents being Salmonella positive</td>
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<td>Min:0.02 Mode:0.37 Max:0.81</td>
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<td>Probability of a head lymph node being Salmonella positive</td>
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<td>Min:0.02 Mode:0.20 Max:0.40</td>
<td>Hurd(2001), Laubach(1998)</td>
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<tr>
<td>Probability of a systemic lymph node being Salmonella positive</td>
<td>Triangular</td>
<td>Min:0.00 Mode:0.04 Max:0.20</td>
<td>Wood (1989, 1991), Larsen(2003), Hurd(2001)</td>
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<tr>
<td>Distribution of Salmonella loads in positive samples</td>
<td>Constant</td>
<td>See figure 2</td>
<td>Gailey(2005)</td>
</tr>
<tr>
<td>Probability of illness with 1 log of Salmonella</td>
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<td>Min:0.00 Mode:0.05 Max:0.05</td>
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<tr>
<td>Probability of illness with 1.5 logs of Salmonella</td>
<td>Triangular</td>
<td>Min:0.00 Mode:0.05 Max:0.10</td>
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<td>Probability of illness with 2 logs of Salmonella</td>
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<td>Min:0.00 Mode:0.07 Max:0.15</td>
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<td>Probability of illness with 2.5 logs of Salmonella</td>
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<td>Min:0.00 Mode:0.12 Max:0.25</td>
<td>Latimer(2001)</td>
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<td>Probability of illness with 3 logs of Salmonella</td>
<td>Triangular</td>
<td>Min:0.00 Mode:0.15 Max:0.30</td>
<td>Latimer(2001)</td>
</tr>
<tr>
<td>Probability of illness with 3.5 logs of Salmonella</td>
<td>Triangular</td>
<td>Min:0.00 Mode:0.17 Max:0.35</td>
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<td>Probability of illness with 4 logs of Salmonella</td>
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<td>Min:0.00 Mode:0.20 Max:0.40</td>
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<td>Probability of illness with 6 logs of Salmonella</td>
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<td>Latimer(2001)</td>
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<td>Triangular</td>
<td>Min:0.10 Mode:0.25 Max:0.50</td>
<td>Latimer(2001)</td>
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Table 1. Description of the variables used in the modeling of potential critical control points in pre-harvest pork production
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<th></th>
<th>Intestinal contamination</th>
<th>Head lymph node contamination</th>
<th>Systemic lymph node contamination</th>
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</thead>
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<td></td>
<td>Carcass Prevalence</td>
<td>Carcass load (log MPN)</td>
<td>Infectious doses per 8000 animals</td>
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<tr>
<td>Baseline</td>
<td>1.33%</td>
<td>1.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Low load intervention</td>
<td>0.34%</td>
<td>3.6</td>
<td>4.7</td>
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<tr>
<td>High load intervention</td>
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<td>1.1</td>
<td>3.2</td>
</tr>
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</table>

Table 2. The effect of interventions on the prevalence, *Salmonella* load, and potential infectious doses, by contamination type
### Table 3. The results of sensitivity analysis, using one-half and double the input probabilities, (27 combinations) by intervention and contamination type

<table>
<thead>
<tr>
<th>Infectious doses</th>
<th>Intestinal Contents</th>
<th>Head lymph node</th>
<th>Systemic lymph node</th>
</tr>
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<td>Mean</td>
<td>10.6ab</td>
<td>190.7a</td>
<td>11a</td>
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<td>Median</td>
<td>7.8</td>
<td>160.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Lower 95%</td>
<td>6.5</td>
<td>121.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Upper 95%</td>
<td>14.7</td>
<td>259.9</td>
<td>15.7</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.9</td>
<td>20.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Maximum</td>
<td>46.6</td>
<td>715.7</td>
<td>55.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salmonella prevalence</th>
<th>Intestinal Contents</th>
<th>Head lymph node</th>
<th>Systemic lymph node</th>
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<tbody>
<tr>
<td>Mean</td>
<td>1.5%a</td>
<td>18.9%ab</td>
<td>1.1%ab</td>
</tr>
<tr>
<td>Median</td>
<td>1.3%</td>
<td>18.6%</td>
<td>0.8%</td>
</tr>
<tr>
<td>Lower 95%</td>
<td>1.1%</td>
<td>14.0%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Upper 95%</td>
<td>2.0%</td>
<td>23.7%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.3%</td>
<td>4.6%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Maximum</td>
<td>4.0%</td>
<td>41.3%</td>
<td>3.2%</td>
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</tbody>
</table>

Means for treatments with similar letters are statistically different (p<0.01) within sample type
Figure 1. A flowchart representing the hazard analysis model with inputs, model calculations, and outputs.
Figure 2. The distribution of *Salmonella* loads for three different sample types collected at three Midwest abattoirs.
Figure 3. Distribution of potential infectious doses for 5000 iterations
GENERAL CONCLUSIONS

There is a great deal of research on *Salmonella* in the farm to fork continuum of pork production. Despite the large amount of research, very little quantitative research currently exists. This research consisted of three general objectives.

The first objective of this research was to improve the most commonly used quantification method (MPN). The “RX tube” was developed to in response to this need. Using common *Salmonella* isolation media, the RX tube detected approximately 1 cell of *Salmonella*, had a sensitivity of 96.9% and a specificity of 93.9%. More importantly, it proved equivalent to the MPN, both in seeded and naturally contaminated samples. As the method requires nearly four times less labor, this method enables the analysis of a greater number of samples. This new technique offers the exciting potential to generate more enumeration data.

Using the RX method, the next objective of this research was to acquire quantitative data for several commonly collected porcine samples. Three types of samples (cecal contents, feces and ileocecal lymph nodes) were collected from 210 animals at 3 Midwest abattoirs. From these 630 samples, 114 were positive for *Salmonella*. The distribution of *Salmonella* loads (from $10^1$ to $10^6$ CFU/g) for each sample type was determined. Cecal contents and feces averaged nearly $10^2$ CFU/g while ileocecal lymph nodes average nearly $10^3$ CFU/g. Also, the distributions for all three sample types were skewed toward lower levels. This intensity of samples at lower quantities indicates that the majority of the *Salmonella* infections are recently acquired. Understanding where or when *Salmonella* infections occur is important in designing potential critical control points in the pre-harvest portion of pork production.

Using this quantitative data, a hazard analysis model was constructed to identify potential critical control points in the pre-harvest portion of pork production. Having quantitative data allowed the prediction of potential infections, something difficult to do using only prevalence data. Analysis of the model found that
interventions aimed at holding and transport are effective in reducing carcass prevalence. This is important as *Salmonella* carcass prevalence is the safety measure used by the FSIS. However, the model illustrates that using prevalence to measure safety is an inaccurate measure of the efficacy of an intervention. Food safety is only improved when illnesses are prevented. This model shows the necessity of having quantitative data in order to predict illnesses. Using the quantitative model, the final weeks of finishing and transport/holding were identified as potential critical control points that could reduce potential human infections and not just reduce the prevalence of *Salmonella*.

Much work remains to be completed in the identification of critical control points in pork production. Perhaps the most pressing need is the collection of quantitative data for other portions of the production process. By collecting quantitative data for the entire process for both the U.S. and other countries, ideal places for the application of critical control points can be identified. Another area for research is to conduct interventions that substantiate or disprove models created using quantitative data. For instance, evaluation of several on-farm interventions implemented in the final weeks prior to slaughter could demonstrate if the conclusions of the model were applicable to real world situations. A final area for potential research is further work in enumeration methods for *Salmonella*. A media or technique that is even more rapid, easier, or less complicated to use would only enhance the collection of quantitative data.

This research developed a less cumbersome quantification method, collected initial quantitative data, and used this data to generate a model identifying potential critical control points. The greatest contribution of this research is the demonstration of the usefulness of quantitative data in understanding the ecology of *Salmonella* and its interaction with the production of food. This demonstration of the importance of quantitative data was conducted with the objective of spurring the collection and utilization of enumeration data. It is hoped that quantification technique and the
model outline will be of use to other researchers seeking to understand the role of Salmonella in the production of pork.
APPENDIX A. A SUMMARY OF OTHER RESEARCH I WAS INVOLVED IN DURING MY DOCTORAL EDUCATION
Salmonella enterica infections in market swine with and without transport and holding.

Hurd HS, McKean JD, Griffith RW, Wesley IV, Rostagno MH.

The objective of this study was to compare, by using identical sample types, the Salmonella enterica prevalences and serovar diversities between pigs necropsied on the farm and those necropsied at the abattoir after transport and holding. We necropsied 567 market weight pigs (>70 kg) from six herds. Pigs were alternately assigned to be necropsied on the farm or at the abattoir. One-half of the group was sent in clean, disinfected trailers to slaughter at a commercial abattoir. After transport (mean distance, 169 km) and 2 to 3 h of holding in antemortem pens, these pigs were necropsied. The 50 pigs remaining on the farm were necropsied the following day. The same sample types and amounts were collected for S. enterica culture at both locations. Results show a sevenfold-higher (P < 0.001) S. enterica isolation rate from pigs necropsied at the abattoir (39.9%; 114 of 286) than from those necropsied on the farm (5.3%; 15 of 281). This difference was also observed for each individual herd. All sample types showed a significantly higher prevalence when comparing abattoir to on-farm collection, respectively: lymph nodes, 9.15 versus 3.6%; cecal contents, 13.6 versus 1.8%; 1 g of fecal matter, 25.2 versus 0.7%. Recovery of additional serovars at the abattoir suggests the pigs are receiving S. enterica from extra-farm sources. This study demonstrates that rapid infection during transport, and particularly during holding, is a major reason for increased S. enterica prevalence in swine. This finding identifies the holding pen as an important S. enterica control point in the pork production chain.

I was introduced to Salmonella in swine during my participation as a laboratory technician in this study. This was perhaps the defining research conducted by our scientific group. The future studies conducted by our group either sought to validate the results of this study sought to find a solution to the great increase of Salmonella seen in swine during transport and holding.
Rapid infection in market-weight swine following exposure to a *Salmonella typhimurium*-contaminated environment.

Hurd HS, Gailey JK, McKean JD, Rostagno MH.

OBJECTIVE: To evaluate the possibility of swine becoming infected with *Salmonella Typhimurium* when housed for 2 to 6 hours in an environment contaminated with *Salmonella*, similar to a lairage situation prior to slaughter.

ANIMALS: 40 crossbred market pigs with an approximate body weight of 92 kg.

PROCEDURE: Five trials were conducted (8 pigs/trial) in simulated lairage conditions. Superficial inguinal, ileocecal, and mandibular lymph nodes, cecal contents, distal portion of the ileum, and fecal samples were obtained from each pig after 2 (n = 10), 3 (10), and 6 (5) hours of exposure to an environment contaminated with feces defecated by 10 pigs intranasally inoculated with nalidixic acid-resistant *Salmonella Typhimurium* (chi4232). In addition, 5 control pigs that were not exposed were also evaluated in the same manner.

RESULTS: Feces deposited on the floor by intranasally inoculated swine were mixed with water to form slurry with a resulting load of approximately 10(3) colony-forming units of *Salmonella Typhimurium*/g of material. Eight of 10, 6 of 10, and 6 of 6 pigs exposed to the slurry for 2, 3, or 6 hours, respectively, had positive results for at least 1 sample when tested for the specific strain of *Salmonella Typhimurium*.

CONCLUSIONS AND CLINICAL RELEVANCE: Pigs can become infected during routine resting or holding periods during marketing when exposed to relatively low amounts of *Salmonella* organisms in the preslaughter environment. Intervention at this step of the production process may have a major impact on the safety of pork products.

This study was conducted to validate the results of the previous study. In this study, I functioned as the research leader. Although Dr. Hurd developed the study design, I was charged with coordinating the sampling, culturing and analysis of the project. Additionally, I wrote the initial manuscripts submitted for publication in which we demonstrated that swine can become infected with *Salmonella* in 2 hours or less.
Experimental rapid infection in market swine following exposure to a *Salmonella* contaminated environment.

Hurd HS, Gailey JK, McKean JD, Rostagno MH.

The objective of these experiments was to evaluate the possibility of swine becoming infected with *Salmonella Typhimurium* after a short time interval in a contaminated environment. Two experiments were conducted. Experiment 1 consisted of five trials with eight market weight swine. Pigs were necropsied at 2 (n = 10), 3 (n = 10) and 6 (n = 5) hours after continuous exposure to an environment contaminated with feces shed by swine intranasally inoculated with nalidixic acid-resistant *Salmonella Typhimurium* (chi 4232). In Experiment 2, pigs were necropsied after 30 minutes (n = 6), 60 minutes (n = 6), 2 hours (n = 6), and 6 hours (n = 3). In addition, control animals with no exposure were also necropsied in both experiments. At necropsy, the superficial inguinal, ileocecal, and mandibular lymph nodes, as well as cecal contents, distal ileum portion, and feces were evaluated. All samples were cultured for the presence of the nalidixic acid-resistant *Salmonella*. Feces deposited on the floor by intranasally inoculated swine were mixed with water to form slurry with a resulting load of 10(3)-10(5) *Salmonella Typhimurium* CFU per gram. In Experiment 1, 80% percent of animals with a 2-hour, 60% of animals with a 3-hour, and 100% of animals with a 6-hour exposure to this slurry had at least one sample test positive for the marked *Salmonella Typhimurium* strain. In Experiment 2, 50% of the 30 minute, 50% of the 60 minute, and 33% of the 2-hour exposed pigs had at least one sample test positive. These experiments show that market swine can become infected during routine resting or holding periods when exposed to relatively low levels (10(3) CFU) of *Salmonella* in the simulated pre-slaughter environment, and that exposure times as short as 30 minutes are sufficient to produce contaminated gastrointestinal tracts. They also demonstrate the high risk of holding pigs longer than six hours. Intervention at this step in the swine production process may have a significant impact on the safety of pork products.

This study was a continuation of the previous study. For this study I maintained my role as research leader as we examined infection trends in times as little as 30 minutes. This work created enough interest that the sponsors of "The 4th International Symposium on the Epidemiology and Control of *Salmonella* and other Food Borne Pathogens of Pork" requested we submit it to a special referred journal edition dedicated to this symposium.
Preslaughter holding environment in pork plants is highly contaminated with *Salmonella enterica*.

Rostagno MH, Hurd HS, McKean JD, Ziemer CJ, Gailey JK, Leite RC.

The objective of this study was to determine whether abattoir pens can provide a *Salmonella enterica* infection source during the 2 to 4 h of preharvest holding. Previous work has suggested that pigs may be getting infected, but little has been reported on the environmental contamination of abattoir holding pens. For 24 groups of pigs studied (approximately 150 animals/group) at two high-capacity abattoirs, six pooled fecal samples (n, 10 per pool) were collected from each transport trailer immediately after pigs were unloaded. Holding pens were sampled (one drinking water sample and six pooled floor samples consisting of swabs, residual liquid, and feces) prior to entry of study pigs for the routine holding period (approximately 2.5 h). After slaughter, cecal contents and ileocecal lymph nodes were collected, on the processing line, from 30 pigs in each studied group. All samples were cultured for the isolation and identification of *S. enterica* by primary enrichment in GN-Hajna and tetrathionate broths, secondary enrichment in Rappaport-Vassiliadis broth, and plating on brilliant green sulfa and xylose-lysine-tergitol-4 agars, followed by biochemical and serological identification. The study pens were highly contaminated with *S. enterica*; all holding pens sampled had at least one positive sample. Additionally, 33% (8 of 24) of drinking water samples were positive for *S. enterica*. All 24 groups of pigs had *S. enterica*-positive cecal contents and ileocecal lymph nodes, including those groups from transport trailers with no positive samples. From pigs, trailers, and pens, 586 isolates representing 36 different *Salmonella* serovars were isolated. Of the 353 isolates from pigs (109 from ileocecal lymph nodes plus 244 from cecal contents), 19% were identified as belonging to the same serovars as those isolated from the respective pens; 27% were identified as belonging to the same serovars as those isolated from the trailers. Sixteen percent of the unique serovars were isolated from both pigs and pens, suggesting that pens served as the infection source. This study demonstrates highly contaminated abattoir holding pens and watering sources. It also demonstrates that holding pens can serve as an infection source. This study identifies the abattoir holding pens as a significant hazard and a potential control point for *Salmonella* contamination in the preharvest pork production chain.
In this research lead by Marcos Rostagno, the impact of the holding pen on *Salmonella* prevalence was further explored. I participated in sample collection, processing, culturing and analysis. During this study, I was exposed to the diversity of *Salmonella* serovars present in the pork production system. At this time, the idea that quantity as well as the prevalence of *Salmonella* may be important began to emerge.

**The association between cleaning and disinfection of lairage pens and the prevalence of *Salmonella enterica* in swine at harvest.**


Schmidt PL, O’Connor AM, McKean JD, Hurd HS.

A series of four field trials were conducted to evaluate the ability of a cleaning and disinfection procedure in swine lairage pens to reduce the prevalence of *Salmonella enterica* in slaughtered pigs. A cleaning and disinfection procedure was applied to lairage pens at a large Midwest abattoir. Each trial consisted of a cleaned (alkaline chloride detergent) and disinfected (H2O2 plus peracetic acid sanitizer) pen (treated) and a control pen, each holding 90 to 95 pigs for 2 to 3 h before slaughter. Ileocecal lymph nodes, cecal contents, and rectal contents were collected from 45 pigs from each study pen at harvest and cultured for *S. enterica*. In all trials, cleaning and disinfection reduced the prevalence of *S. enterica*-positive floor swabs in the treated pen (P < 0.05). However, the postharvest prevalence of *S. enterica*-positive pigs varied between trials. In trial 1, there was no significant difference in the prevalence of *S. enterica* in pigs between treatment and control groups. In trials 2 and 3, the prevalence of *S. enterica* was higher in pigs from treated pens versus pigs from control pens (91% versus 40%, P < 0.0001, and 91% versus 24%, P < 0.0001, respectively). In trial 4, the prevalence of *S. enterica* was lower in pigs from treated pens compared with pigs from control pens (5% versus 42%, P < 0.0001). This study indicates that cleaning and disinfection effectively reduces the amount of culturable *S. enterica* in lairage pens, but the ability of cleaned and disinfected pens to reduce the prevalence of *S. enterica* in market-weight pigs remains inconclusive.
This study focused on pen sanitation as a potential intervention for Salmonella in pork production. I served as a technical advisor for Dr. Schmidt for this study, particularly mentoring her in Salmonella isolation technique and pen sampling technique. Additionally, this study was important as some of my enumeration samples were collected during this study.

**Culture methods differ on the isolation of Salmonella enterica serotypes from naturally contaminated swine fecal samples.**


Rostagno, MH, Gailey, JK, Hurd, HS, Mckean, JD, Leite, RC

Four culture methods (A, B, C, and D) were comparatively evaluated for their ability to isolate Salmonella enterica from pooled swine fecal samples (n=100). None of the methods was able to isolate Salmonella from all positive samples. The relative sensitivity of the culture methods evaluated was 82%, 94%, 95%, and 78% for methods A, B, C, and D, respectively. The comparison of sensitivities showed that methods B and C performed significantly better (p<0.05) than methods A and D. Although relative sensitivities of methods B and C were equal, from the 89 positive samples concomitantly detected by both, 35 (39.3%) had different serotypes (no match) isolated by each method. Based on the results of this study, it was concluded that culture methods differ on the isolation of Salmonella enterica serotype from naturally contaminated swine fecal samples. Depending on the objective(s) of investigations on the ecology and epidemiology of Salmonella enterica in swine populations, a method or a combination of methods should be considered for more reliable results.

This evaluation of culture methods for Salmonella was joint project conducted with Marcos Rostagno. We both realized that culture technique can greatly influence Salmonella isolation. We conducted this study to examine the effect of different culture methods. During the conduction of this study, I first began to experiment with the idea for the "RX tube" as a Salmonella isolation method.
Variable abattoir conditions affect *Salmonella enterica* prevalence and meat quality in swine and pork

Submitted to Foodborne Pathogens & Disease, October 2004
Hurd, HS, Gailey, JK, McKean, J.D., Griffith, R.W.

Research suggests that abattoir holding pens pose significant *Salmonella enterica* risk to swine immediately pre-harvest. The goal of this study was to evaluate those factors related to holding that increased the prevalence of *S. enterica* in swine at slaughter. To accomplish this goal, we focused holding time and flooring. Our objectives were to 1) compare *Salmonella enterica* prevalence among pigs held for short (15-45 minutes) vs. long (up to 4 hours) periods before slaughter; and 2) determine the impact of flooring (slatted vs. concrete) as it relates to the prevalence of *S. enterica*. The study consisted of seven repetitions at a large volume (11,000 head/day) Midwest abattoir. Each repetition consisted of one truck load of pigs (n=170) sorted into one of three groups: 1) animals held for a short time (15-45 min) on solid floors (short-hold); 2) animals held for 4 ± 0.5 h on slatted floors; and 3) animals held for 4 ± 0.5 h on solid concrete floors. At slaughter, samples were collected from 30 pigs in each group. Cecal contents (20 ml), feces (20 g), and the ileocecal lymph node were cultured for *S. enterica*. Additionally, the effect of holding time on meat quality parameters (loin pH at 35 min and 6 h, color, drip loss) was evaluated for the first four replicates. The proportion of *S. enterica*-positive samples was highest (*P < 0.05*) in the cecum of pigs held on solid concrete floors (72.4%), and slightly less for pigs held on slatted floors (63.3%). Animals held for less than 45 minutes before slaughter demonstrated the lowest proportion of *S. enterica*-positive samples (52.9%). The pig prevalence, as measured by any one of the three samples being positive, was significantly different (*P < 0.05*) between animals held on solid floors (81%) and those animals held for 45 minutes or less before slaughter (69%). Meat quality, as measured by multiple parameters, was adversely affected by lack of a rest period. The mean 24-h pH was significantly lower for the short-hold group compared to the other two groups. The mean Minolta L and the drip loss were significantly higher in the short-hold group. From this and other studies it appears that elimination of the holding process is not feasible *S. enterica* control option, given current U.S. harvesting systems.

This project was designed to examine short holding times and slatted pen floors as holding pen interventions. I served as the research leader for this project, in which I coordinated extensively with the abattoir to conduct the research. Additionally, I made many inputs into the study design and the practical aspects of
data collection. I participated extensively in data analysis and learned much about data organization and analysis. I also helped write many sections of the initial manuscript. Many of the enumeration samples used in my research were collected during this study.
APPENDIX B. FORMAT AND SOURCE CODE USED IN THE
@ RISK MODEL
## Model Result Reporting Worksheet

| For animals | Cancer prob | Case/No. | Head/tons | Lymph |  |
|-------------|-------------|----------|-----------|-------|-
| 6000        |             |          |           |       |  |

### Case 1

- **Cancer prob**: 0.999
- **Case/No.**: 0.9
- **Head/tons**: 0.9
- **Lymph**: 0.9

### Case 2

- **Cancer prob**: 0.99
- **Case/No.**: 0.9
- **Head/tons**: 0.9
- **Lymph**: 0.9
Model result reporting worksheet continued

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Diagram showing model results with various parameters and values.
## Illness Calculating Worksheet

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