The Isolation and Characterization of *Salmonella* from Swine Feces in Kenya

THESIS

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Abstract

Nontyphoidal *Salmonella* (NTS) is an important pathogen that causes foodborne diseases in both humans and also gastrointestinal illness in animals. NTS causes considerable morbidity and mortality worldwide. Since the 1990s, antimicrobial resistance in NTS has become a global concern. In Africa, NTS is consistently a leading cause of bacteremia among immunocompromised people. Yet, the sources and transmission routes of *Salmonella* in developing countries are poorly understood. Antimicrobial resistance reduces treatment options for physicians and veterinarians, and is associated with higher mortality, invasiveness, and increased hospital costs. Bacterial plasmids encode for many influential properties including resistance to many antibiotics. Plasmids are transposable and can therefore be shared among bacteria, allowing them to become more virulent or evade normal host defenses for example. Antimicrobial use is an important selective pressure for emergence and persistence of resistance in the ecosystem. In addition, ecologic factors other than antimicrobial use may play a significant role in dissemination of antimicrobial resistance. In Kenya, swine production is one of the fastest growing food animal industry systems. We hypothesized that herd-level ecologic factors will have an impact on the prevalence and transmission of *Salmonella* in swine and these factors may contribute to the persistence of antimicrobial resistant strains. A total of 195 samples were collected from 30 farms located around Nairobi, Kenya. First, isolation,
identification and serogrouping were carried out. Further phenotyping and genotyping were done using Kirby-Bauer disc diffusion and pulsed field gel electrophoresis methods respectively. We found 99 isolates (17% prevalence) from 10 of the 30 (33% farm level prevalence) farms. All farms were classified as semi-intensive swine production systems. Surveys were completed at each farm that samples were collected through an interview using MagPi software on a smartphone. Logistic regression analyses indicated that the herd-level data were not significant predictors of being *Salmonella* positive at the 0.05 confidence level. The isolates were first phenotypically classified based on their O antigen using Somatic (O) Antigen Agglutination Tests. We found a total of 4 groups among the 99 isolates including: B; C; +(A-I) -(B, C, E, G, D1, and D2); -(A-I). Most isolates (n=65) were found to belong to sergroup C. The antimicrobial susceptibility test was done using the Kirby-Bauer disk diffusion method with a panel of 12 antimicrobials. Most isolates (n=55) were pansusceptible. The second most frequent pattern was resistance to sulfisoxazole and ciprofloxacin (R-type SuCip) with a total of 19 isolates. Approximately 40% of the isolates (40 of 99) were found to be resistant to sulfisoxazole and 20% (20 of 99) were resistant to ciprofloxacin. Genotypically, pulsed-field gel electrophoresis (PFGE) was used to assess the persistence and transmission of the same strains within and across pig populations in this study. Dendrogram analysis of the PFGE profiling resulted in 18 genotypic clusters and nine sporadic clones. Most clusters showed a cohesive phenotype within. Clusters H, J, M, N, and R had multiple farms within each
cluster and/or multiple resistance patterns within each cluster. The outcomes of this research might be useful as a baseline for a larger longitudinal study to better understand any ecological management factors that are playing a role in the transmission of *Salmonella*. 
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# Table of Contents

Abstract .................................................................................................................. ii

Acknowledgments .................................................................................................... v

Vita ............................................................................................................................ vi

List of Tables .......................................................................................................... ix

List of Figures ......................................................................................................... x

Chapter 1: Literature Review .................................................................................... 1

A. Foodborne Illness and Public Health ................................................................. 1

B. *Salmonella* the Organism ............................................................................... 2

C. Epidemiology of Salmonellosis in Humans ....................................................... 5

D. *Salmonella* in Swine ....................................................................................... 10

E. Antimicrobial Resistance in *Salmonella* ......................................................... 13

F. Virulence Plasmids ........................................................................................... 16

G. MDR and Virulent Strains ................................................................................. 17

H. Risk Factors and Control Measures ................................................................. 20

I. Future Needs ...................................................................................................... 23

Chapter 2: Introduction .......................................................................................... 24

A. Background ....................................................................................................... 24

B. Goals and Objectives ....................................................................................... 25

Chapter 3: Materials and Methods ....................................................................... 26
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Source of Isolates</td>
<td>26</td>
</tr>
<tr>
<td>B. MagPi</td>
<td>28</td>
</tr>
<tr>
<td>C. <em>Salmonella</em> Isolation</td>
<td>28</td>
</tr>
<tr>
<td>D. Somatic (O) Antigen Agglutination Testing</td>
<td>29</td>
</tr>
<tr>
<td>E. Antimicrobial Susceptibility Testing</td>
<td>29</td>
</tr>
<tr>
<td>F. Pulsed-Field Gel Electrophoresis (PFGE) Genotyping</td>
<td>30</td>
</tr>
<tr>
<td>G. Statistical Analysis</td>
<td>31</td>
</tr>
<tr>
<td>Chapter 4: Results</td>
<td>33</td>
</tr>
<tr>
<td>A. Prevalence and Risk Factors</td>
<td>33</td>
</tr>
<tr>
<td>B. Serogroups</td>
<td>33</td>
</tr>
<tr>
<td>C. Antimicrobial Resistance Profiles</td>
<td>36</td>
</tr>
<tr>
<td>D. Pulsed-field Gel Electrophoresis (PFGE) Analysis</td>
<td>39</td>
</tr>
<tr>
<td>Chapter 5: Discussion</td>
<td>43</td>
</tr>
<tr>
<td>References</td>
<td>52</td>
</tr>
<tr>
<td>Appendix A: Sample MagPi Questionnaire</td>
<td>67</td>
</tr>
</tbody>
</table>
List of Tables

Table 1. Sample Collection.................................................................27
Table 2. Summary of odds ratios at the farm level..............................34
Table 3. Median and range for continuous farm management practices...35
Table 4. Serogrouping percentages by farm......................................36
Table 5. Antibiotics each farm showed resistance to............................39
Table 6. Clustering Pattern...............................................................40
Table 7. Farms genotypically and phenotypically described..................41
List of Figures

Figure 1. Number of isolates resistance to each antibiotic..............................37
Figure 2. Resistance Patterns .................................................................38
Figure 3. Dendogram ..........................................................................42
Chapter 1: Literature Review

A. Foodborne Illness and Public Health:

Every year, approximately one in six Americans get sick by one of the most costly, yet preventable public health problems, foodborne illnesses. There are more than 250 different foodborne diseases causing nausea, vomiting, abdominal cramps, and diarrhea\textsuperscript{1,2}. Foodborne illness is caused by viruses, bacteria, and parasites\textsuperscript{3}. These illnesses can lead to hospitalization and even death. According to a report published in 2011, approximately 48 million people got sick, 128,000 were hospitalized, and 3,000 died from foodborne related illnesses in the US\textsuperscript{1}. It is estimated that foodborne diseases cost the US 5-6 billion dollars in medical expenses and lost productivity annually\textsuperscript{4}. It is difficult to get a true grasp of the incidence of these illnesses because they are often underreported and go unrecognized\textsuperscript{2,3}. If foodborne illness could be reduced by just 10%, approximately 5 million Americans would be kept from getting sick each year\textsuperscript{1}. It is important to consider that everyone is at risk for getting a foodborne illness; yet, some individuals are at greater risk and may experience more serious complications and death from a foodborne disease\textsuperscript{5}.

According to the Centers for Disease Control and Prevention (CDC), nontyphoidal \textit{Salmonella} is the second most common cause of domestically acquired foodborne disease and is the most common cause of foodborne-related hospitalizations and death\textsuperscript{1}. Every
year in the US, approximately 40,000 cases of salmonellosis are reported to the CDC, and up to 4 million additional cases likely go unreported. Approximately 400 people in the US die yearly with acute salmonellosis\textsuperscript{6,7}. In a recent report, Scallan et al. estimated that 28\% of deaths caused by foodborne illnesses in the United States could be attributed to nontyphoidal \textit{Salmonella} (NTS) species\textsuperscript{3}.

Of recent concern, there has been an increasing prevalence of multi-drug resistance (MDR) among \textit{Salmonella}\textsuperscript{8-13}. Resistance has been shown to clinically important agents including fluoroquinolones and cephalosporins\textsuperscript{10-13}. It is estimated that 95\% of human \textit{Salmonella} infections can be attributed to foodborne exposure\textsuperscript{7} and several studies have documented farm animals as a major reservoir for NTS in industrialized countries. However, there is a lack of data on sources of NTS that are causing infections in humans in developing countries\textsuperscript{14}.

\textbf{B. \textit{Salmonella} the Organism}

Named after the American veterinarian scientist Daniel Salmon, Salmonellosis is an illness caused by the bacteria \textit{Salmonella}\textsuperscript{6,7}. Karl Eberth first observed the rod-shaped organisms that we associate with the serotype Typhi organism in 1880\textsuperscript{15}. This gram-negative, flagellated facultative anaerobe belongs to the family Enterobacteriaceae\textsuperscript{16}. Since the discovery of \textit{Salmonella}, more than 2557 different serotypes have been isolated from different animal species\textsuperscript{17-19}. The bacteria normally reside in the digestive tract of many wild and domestic animals including: cattle, sheep, pigs, fowls, and reptiles. Many
of these serotypes cause infection in humans\textsuperscript{2,20-22}. \textit{Salmonella} is subdivided into two species including \textit{enterica} and \textit{bongori}. Specifically, \textit{Salmonella enterica} is further subdivided into the six subspecies of \textit{enterica}, \textit{salamae}, \textit{arizonae}, \textit{diarizonae}, \textit{houtenae}, and \textit{indica}. In order to differentiate isolates of \textit{Salmonella} beyond the subspecies level, serotyping can be performed\textsuperscript{17,18}. Serotypes of \textit{Salmonella} are differentiated based on their somatic (O) and flagellar (H) antigens\textsuperscript{19,23}. The serotypes can also be classified by whether: (1) the serotype is capable of causing a typhoid-like disease in a single host species (host-restricted serotypes); (2) the serotype is associated with one host species, but can also cause disease in other hosts (host-adapted serotypes)\textsuperscript{19,24,25} (3) the serotype rarely produces systemic infections but is able to colonize the alimentary tract of a wide range of animals (broad host range serotypes)\textsuperscript{26}. In the United States, serotypes Typhimurium and Enteritidis are the most common\textsuperscript{6}.

The O antigen is the outermost component of the cell surface lipopolysaccharide. An agglutination assay using antisera that reacts with groups of related antigens or a single antigen is used to detect the O antigen\textsuperscript{18}. For the isolation and identification of \textit{Salmonella}, the United States Department of Agriculture (USDA) recommends testing of isolates with polyvalent O antiserum reactive with serogroups A through I. These groups encompass the majority of \textit{Salmonella} serotypes that are commonly recovered from meat and poultry products. Periodically, an isolate may be typical of \textit{Salmonella} biochemically, but non-reactive with available O group antisera\textsuperscript{27}. 
Because of the emergence of antimicrobial resistance, the performance of antimicrobial susceptibility testing is critical to determine the extent of antimicrobial resistance (AMR) of *Salmonella* to various antimicrobial agents. Widely used test methods include: broth microdilution, agar dilution, and Kirby-Bauer disk diffusion\textsuperscript{28, 29}. Disk diffusion was used in this project because of the test simplicity, it is the least costly method, it could be used to screen a large number of isolates, and it does not require any special equipment.

Disk diffusion tests are interpreted based on the zones of growth inhibition around each of the antibiotic disks. The diameter of the zone correlates to the diffusion rate of the drug through the agar and susceptibility of the isolate\textsuperscript{28-30}. The zone is demarcated when the concentration of the antimicrobial can no longer inhibit the growth of the test bacterium because it has become so diluted\textsuperscript{28,30}. Data collected based on these tests can aid the clinician in the selection of the appropriate antimicrobial agent, provide data for epidemiological surveillance, and aid in the development of policies on antimicrobial use. Successively, the epidemiological data can assist in decisions regarding first-line therapy and help to detect the emergence and the propagation of resistant bacterial\textsuperscript{30}. Antimicrobial susceptibility testing has also been used as a way of phenotypic subtyping of isolates based on their resistance pattern, often referred as R-types\textsuperscript{28}.

Pulsed-field gel electrophoresis (PFGE) provides further subtyping using a genotypic approach\textsuperscript{18}. Other methods similarly used for genotyping of Salmonella include amplified fragment length polymorphism, repetitive palindromic extragenic-PCR\textsuperscript{31}, multi-locus
sequence testing\textsuperscript{32}, and multilocus variable-number tandem-repeat analysis (MLVA)\textsuperscript{33}. Bacteria are first loaded in an agarose suspension (plug), the bacterial cell is lysed open, and the intact genomic DNA is treated with restriction enzymes to cut the bacteria into DNA pieces. The plugs are then loaded onto an agarose gel and placed into an electric field that is constantly changing direction\textsuperscript{34, 35}. As the electric field changes direction smaller DNA is pushed across the gel while larger DNA fragments lag behind. The DNA is separated into bands as it runs across the gel\textsuperscript{34}. The restriction patterns created by PFGE are stable and reproducible\textsuperscript{35}.

C. Epidemiology of Salmonellosis in Humans

\textit{NTS Salmonella} is usually transmitted to humans when individuals consume food contaminated with animal feces. Although contaminated foods are often of animal origin, any food, including vegetables can become contaminated. Thorough cooking kills the bacteria, but food can also become contaminated by the hands of an infected food handler who did not wash their hands properly\textsuperscript{36}. It is estimated that 95\% of human \textit{Salmonella} infections can be attributed to foodborne exposure\textsuperscript{2}. A farm-to-fork model predicted that 99,430 human cases of salmonellosis are associated with pork. The same model approximated that salmonellosis associated with pork has social costs totaling $81.53 million\textsuperscript{37}.

\textit{After Salmonella} is ingested, symptoms may begin within 12 to 48 hours\textsuperscript{6,7,20}. Nausea and abdominal cramping occur first, followed by watery diarrhea, fever and vomiting.
Most individuals recover without treatment and symptoms conclude within a week\textsuperscript{6}. Other symptoms may result from bacteremia if the infection spreads\textsuperscript{20}. As mentioned throughout the review, clinical presentation is often more severe in developing countries. In a study conducted in rural Kenya, 26\% of all inpatient childhood deaths were associated with bacteremia. This was higher than the number of deaths associated with malaria\textsuperscript{38,39}.

Diagnosis is made through culture of samples of stool, pus, blood or a swab used to obtain a sample from the rectum\textsuperscript{20}. To diagnose bacteremia, blood culture facilities are needed. Sadly, the infrastructure necessary for adequate clinical laboratory diagnosis is lacking in the resource-poor countries in Africa\textsuperscript{38,40}.

Enteric infections of \textit{Salmonella} are treated with supportive treatments given orally or intravenously. Antibiotics are not prescribed in most cases as they do not shorten recovery time and can result in bacteria being excreted for a longer period of time in the stool. The exceptional use of antibiotics is for individuals at risk of bacteremia and those with implants. Antibiotics are also given to people that continue to excrete the bacteria in the stool after symptoms have disappeared\textsuperscript{20,36}. The World Health Organization advises that if there is known substantial antimicrobial resistance to traditional first-line antimicrobial agents, then the use of a third-generation cephalosporin may be appropriate\textsuperscript{38,41}. The CDC recommends that when treating NTS bacteremia in HIV-infected adults, ciprofloxacin is used if the CD4 cell count is less than 200 cells/mm\textsuperscript{3},
followed by long-term secondary prophylaxis$^{38,42}$. There are not sufficient data for
duration of therapy or secondary prophylaxis for neonates, patients with meningitis, and
people infected with HIV in Africa$^{38}$.

*Salmonella* may also cause bacteremia and spread causing abscess at distant sites. These
sites include but are not limited to bones, joints, along the urinary tract, lungs, and may
also cause infection on prosthetic joints or heart valves, on a blood vessel graft, or on
tumors$^{20,43}$. Reiter’s syndrome may develop in a small number of persons, whereby the
individual develops pain in their joints, painful urination, and irritation of the eyes. This
can last for months to years and can lead to difficult to treat chronic arthritis$^{44}$. Abscesses
and infected arteries may cause chronic bacteremia. *Salmonella* is more likely to spread
through the bloodstream in infants, older people, individuals that are immune
compromised with diseases such as HIV, individuals with disorders that affect red blood
cells such as sickle cell anemia, and individuals who take drugs that suppress the immune
system, such as those that are used to treat cancer or prevent rejection of an organ after
transplantation$^{20,43,45}$. Specifically for children, the rate of diagnosed infections in those
less than five years of age is approximately five times higher than the rate in all other
people$^{6}$. Despite the fact that *Salmonella* infections caused by nontyphoidal serotypes are
often self-limiting in humans, if systemic spread occurs, effective antimicrobial therapy
may become necessary$^{8,46,47}$.
Of bacterial pathogens involved in invasive disease, NTS is among the most common in sub-Saharan Africa. Here, infections are much more severe, causing bacteremia and meningitis with a mortality rate of 20-25%\textsuperscript{43,48-53}. This is especially true among young children with malaria and malnutrition, and among adults with HIV\textsuperscript{54-56}. More specifically, *Salmonella enterica* serotype Typhi and nontyphoidal *S. enterica* (NTS) are leading causes of bacteremia. All NTS isolates collected during a study in the DRC were MDR and most of these isolates were of the serotypes Enteritidis and Typhimurium\textsuperscript{38,57}. Enteritidis and Typhimurium are also the most common serotypes in the US\textsuperscript{6}. Notably, these serotypes are shown to be the most common serotypes of NTS causing human disease in all of sub-Saharan Africa\textsuperscript{38,39,43,51,56,58,59}.

Because those that are infected with NTS often present with a non-specific febrile illness, diagnosis and treatment is an extremely difficult, made worse by a lack of resources\textsuperscript{54-56}. In Africa, invasive NTS (iNTS) has an estimated annual incidence of 175-388/100,000 among children less than 5 years old\textsuperscript{50,52,54,60}, and 1800-9000/100,000 per person years of observation among non-ART treated HIV prevalent cohorts in Africa\textsuperscript{54,61-63}. NTS also remains an important cause of neonatal sepsis in Africa\textsuperscript{54,64}. Intracellular persistence and recrudescence is suggested by the high rate of bacteraemic recurrence of iNTS in HIV-infected adults. It has been shown that iNTS persists and replicates in the bone marrow following an index event\textsuperscript{54,65}.
For the continent as a whole, iNTS is considered endemic to rural and urban sub-Saharan Africa\textsuperscript{38,50}. For the country that this study was conducted in (Kenya) the estimated minimum incidence of bacteremia was 505 cases per 100,000 person-years in the children less than 5 years old. Of these cases, 88 per 100,000 person-years were NTS bacteremia\textsuperscript{38,52}. Arguably, the true incidence of bacteremia is probably 2-3 times this figure. This is because the children that died before reaching the hospital weren’t tested\textsuperscript{38,50,52}.

It is important to note that in Africa, most studies on iNTS have focused on high-risk groups. True incidence is likely to vary by how prevalent HIV is in the population, local conditions, and the age distribution. Also, the incidence of iNTS in sub-Saharan Africa is likely higher than the incidence of typhoid fever\textsuperscript{38,66}.

Comparatively, iNTS is fairly rare in industrialized populations\textsuperscript{54}. NTS disease in developed countries is usually a self-limited diarrhea and the mortality rate is much lower\textsuperscript{38}. The International Bacteremia Surveillance Collaborative reported an overall crude annual incidence of invasive \textit{Salmonella} infections in Finland, Australia, Denmark, and Canada from 2000-2007 to be 1.02/100,000 population. It was noted that there was a gradual overall increase in iNTS, a seasonal pattern was observed (increase in autumn), and the strongest risk factors were male gender and older age\textsuperscript{54,67}.
D. *Salmonella* in Swine:

*Salmonella* Choleraesuis was the first serotype of *Salmonella* to be isolated from pigs. This serotype was isolated just two years after the first isolation of *Salmonella*\(^{68}\). Even though pigs infected with *Salmonella* can develop enteric and fatal systemic disease, pigs that are infected often carry *Salmonella* asymptomatically in the tonsils, the intestines and the gut-associated lymphoid tissue (GALT)\(^{26,69,70}\). Such carriers are a considerable reservoir of *Salmonella* and pose a threat to both human and animal health\(^{71}\).

Worldwide, during the 1950s and 1960s, *Salmonella* Choleraesuis, including variant Kuzendorf, was the leading serotype isolated from swine\(^{26}\). Currently, *Salmonella* Choleraesuis is highly prevalent in North America and Asia\(^{26,72-76}\). Throughout the world, *Salmonella* Typhimurium, including variant Copenhagen and *Salmonella* Derby, are the most commonly isolated nontyphoidal serotypes in swine\(^{10,74,77}\).

Statistical models have predicted that in the US an estimated 100,000 human cases of salmonellosis are related to the consumption of pork. This has an estimated social cost of roughly 80 million dollars per year\(^{78}\). The public health risk of *Salmonella* infection from eating pork contaminated with *Salmonella* depends on multiple factors. These factors include: the level of infection in the pig herd\(^{72,79}\), hygiene during slaughterhouse processing\(^ {80}\), conditions of meat storage and distribution\(^ {81}\), and how the consumer handles the undercooked pork\(^ {72}\). Cross-contamination from hands from preparing pork cuts has been shown to pose the highest risk to the consumer\(^ {72}\).
Transmission is thought to occur mainly by way of the feco-oral route between pigs. Clinical signs and fecal excretion of high numbers of bacteria depend on the inoculating dose\textsuperscript{82,83}. Other studies have shown that the upper respiratory tract and lungs may also be a port of entry\textsuperscript{26,84}. In addition, reports have found that airborne Salmonella Typhimurium transmission over short distances in weaned pigs is possible. Nose-to-nose transmission is also possible and should be evaluated in intensive swine raise systems\textsuperscript{85}. Salmonella contamination at slaughter may come from palatine tonsils in heavily infected pigs\textsuperscript{69}. Upon ingestion, Salmonella enters the soft palate tonsils and persists in the tonsillar crypts\textsuperscript{26,70}. The bacteria can persist asymptotically within the tonsils. This makes identification of swine that are carrying the disease difficult and makes disease control and pathogen elimination challenging\textsuperscript{70}.

In order to survive after ingestion, Salmonella must endure the low pH of the stomach. By producing acid shock proteins, Salmonella can adapt to and survive in acidic environments up to a pH of 3\textsuperscript{86-88}. Studies have shown that pigs being fed a coarse nonpelleted diet have reduced pH in their stomach and an increased in vitro death rate of Salmonella\textsuperscript{89}. Bacteria that are able to survive the acid environment of the stomach can pass into the intestinal tract, where they can cause gastroenteritis\textsuperscript{87}.

After passage through the stomach, bacteria that survive will encounter other antibacterial factors in the small intestine including bile salts. Salmonella can sense and
respond to bile and therefore survive in a normally bactericidal environment by altering protein expression\textsuperscript{90}. \textit{Salmonella} may use bile salts as a signal allowing them to know where they are in the digestive tract and what invasion factors they must manufacture to survive\textsuperscript{91}.

The subepithelial layer of Peyer’s patches is the main portal of entry in early \textit{Salmonella} infection. Invasion of the porcine jejunum is not limited to any specific epithelial cell type\textsuperscript{92}. \textit{Salmonella} Typhimurium is often confined to the intestines in pigs because it rapidly grows in the pig’s gut and creates a pro-inflammatory response. \textit{Salmonella} Choleraesuis may spread beyond the intestinal boundaries because it slowly replicates which may enable it to evade host immunity and disseminate within the intestinal mucosa\textsuperscript{93}.

It is possible for pigs that are infected with \textit{Salmonella} Typhimurium to asymptptomatically carry these organisms long-term\textsuperscript{69}. These pigs may bias monitoring programs because asymptomatic shedders are difficult to detect in live animals\textsuperscript{79}. These undetected pigs may cause contamination of shipping equipment and holding areas, and result in pre-slaughter transmission of \textit{Salmonella} to non-infected pigs, especially during times of stressed induced shedding\textsuperscript{94,95}. A Dutch study showed that during transport and holding times the number of \textit{Salmonella} shedders can double within 2-6 hours. This increase in shedding animals can be caused by both pigs already excreting \textit{Salmonella} and pigs with reactivated latent infections\textsuperscript{96}.
E. Antimicrobial Resistance in *Salmonella*

In the early 1990s, there was a substantial increase in antimicrobial resistance in nontyphoidal *Salmonella*, and it has since become a global problem\textsuperscript{8,46}. The rate of drug resistance varies between different serotypes. For example, *Salmonella* Enteritidis shows less acquired resistance compared to other nontyphoidal serotypes\textsuperscript{46}. In *Salmonella* Typhimurium, the prevalence of acquired antimicrobial resistance is much higher\textsuperscript{97}. There are also strains of Typhimurium that are resistant to ten or more antimicrobial agents\textsuperscript{13}.

Because resistance to conventional antibiotics is spreading in humans, extended spectrum cephalosporins and fluoroquinolones have become the drugs of choice\textsuperscript{98}. In recent years, there has been an increasing prevalence of resistance to these antimicrobials as well\textsuperscript{9-11,99}. The prevalence of drug resistant *S*. Typhimurium in the US has been estimated to be approximately 40\%\textsuperscript{100}. In different animal species, fluoroquinolones are often used to treat the severe enteric forms of salmonellosis\textsuperscript{101-103}. These antibiotics are also showing resistance in pigs and pork\textsuperscript{12,104-108}. Multidrug resistant *Salmonella* Typhimurium strains are also prevalent in antimicrobial free swine production systems, despite the absence of antimicrobial selection pressure\textsuperscript{109}.

During a three year study on *Salmonella* serotypes in swine in North Carolina, Gebreyes et al. found 86\% of isolates showed resistance to at least one antimicrobial and among resistance isolates, 56\% were found to be multi-drug resistant. Resistance was shown to
widely used tetracycline and β-lactams. Notably, a high frequency of resistance was seen to chloramphenicol despite phenicols not being used in swine production for more than a decade before the study was conducted10.

The increasing multiple antimicrobial resistance associated with *Salmonella* Typhimurium, *Salmonella* Derby, and other pork-related serotypes may quickly become a serious human health hazard8,110-113. Additionally, *Salmonella* Choleraesuis and *Salmonella* Typhimurium have been reported to be able to generate hybrid plasmids that consist of virulence and antimicrobial resistance genes. This may pose a larger threat to public health114.

During a study examining possible clonal relationships of NTS, Kariuki et al found the predominant strain among the pigs they tested in Kenya to be *S.* Agona14. A study by Kagambega in Burkina Faso found 16% of samples from swine contained *Salmonella*115. Because *Salmonella* infection persists in pig herds sub-clinically and the pigs are often asymptomatic, it is still possible to isolate *Salmonella* from apparently healthy pigs116. Kikuvi et al. collected samples from random pigs in a slaughter house in Nairobi and found 20.7% prevalence of *Salmonella* in pigs117,118. The three serotypes that were identified include: *S.* Saintpaul (9), *S.* Heidelberg (3), *S.* Braenderup (2). This was the first report of *S.* Heidelberg being found in food animals in Kenya117. In a study conducted in Burkina Faso, *S.* Muenster was the predominate serotype found in pigs115.
Disparities in the serotypes found may be due to differences in the period of sampling, the location of sampling, or the origin and number of infected pigs\textsuperscript{119}.

During Kariuki’s study, all of the NTS isolated from pigs were fully susceptible to all 11 antimicrobials tested (ampicillin 10µg, co-amoxiclav 10:20µg, cefuroxime 30µg, ceftrazidime 30µg, co-trimoxazole 25µg, chloramphenicol 30µg, ciprofloxacin 5µg, gentamicin 10µg, nalidixic acid 10µg, streptomycin 10µg, and tetracycline 30µg)\textsuperscript{14}. Kikuvi et al. found resistance to ampicillin, tetracycline, and streptomycin in a S. Braenderup isolate. Three S. Saintpaul isolates were resistant to one of chloramphenicol, streptomycin or ampicillin, and the fourth isolate was intermediately resistant to tetracycline. The other isolates were susceptible to all antimicrobials tested. All isolates were susceptible to gentamicin, kanamycin, sulphamethoxazole/trimethoprim, and nalidixic acid\textsuperscript{117}. Other studies have found that \textit{Salmonella} isolates from swine were susceptible to the tested antimicrobials\textsuperscript{115}.

Examining the dynamics of \textit{Salmonella} in swine populations reared in antibiotic free (ABF) production systems, Thakur et al. found a high frequency of antimicrobial resistance without antimicrobial selection pressures\textsuperscript{109}. This study highlighted the need for epidemiological based studies to determine the role played by the environment in the dissemination of \textit{Salmonella} in swine where the selection pressure is absent\textsuperscript{109}.
F. Virulence Plasmids

Bacterial plasmids are described as extrachromosomal, circular, double-stranded DNA, genetic accessory elements. They encode for many influential properties including resistance to many antibiotics\textsuperscript{120}. Eight serotypes, out of the more than 2500 serotypes of *Salmonella*, carry a virulence plasmid. This includes serotypes Choleraesuis, Dublin, Enteritidis, and Typhimurium\textsuperscript{114}, all of which have been isolated in swine\textsuperscript{26,121-123}. Each serotype contains a serotype-specific virulence plasmid that contains the spv (*Salmonella* plasmid virulence) operon\textsuperscript{114,124}. The spv operon assists in the full expression of virulence of the serotype in its specific host\textsuperscript{124-126}. Some virulence plasmids are conjugally self-transmissible. On these, some of the virulence traits are part of a small transposable DNA unit\textsuperscript{127}. Because of these transposable virulence traits, bacteria can share traits that may allow them to become more virulent or evade normal host defenses for example\textsuperscript{120}.

Resistance genes carried on plasmids has led to the emergence of strains resistant to conventional antibiotics including ciprofloxacin and ceftriazone\textsuperscript{114,128}. The recombination of virulence plasmids with resistance plasmids afford *Salmonella* with both a survival advantage against many antibiotics and the ability to proliferate into a new genetic lineage\textsuperscript{114}. These recombinant plasmids may also extend the host range of these plasmids\textsuperscript{129}. Collectively, these trends could lead to the existence and spread of more virulent and resistant nontyphoidal *Salmonella*\textsuperscript{114}. 
G. MDR and Virulent Strains

The incidence of MDR serotypes, including Newport and Typhimurium, are reported to be increasing throughout the globe\textsuperscript{10,13}. First recognized in the UK in 1984\textsuperscript{11}, Definitive Type 104 (DT104) has become a major concern to public health as it too is being identified in other parts of the world\textsuperscript{12,130-132}. This phage type exhibits resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline\textsuperscript{131}. Additionally, this phage type has been shown to acquire additional resistance to fluoroquinolones\textsuperscript{11} and higher generation cephalosporins\textsuperscript{9,99}. Other MDR strains becoming globally established include the highly-fluoroquinolone resistant \textit{Salmonella enterica} serotype Kentucky ST198\textsuperscript{108,133}. High rates of resistance to tetracycline, sulfonamide, and streptomycin have been found in Southern Brazil in phage types DT177, DT194, and DT 192\textsuperscript{134}.

Recently, a multidrug-resistant \textit{Salmonella enterica} serotype Typhimurium ST313 has emerged in sub-Saharan Africa causing severe infections in humans\textsuperscript{48,53,135,136}. This disease caused by ST313 has been characterized by bacteremia, meningitis, and septic arthritis. Often, a fever is the only clinical sign which makes microbiological confirmation necessary for making a diagnosis. Case-fatality rates are between 20-25\% for children and up to 50\% in adults\textsuperscript{50,51,65,135}.

Studies have hypothesized that this specific sequence type is carrying factors that are associated with an increased ability to cause disease. Additionally, Next Generation
Sequencing (NGS) studies have shown that *S. Typhimurium* ST313 is a clonal clade presently circulating in sub-Saharan Africa and was introduced by a common ancestry more than 50 years ago\(^{48,137}\). This clade has been divided into two lineages which have acquired resistance genes on separate occasions\(^{48,138}\). These two phylogenies have evolved sequentially, likely being driven by the use of antimicrobials and the emergence of HIV\(^{138,139}\). Conversely, ST313 is also driving the use of expensive antimicrobial drugs in countries with the poorest health services in the world\(^{53}\).

Studies hoping to elucidate the increased pathogenicity have suggested that *S. Typhimurium* ST313 could have adapted to occupy an ecological and immunological niche. This niche is created by HIV, malaria, and the malnutrition that is ever-present in Africa\(^{48,53}\). Because epidemiological investigations have been unable to determine an environmental or zoonotic source and because of the niche created in sub-Saharan Africa, it has been suggested that ST313 is restricted in human infections\(^{53,138,139}\). Transmission is thought to occur through direct or indirect human-to-human routes with asymptomatic carriage possibly playing a role.\(^{14,53,138,139}\) ST313 has also been demonstrated to carry virulence mechanisms that allow for intracellular survival inside macrophages and infection of the intestinal epithelial layer\(^{48}\).

During a study conducted on ST313 in Nigeria and the Democratic Republic of the Congo, the most common resistance profile exhibited resistance to ampicillin, chloramphenicol, spectinomycin, streptomycin, sulfamethoxazole, and trimethoprim.
Other profiles showed additional resistance to tetracycline\textsuperscript{136}. Bacterial strains from Kenya used by Kingsley et al. showed resistance to ampicillin, chloramphenicol, kanamycin, streptomycin, sulphonamide, and trimethoprim\textsuperscript{135}.

Currently, there is no epidemiological evidence that birds are a source of ST313 infection, though studies have shown that they are a likely zoonotic source. Domestic chickens in Africa live in close contact with humans in urban and village communities\textsuperscript{139,140}. Other pathovars of \textit{S. Typhimurium} can persist within the gastrointestinal tract of chickens. This leads to fecal shedding into the environment and the contamination of products from the chicken, including meat and eggs\textsuperscript{139,141}. Wild birds have been sources of major \textit{Salmonella} outbreaks in both the UK and USA\textsuperscript{139,141}. The Kingsley et al. study noted that most of the ST313 isolates in their study belonged to the phage type DT56var\textsuperscript{136}. The finding of this phage type may also raise the possibility of wild birds as a source of this sequence type\textsuperscript{136,137}. Also, ST313 was proven to cause invasive infection in the chicken with intestinal inflammation and colonization of the chicken’s gastrointestinal tract\textsuperscript{139}.

Likely, ST313 has an original zoonotic source, but once it entered a susceptible population, human-to-human transmission became more critical to the spread of the disease. Alternatively, zoonotic transmission from infected feces or via contaminated food may remain an important source of infection\textsuperscript{139}.
H. Risk Factors and Control Measures

Because risk factors for NTS infection in Africa have not been well characterized, evidence based prevention studies conducted in more-developed countries should be examined. Environmental risk factors that should be examined include food and water, hospital-acquired infection, direct and indirect animal contact, and transmission between humans. Seasonal trends, with peaks during the rainy season, may correlate with fecal organisms being found at their highest concentrations in drinking water sources in Africa. This could be mitigated by protection of source water, increased access to treated safe water, use of narrow-mouth spigoted containers for water storage; and the treatment of water at home with chlorine, solar disinfection, filtration, flocculation, or a combination.

Considering NTS infects or colonizes most mammalian species, food animals have been a focus of efforts to reduce transmission in developed countries. It is recommended that meat and other animal products are cooked thoroughly. It is also essential that hands are regularly washed after using the bathroom and after handling raw meat. Dairy products that have not been pasteurized and have not been kept refrigerated should be avoided. Salmonellosis caused by contaminated foods may be prevented with improvements in farm animal hygiene, in practices in the slaughter plant, and in fruit and vegetable harvesting and packing operations. It is important to educate food industry
workers. Wider use of pasteurized eggs should also be considered in restaurants, hospitals, and nursing homes\textsuperscript{146}. Studies suggest that changes made during processing are more important for human health risk when they are compared with on-farm strategies for the control of \textit{Salmonella}\textsuperscript{72,78,119}. By reducing the prevalence of \textit{Salmonella} during processing and slaughter by 10\% the number of human salmonellosis would decrease by approximately 75\%\textsuperscript{78}. Results have suggested that when a control strategy is employed closer to the consumer there will be a greater impact on human cases of \textit{Salmonella}. By using these strategies in plants, there is a lower per-pig cost than on-farm strategies such as vaccination\textsuperscript{78}. Though not all of these prevention strategies are practical in developing countries, where the infrastructure needed for the strategy is in place or could be realistically developed, they should be implemented.

Nosocomial NTS disease has been reported in many parts of the world. These outbreaks can be particularly severe in pediatric wards in developing countries. Children in these wards often have other host risk factors and may be malnourished. When the outbreaks are caused by strains that are resistant to the local empirical therapy, high death rates are frequently observed\textsuperscript{38,147}. A study in Kenya found that adults with a hospital acquired infection of \textit{Salmonella} or Shigella diarrhea were associated with sharing a hospital room with someone who had diarrhea and a history of previous hospitalization\textsuperscript{38,148}. To prevent these hospital acquired infections strategies should be implemented that include patient and visitor education, provision of safe drinking water, hand washing before and after patient contact, thorough cleaning of the environment, reduction in crowding, increasing
the number of health care workers, adequate disinfection of reusable equipment, and thorough surveillance\textsuperscript{38,147,149}. If in place, public health departments should know about cases of salmonellosis and clinical laboratories should send isolates of \textit{Salmonella} to the appropriate public health lab so that the specific types can be determined and compared with other \textit{Salmonella} in the community\textsuperscript{146}.

In developed countries, animal contact is a well-established risk factor for acquiring NTS. This is particularly true of children handling young chickens\textsuperscript{38,150}. One study estimated that over 95\% of NTS infections in the US are related to food-borne transmission\textsuperscript{2}. In part, because of the asymptomatic carriers of NTS in Africa\textsuperscript{38,151}, transmission between humans has been suggested to be relatively more important\textsuperscript{152}. In a study conducted in 2002, Kariuki et al. concluded that NTS from animal and environmental sources are not closely related to NTS isolated from humans living in close contact to these animals\textsuperscript{14}. A similar study in Gambia had comparable findings, but believes other data suggest that poultry may play an important part in the epidemiology of NTS\textsuperscript{32}.

In addition, host risk factors should be examined. These include: age, HIV infection, malnutrition, sickle cell disease, malarial anemia, and recent antimicrobial use\textsuperscript{38}. Children less than 3 years old are particularly at risk for iNTS disease\textsuperscript{38,39,50,51,56,60,153}. NTS bacteremia is markedly more common among those infected with HIV\textsuperscript{43}. In developed countries, combination ART has been shown to reduce the incidence of NTS diarrhea and NTS bacteremia among HIV-infected persons\textsuperscript{154}. There is an association between
malnutrition and NTS bacteremia among children in Kiliki, Kenya\textsuperscript{39,50}. Interestingly, children less than four months of age appear to be relatively protected, possibly by both maternal antibodies\textsuperscript{58} and by exclusive breast-feeding, which would limit exposure to unsafe water and food\textsuperscript{38}. Although the mechanism underlying the association between malaria and NTS is not fully understood, malaria is suspected to increase the risk of iNTS\textsuperscript{38,39,155,156}. Therefore, the control of malaria may lead to a reduction in the incidence of iNTS\textsuperscript{38}. The use of antimicrobial agents contributes to abnormal gastrointestinal flora and is an established risk factor for development of NTS diarrhea\textsuperscript{38,148,157}.

I. Future Needs

As other invasive diseases are controlled by vaccine strategies, iNTS may assume position as the leading cause of community-acquired bloodstream infection in sub-Saharan Africa\textsuperscript{38}. There is an urgent need for improved diagnostic tools and vaccine development. Understanding how NTS is transmitted and the nature of the relationship between the disease and its invasiveness in Africa is critical in the development of diagnostic and prevention tools\textsuperscript{54}. A clearer understanding will also contribute to how health care resources should be prioritized\textsuperscript{38}. Additionally, algorithms for the management of febrile illness need to continually be reevaluated so that invasive bacterial infections such as NTS won’t be misdiagnosed as malaria\textsuperscript{38}. Because the food supply is so globalized, national and international health, food, and agricultural authorities should monitor for \textit{Salmonella}, especially strains that have shown antibiotic resistance\textsuperscript{108}. 

23
Chapter 2: Introduction

A. Background:

Following norovirus, nontyphoidal *Salmonella* caused the most illnesses in the United States. Additionally, NTS is the leading cause of hospitalizations and deaths caused by foodborne pathogens in the US\(^3\). In developed countries NTS is usually a self-limited diarrhea and the mortality rate is much lower\(^{38}\). Discordantly, of the bacterial pathogens involved in invasive disease, NTS is among the most common in sub-Saharan Africa and here infections are much more severe\(^{43,48-53}\). The severity of infections is especially apparent among young children with malaria and malnutrition, and among adults with HIV\(^{54-56}\).

Of late, it has been suggested that a highly invasive sub-type of *Salmonella, S. Typhimurium* ST313 may be contributing to the high incidence of invasive salmonellosis in sub-Saharan Africa\(^{48,53,135}\). Those that are infected with NTS often present with a non-specific febrile illness. This makes diagnosis and treatment extremely difficult and is made worse by a lack of resources\(^{54-56}\). This disease has been characterized by bacteremia, meningitis, and septic arthritis with case-fatality rates are between 20-25% for children and up to 50% in adults\(^{50,51,65,135}\).
Likely, ST313 has an original zoonotic source, but once it entered a susceptible population, human-to-human transmission became more critical to the spread of the disease. Alternatively, zoonotic transmission from infected feces or via contaminated food may remain an important source of infection\textsuperscript{139}. Understanding how NTS is transmitted and the nature of the relationship between the disease and its invasiveness in Africa is critical\textsuperscript{54}.

B. Goals and Objectives:

The underlying goal of this study was to understand the various biotic and abiotic ecologic factors that contribute to emergence, transmission, and persistence of Salmonella and antimicrobial resistance in Salmonella in food production systems, specifically swine. By looking at farm management and other herd-level ecologic factors it was hoped that some direction would be provided regarding the mechanisms by which transmission varies for different strains of Salmonella in swine herds in Kenya. Objectives included estimating the prevalence of Salmonella serotype Typhimurium in pig production systems around Nairobi, collecting baseline data on heavy metal exposure and ecological management factors that contribute to the prevalence and persistence of different Salmonella Typhimurium strains, and determining the antimicrobial resistance of the Salmonella we collected.
Chapter 3: Materials and Methods

A. Source of Isolates:

During the summer of 2013, 99 isolates of *Salmonella* were obtained from swine feces in Kabete, Zambezi-Kiambaa, Dagoretti South Constituency, Kajiado, and other sub-locations in and around the capital city of Nairobi, Kenya. Sample collection is summarized in table 1. It should be noted that because Kenyans do not have local addresses, farms 13 through 30 were all classified from the Kiambu County and within the Kiambaa constituency. Directions to each farm were based on word of mouth and door-to-door inquires on whether the owner of the plot kept swine on his or her property. The vehicle was parked within one section of the village and the farms for the day were traveled to by foot. Each day a new section or village was covered. Samples were collected from each stall that housed swine up to 15 samples. A total of 195 samples were collected from 30 separate farms.
<table>
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<th>Number of samples taken</th>
</tr>
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<tr>
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<td>Kiserian</td>
<td>3</td>
<td>2</td>
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<tr>
<td>June 4th</td>
<td>Kiserian</td>
<td>4</td>
<td>5</td>
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<tr>
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<td>3</td>
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<tr>
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<td>11</td>
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<tr>
<td><strong>TOTAL:</strong></td>
<td></td>
<td>30</td>
<td>195</td>
</tr>
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Table 1: Sample Collection
B. MagPi

MagPi software was downloaded onto a smartphone and used for all data collection. The software allows the user to design a form and create a list of questions with various prompts to ensure that data are collected in a uniform fashion. The phone was carried into the field and a form was completed through communication with the owner of the property at each farm where samples were collected. Thirty forms were completed in this study. The survey designed in MagPi can be found in appendix A.

C. *Salmonella* Isolation:

Isolation of *Salmonella* was conducted at KEMRI’s lab facilities in Nairobi. Standard *Salmonella* isolation using buffered peptone water (BPW), rappaport-vassiliadiis (RV), and xylose lysine tergitol 4 agar (XLT4) was used. This protocol was directed and authorized by Dr. Bayleygen Molla at The Ohio State University. In short, fecal samples were collected by hand from each stall and stored in a whirl pack bag over ice. BPW was added to each sample and incubated at 37 degrees C for 24 hours. A small sample of this mixture was transferred to RV broth and incubated at 42 degrees C for 24 hours. This broth is used for the enrichment and selective isolation of *Salmonella*. A loop-full of RV was then streaked onto an XLT4 selective plate and incubated at 37 degrees C for 24 hours. Presumptive *Salmonella* positive plates had black colonies. If possible, three random colonies were chosen from each positive plate and stored on Mueller Hinton
slants until biochemical testing was performed. The colonies were refreshed on XLT4 before performing biochemical tests.

Biochemical testing was performed to confirm that isolates were *Salmonella*. *Salmonella* positive samples showed a positive reaction to triple sugar iron (TSI), a negative reaction to urease, and a negative reaction to lysine.

Samples were then regrown on XLT4 to reconfirm each sample was a pure isolate of *Salmonella*. Each isolate was transferred on Mueller Hinton slants in cryo tubes, sealed with parafilm and shipped from Nairobi to The Ohio State University for further testing.

D. Somatic (O) Antigen Agglutination Tests.

After being refreshed on Muller Hinton plates, isolates were first tested with polyvalent O antiserum reactive with groups A through I. A saline control was included with each isolate. Following a positive reaction with polyvalent O antiserum, the isolates were tested with individual *Salmonella* antisera for O groups B, C, D1, D2, E, and G. If a negative reaction was observed after testing with the polyvalent O antiserum, the isolate was tested with *Salmonella* antisera for O group R.

E. Antimicrobial susceptibility testing:

The *Salmonella* isolates were tested for antimicrobial susceptibility to a panel of 12 antibiotics using disk diffusion method. The twelve antimicrobials and disc potencies
included: ampicillin (Am, 10 μg), amoxicillin/clavulanic acid (Ax, 20/10 μg), ceftiofur (Cf, 30 μg), ceftriaxone (Ce, 30 μg), cephalothin (Ch, 30 μg), chloramphenicol (Cl, 30 μg), ciprofloxacin (Cip, 5 μg), gentamicin (Gm, 10 μg), kanamycin (Km, 30 μg), streptomycin (St, 10 μg), sulfisoxazole (Su, 250 or 300 μg), and tetracycline (Te, 30 μg). The interpretations follow the recommendation of the Clinical and Laboratory Standards Institute (CLSI). *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains on Mueller-Hinton agar according to the standards. The results were classified as either susceptible or resistant based on the zone diameter. The isolates with resistance to two or more classes of antibiotics were classified as multidrug resistant (MDR).

F. Pulsed-Field Gel Electrophoresis (PFGE) Genotyping:

The method used for Pulse-Field Gel Electrophoresis (PFGE) followed the PulseNet protocol from the Center for Disease Control and Prevention (CDC). *Salmonella* isolates were first grown on TSA (Trypticase Soy Agar) at 37 °C for 14-18 hours. Cell suspension buffer (100 mM Tris: 100 mM EDTA, pH 8.0) was used to suspend and adjust the bacterial concentration found by using a spectrophotometer at 610 nm wavelength with an OD 1.35 and no greater than OD 1.4. TE buffer (10 mM Tris: 1 mM EDTA, pH 8.0) was used to prepare agarose embedded cells. These cells were lysed by cell lysis buffer (50 mM Tris: 50 mM EDTA, pH 8.0 + 1% Sarcosyl) and proteinaseK. The intact genomic DNA that was embedded in the cells, was digested with 20 μl of XbaI restriction enzyme (New England Biolabs, Ipswich, MA, USA) at 37°C for 4 hours. The
PulseNet universal strain *Salmonella enterica* serotype Braenderup H9812 was used as a molecular standard marker and was prepared in the same manner. The DNA fragments were separated by CHEF-DR® III Pulsed-Field Electrophoresis System (Bio-Rad Laboratories, Hercules, CA, USA) on 1% SeaKem Gold (Lonza, Rockland, ME, USA) agarose in 0.5X Tris-borate EDTA (TBE) buffer. The machine was set with the following conditions: temperature - 14°C, voltage - 6 V, run time - 18 hours, initial switch time - 2.2s, final switch time - 63.8s, and included angle - 120°. After the gel was finished running, it was dyed with ethidium bromide and the DNA fragment bands were observed under UV trans-illumination (Gel Doc™ 2000, Bio-Rad Laboratories, Hercules, CA, USA). Quantity one 1-D analysis software (Bio-Rad Laboratories, Hercules, CA, USA) was used to capture the image. Bionumerics software V. 4.61 (Applied Maths NV, Belgium) was used to analyze the PFGE gels by cleaning the images, banding the patterns, and grouping using the Dice similarity index. The dendogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA). The isolates with PFGE banding patterns showing more than 87% similarity were categorized in the same cluster. The banding patterns were compared using a 0.5% optimization and 1% tolerance.

G. Statistical Analysis:

We used STATA® software to complete the statistical analyses. Logistic regression was used to calculate the strength of the association (in terms of an Odds Ratio (OR)) between a farm testing positive for *Salmonella* and various farm management practices that were
sampled during the survey collection. The unit of analysis was the farm. The same
calculations were made controlling for the number of stalls on each farm in our logistic
regression models to avoid possible confounding created because larger farms had a
greater possibility of testing positive for *Salmonella*. The OR was calculated with a 95%
confidence interval and a value of P<0.05 was considered statistically significant.
Chapter 4: Results

A. Prevalence and Risk Factors:

Of the 195 stalls sampled, 37 were positive for *Salmonella* (19.97% prevalence). The prevalence of *Salmonella* at the level of the farm was 33.33% (10/30).

Odds ratios relating farm characteristics to odds of *Salmonella* positive are provided in table 2. No values were statistically significant at the 0.05 level. The p-value for additives being added to the water and/or feed was marginally significant at 0.08, controlling for the number of stalls and the OR indicated that the odds of testing positive for *Salmonella* among farms that used additives in water and/or feed were 4.09 times the odds among farms that didn’t introduce additives to the water and/or feed, controlling for the number of stalls on the farm.

Odds ratios for some of the farm management practices we investigated with the survey could not be calculated. This was true of the type of facility that was used because nearly all thirty farms were considered an open building with outside access for hogs and pigs. Similarly, the impact of owners or workers visiting farms could not be examined because nearly all households in these communities had farm animals on their property and
visiting neighbors was commonplace. We also couldn’t study the impact of using heavy metals in swine feed since none of the farms we sampled indicated that they did this.

<table>
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<th></th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
<th>OR controlling for # stalls</th>
<th>95% CI</th>
<th>P-value</th>
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<tr>
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<td>0.4286</td>
<td>0.08955-2.051</td>
<td>0.2874</td>
<td>0.2973</td>
<td>0.05131-1.723</td>
<td>0.1344</td>
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<tr>
<td>Number of sows</td>
<td>0.9766</td>
<td>0.8213-1.161</td>
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<td>0.8980</td>
<td>0.7033-1.1465</td>
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<tr>
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<td>0.9527-1.045</td>
<td>0.9203</td>
<td>0.9777</td>
<td>0.9197-1.039</td>
<td>0.2548</td>
</tr>
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<td>Number of growers</td>
<td>1.003</td>
<td>0.9724-1.0348</td>
<td>0.8472</td>
<td>0.9776</td>
<td>0.9335-1.024</td>
<td>0.2112</td>
</tr>
<tr>
<td>Number of males</td>
<td>1.205</td>
<td>0.2827-5.1324</td>
<td>0.8026</td>
<td>0.6549</td>
<td>0.1199-3.577</td>
<td>0.3139</td>
</tr>
<tr>
<td>Pigs fed scraps</td>
<td>1.5</td>
<td>0.3219-6.991</td>
<td>0.6038</td>
<td>1.250</td>
<td>0.2501-6.249</td>
<td>0.3421</td>
</tr>
<tr>
<td>New animals kept in isolation</td>
<td>0.8148</td>
<td>0.1744-3.807</td>
<td>0.7941</td>
<td>0.6374</td>
<td>0.1223-3.222</td>
<td>0.3068</td>
</tr>
<tr>
<td>Number of farms within 5 km</td>
<td>0.9167</td>
<td>0.7881-1.066</td>
<td>0.2340</td>
<td>0.9367</td>
<td>0.8000-1.097</td>
<td>0.2506</td>
</tr>
<tr>
<td>Insect Control Used</td>
<td>0.2593</td>
<td>0.0266-2.526</td>
<td>0.1976</td>
<td>0.1869</td>
<td>0.1570-2.226</td>
<td>0.1126</td>
</tr>
<tr>
<td>Additives in water and/or feed</td>
<td>3.500</td>
<td>0.7166-17.09</td>
<td>0.1149</td>
<td>4.0933</td>
<td>0.7524-22.27</td>
<td>0.0857</td>
</tr>
<tr>
<td>Dewormer Used</td>
<td>0.6667</td>
<td>0.1430-3.107</td>
<td>0.6038</td>
<td>0.5595</td>
<td>0.1105-2.8344</td>
<td>0.2761</td>
</tr>
<tr>
<td>Reported Diarrhea</td>
<td>2.667</td>
<td>0.5002-14.22</td>
<td>0.2508</td>
<td>2.297</td>
<td>0.4122-12.80</td>
<td>0.2267</td>
</tr>
</tbody>
</table>

Table 2: Summary of odds ratios at the farm level

Histograms were constructed for all continuous variables. Because none of the histograms were found to be symmetric the median and range were calculated for each and can be found in table 3. This table demonstrates the diversity of the size of farms we sampled. Additionally, the number of farms within 5km is fairly large indicating that these farms were small in size.
B. Serogroups:

Of the 99 isolates tested, eight tested negative for the polyvalent O antiserum and groups G and R. Five isolates tested positive for the polyvalent O antiserum, but negative for groups B, C, E, G, D1, and D2. Twenty-four isolates tested positive for group B and sixty-two tested positive for group C.

Examining serogroups within farms, farms 18, 28, and 30 showed some variation. All other farms had only one serogroup. The serogroups for each farm are provided in table 4.
Table 4: Serogrouping by farm (percentage of isolates)

<table>
<thead>
<tr>
<th>Farm</th>
<th>Group B</th>
<th>Group C</th>
<th>+(A-I)</th>
<th>-(A-I) and -R</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td>3 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td>1 (100%)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>8 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>10 (45%)</td>
<td>12 (55%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>31 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>5 (28%)</td>
<td>7 (39%)</td>
<td>5 (28%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td>1 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1 (14%)</td>
<td></td>
<td></td>
<td>6 (86%)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (24%)</td>
<td>62 (63%)</td>
<td>5 (5%)</td>
<td>8 (8%)</td>
</tr>
</tbody>
</table>

C. Antimicrobial resistance profiles

Although most isolates (55) were pan susceptible to all drugs tested, the other isolates had varying degrees of susceptibility to the twelve antibiotics tested. The second most frequent pattern was resistance to Su and Cip (19 isolates). Interestingly, one isolate from farm 28 showed resistance to Am, St, Su, Ch, and Cip. All other isolates from this farm were pan-susceptible. Patterns of resistance can be seen in figure 2. Resistance to each of the antimicrobials was noted and can be found in figure 1. Notably, 40% of the isolates were resistant to sulfisoxazole (Su, 250 or 300 μg). Five of the ten farms were susceptible to all 12 antibiotics tested. A summary of resistance patterns across farms can be found in table 5.
Figure 1: Number of isolates resistant to each antibiotic
Figure 2: Resistance Patterns
Table 5: Antibiotics each farm showed resistance to

<table>
<thead>
<tr>
<th>Farm</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>PanS</td>
</tr>
<tr>
<td>6</td>
<td>Te</td>
</tr>
<tr>
<td>8</td>
<td>PanS</td>
</tr>
<tr>
<td>16</td>
<td>PanS</td>
</tr>
<tr>
<td>17</td>
<td>St, Su, Te</td>
</tr>
<tr>
<td>18</td>
<td>Su, Cip, Te, Ax</td>
</tr>
<tr>
<td>22</td>
<td>Su, Cip, St, Te</td>
</tr>
<tr>
<td>28</td>
<td>Am, Su, St, Ch, Cip</td>
</tr>
<tr>
<td>29</td>
<td>PanS</td>
</tr>
<tr>
<td>30</td>
<td>PanS</td>
</tr>
</tbody>
</table>

D. Pulsed Field Gel Electrophoresis (PFGE) Analysis:
In order to understand the genetic relatedness of the *Salmonella* isolates collected in Kenya, a total of 18 distinct clusters were identified among the 99 isolates. Clusters were labeled A-R and there were 9 sporadic clones whose pattern did not match with any of the clusters. Figure 3 shows the dendogram formed using Bionumerics. We summarized each cluster in table 6. In addition, table 7 summarizes clusters found within each farm.
<table>
<thead>
<tr>
<th>Cluster</th>
<th>Farm numbers</th>
<th>R-type</th>
<th>O-antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>PanS</td>
<td>C</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>PanS</td>
<td>+(A-I)</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>PanS</td>
<td>-(A-I), -R</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>PanS</td>
<td>-(A-I), -R</td>
</tr>
<tr>
<td>E</td>
<td>22</td>
<td>PanS</td>
<td>C</td>
</tr>
<tr>
<td>F</td>
<td>18</td>
<td>PanS</td>
<td>C</td>
</tr>
<tr>
<td>G</td>
<td>18</td>
<td>PanS</td>
<td>C</td>
</tr>
<tr>
<td>H</td>
<td>22, 28</td>
<td>SuCip, AmStSuChCip</td>
<td>C</td>
</tr>
<tr>
<td>I</td>
<td>22</td>
<td>SuCip</td>
<td>C</td>
</tr>
<tr>
<td>J</td>
<td>22, 28</td>
<td>PanS</td>
<td>C</td>
</tr>
<tr>
<td>K</td>
<td>18</td>
<td>Su</td>
<td>C</td>
</tr>
<tr>
<td>L</td>
<td>28</td>
<td>PanS</td>
<td>C</td>
</tr>
<tr>
<td>M</td>
<td>17, 18, 22</td>
<td>StSuTe, SuTeAx</td>
<td>C</td>
</tr>
<tr>
<td>N</td>
<td>6, 30</td>
<td>PanS, Te</td>
<td>B</td>
</tr>
<tr>
<td>O</td>
<td>28</td>
<td>PanS</td>
<td>B</td>
</tr>
<tr>
<td>P</td>
<td>28</td>
<td>PanS</td>
<td>B</td>
</tr>
<tr>
<td>Q</td>
<td>18</td>
<td>PanS</td>
<td>B</td>
</tr>
<tr>
<td>R</td>
<td>18</td>
<td>PanS, SuCip</td>
<td>B</td>
</tr>
</tbody>
</table>

Table 6: Clustering patterns
<table>
<thead>
<tr>
<th>Farm</th>
<th>Serogroups (n)</th>
<th>R-type (n)</th>
<th>Cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>C (3)</td>
<td>PanS (3)</td>
<td>A, sporadic clone</td>
</tr>
<tr>
<td>6</td>
<td>B (7)</td>
<td>PanS (4), Te (3)</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>B (1)</td>
<td>PanS (1)</td>
<td>sporadic clone</td>
</tr>
<tr>
<td>16</td>
<td>-(A-I) (1)</td>
<td>PanS (1)</td>
<td>sporadic clone</td>
</tr>
<tr>
<td>17</td>
<td>C (8)</td>
<td>StSuTe (8)</td>
<td>M</td>
</tr>
<tr>
<td>18</td>
<td>B (10), C (12)</td>
<td>Su (8), SuTeAx (1), PanS (11), SuCip (1), SuTe (1)</td>
<td>F, G, K, M, Q, R, sporadic clone</td>
</tr>
<tr>
<td>22</td>
<td>C (31)</td>
<td>StSuTe (3), PanS (10), SuCip (18)</td>
<td>E, H, I, J, M</td>
</tr>
<tr>
<td>29</td>
<td>C (1)</td>
<td>PanS (1)</td>
<td>sporadic clone</td>
</tr>
<tr>
<td>30</td>
<td>-(A-I) (6), B (1)</td>
<td>PanS (7)</td>
<td>C, D, N</td>
</tr>
</tbody>
</table>

Table 7: Farms genotypically and phenotypically described
Chapter 5: Discussion

Despite being a common bloodstream isolate in febrile patients, little is known about the environmental reservoirs and how NTS is being transmitted\textsuperscript{117}. Animals raised for food play an important role in the transmission of antimicrobial resistant \textit{Salmonella} to humans\textsuperscript{115}. In Africa, humans and animals often live in close proximity to one another, and thus there is the potential for these \textit{Salmonella} serotypes to be transmitted. This makes it necessary for a joint and coordinated surveillance and monitoring program for \textit{Salmonella} in Africa\textsuperscript{115}. Pointedly, identification of \textit{Salmonella} in the pork production chain is essential\textsuperscript{117,119}.

The sources and transmission routes of \textit{Salmonella} in developing countries are poorly understood due to the lack of coordinated national epidemiological surveillance systems. Presently, few studies have evaluated the levels of resistance to antimicrobial agents in \textit{Salmonella} serotypes from pigs in Kenya. The mechanisms of resistance remain unknown\textsuperscript{14}. It is important to consider that exposure to antibiotics is not the only factor that influences antibiotic resistance and considerable research is needed to understand proliferation of antimicrobial resistance\textsuperscript{159}.

Based on the odds ratios we calculated, no statistically significant conclusions could be drawn from the data collected during the survey at the 0.05 confidence level. This is in
part due to the small sample size (number of herds). During this study we took a convenience sample, mostly sampling farms based on word of mouth where individuals dictated which farms owned pigs in a particular area. Because of this we cannot assume that our sample was representative of the actual population. Additionally, because we took a convenience sample, farms we sampled may have biased our results. In order to have more statistical power and avoid bias, it is recommended that future studies conduct investigations with an adequate sample size and with a more systematic sampling technique. Using the prevalence of *Salmonella* we calculated in our study, it is possible to calculate the sample size needed to design a study with systematic sampling and sufficient statistical power.

Kikuvi et al. collected samples from random pigs in a slaughter house in Nairobi and found 20.7% prevalence of *Salmonella* in pigs\textsuperscript{117,118}. A study by Kagambega in Burkina Faso found 16% of samples from swine contained *Salmonella*\textsuperscript{115}. The prevalence of *Salmonella* found in our study is in line with these previous studies. At the level of the stall we found 19.97% prevalence and at the level of the farm we found a 33.33% prevalence. Because *Salmonella* infection persists in pig herds sub-clinically and the pigs are often asymptomatic, it is still possible to isolate *Salmonella* from apparently healthy pigs\textsuperscript{116}. It should be noted that Kagambega et al. only collected samples from animals that appeared healthy\textsuperscript{115}. Additionally, both of these studies sampled at the level of the animal\textsuperscript{115,118}, while we sampled at the level of the stall. This may account for differences in the prevalence found at each farm and between each study. Thakur et al found a
higher prevalence of Salmonella from swine reared in intensive (indoor) production systems compared to extensive (outdoor) production systems. Intensive farms in their study were all-in all-out based systems of production with the goal of reducing transmission of infectious agents\textsuperscript{109}. Yet, \textit{Salmonella} has been shown to persist on the farm floor, even after cleaning with disinfectants\textsuperscript{160}. Because all of the farms in our study were classified as semi-intensive, it is likely that \textit{Salmonella} is persisting on the floor of the pigs’ stalls and is potentially infecting the pigs.

During the study by Kikuvi et al., the three serotypes that were identified include: \textit{S}. Saintpaul (9), \textit{S}. Heidelberg (3), \textit{S}. Braenderup (2). This was the first report of \textit{S}. Heidelberg being found in food animals in Kenya\textsuperscript{117}. These findings are contrary to a study performed by Kariuki et al. that found \textit{S}. Agona to be the main serotype in pigs\textsuperscript{14}. In a study conducted in Burkina Faso, \textit{S}. Muenster was the predominate serotype found in pigs\textsuperscript{115}. Disparities in the serotypes found may be due to differences in the period of sampling, the location of sampling, or the origin and number of infected pigs\textsuperscript{119}. Based on somatic O antigen agglutination testing, group C was the most commonly identified serogroup (n=62) and B was identified in twenty-four isolates. Phase 1 and phase 2 flagellar (H) antigens should be identified to further serotype the isolates and name them following the Kauffmann-White Scheme\textsuperscript{17,18}. Because most of our isolates were classified as having groups C or group B somatic antigens, it is possible that we have isolated \textit{S}. Agona, \textit{S}. Heidelberg, \textit{S}. Saintpaul (all with B O antigens) or \textit{S}. Braenderup (C O antigen) because they were identified in previous studies in swine in the same
Though it is feasible that we isolated the highly invasive multidrug-resistant \textit{S. Typhimurium} ST313 because it is classified as having an O antigen in group B and some of our group B isolates were multi-drug resistant, our isolates classified as having a group C O antigen showed the most resistance. Multilocus sequence testing should be performed to confirm that indeed any of our isolates are ST313\textsuperscript{48}.

During the Kikuvi et al study, a \textit{Salmonella} Braenderup isolate had resistance to ampicillin, tetracycline, and streptomycin. Three \textit{S. Saintpaul} isolates were resistant to one of chloramphenicol, streptomycin or ampicillin, and the fourth isolate was intermediately resistant to tetracycline. The other isolates were susceptible to all antimicrobials tested. All isolates were susceptible to gentamicin, kanamycin, sulphamethoxazole/trimethoprim, and nalidixic acid. They did not test for resistance to ciprofloxacin or sulfisoxazole\textsuperscript{117}. Other studies have found that \textit{Salmonella} isolates from swine were susceptible to all of the tested antimicrobials (neither tested for resistance to sulfisoxazole)\textsuperscript{14,115}. In contrast, over 44\% of our isolates showed resistance to at least one antimicrobial and 55.5\% of our isolates were pan-susceptible. We noted resistance to Am, Ax, Ch, St, Te, Cip, and Su. Of the isolates that showed resistance, nearly 91\% were resistant to sulfisoxazole. The second and third most common resistances were ciprofloxacin (n=20 of 99 isolates) and tetracycline (n=16 of 99 isolates). The amount of resistance we found was surprising considering few farmers reported use of antimicrobials on their farms. We must also consider that not all antibiotics that we tested were tested in the other studies that we examined.
Despite antimicrobial use for animals being under veterinary prescription control, farmers still use unprescribed antimicrobials as growth promoters and as treatment for various afflictions. This practice may lead to bacterial resistance developing in food animals and transferring to the human population. Resistance in ampicillin, tetracycline, and streptomycin may be due their availability and relatively low cost. Farmers often use these drugs for both therapeutic and prophylactic purposes.

Although few farmers admitted to using antimicrobials on their farms during the survey, we observed individuals purchasing antibiotics at market feed stores without prescriptions or without authorization of use by a veterinarian. Farmers using antimicrobials without the consent of a veterinarian may have been less likely to admit to their use during the survey. We also noted that on numerous occasions, during the course of the survey, farmers stated that they did not use antibiotics, but when asked to see any bottles they use for their pigs we discovered use of dexamethasone and ivermectin. We made sure to ask to see the packaging of all injectables or additives given to the pigs, but it is probable that recently used antibiotics and their packaging were thrown away after they were completely used. Additionally, no farm that was visited kept records of medications given. This may explain resistance to antimicrobials when there was no record of their use.
Though resistance to sulfisoxazole has not been previously seen in swine in Kenya, our isolates showed the most resistance to this antibiotic. Resistance to this antibiotic has been seen among pig isolates tested from a pan-European survey, in the US, and in Canada\textsuperscript{9,13,116,161}.

Most surprising, was the resistance found to ciprofloxacin. All 1,090 \textit{Salmonella} isolates from swine production systems in the U.S. tested by Keelara et al. were susceptible to ciprofloxacin\textsuperscript{161}. Contrasting this finding, a study conducted in Taiwan found closely related ciprofloxacin-resistant isolates from humans and pigs. They suggest a nationwide dissemination of \textit{S. Choleraesuis} isolates from pigs to humans occurred from 1999 to 2002\textsuperscript{162}. These findings and the ciprofloxacin resistance found in our isolates highlight the need for active surveillance of \textit{Salmonella} in swine in Kenya.

Resistance to ampicillin, co-trimoxazole, tetracycline, and streptomycin has been found in NTS from chickens in the region\textsuperscript{14}. We observed that chickens were sometimes housed near or on top of the pig stalls. Additionally, nearly all the farms had stalls that were at least partially exposed to the outdoors. It is possible that resistance found in chickens may be spilling over to the swine through shared resistance mechanisms.

Gordon et al., described NTS infections associated with MDR in Malawi. They note that by 2002 isolates from humans that were previously susceptible to chloramphenicol became resistant to the drug and were already resistant to co-trimoxazole and ampicillin.
By 2003, alternative therapies such as ciprofloxacin and ceftriaxone were being used. The close proximity that farmers in this study lived with their pigs may explain the high amount of resistance observed with ciprofloxacin: either through shared serotypes or shared resistance genes.

During a study on the occurrence of MDR Salmonella in antimicrobial-free swine production systems, a higher prevalence of Salmonella and higher frequency of multidrug resistance was seen among intensively reared herds. This may be explained by the fact that strains persist in the farm environment for long periods of time and because multiresistance may build up through time due to co-selection. Though all of our herds were considered semi-intensive, they were housed in contained environments, and resistance seen on these farms may be explained in a similar fashion.

Thakur et al. also demonstrated that antimicrobial resistant Salmonella can exist in the environment even in the absence of selection pressure and have the potential to spread to other swine over long periods of time. This should be recognized when considering none of the farms used adequate cleaning methods and farmers often traveled to multiple farms in short periods of time.

PFGE analysis showed that five of the ten farms that tested positive for Salmonella had multiple clusters or at least one cluster and a sporadic clone. The diversity of the isolates found on these farms differed greatly from previous studies. Kariuki et al., found
that domestic animals produced indistinguishable patterns. They concluded that single
common strain type caused infections within the farms they tested. However, *S. Agona*
isolated from two farm workers and from animals had indistinguishable patterns.\textsuperscript{14}

Gebreyes et al. detected a widespread similarity of isolates within a production system\textsuperscript{31}.
A study by Fey et al., demonstrated by PFGE that a ceftriaxone-resistant strain of *S.
enterica* that caused illness in a child was acquired from cattle\textsuperscript{99}. This demonstrates the
possibility of zoonotic transmission and is contrary to other studies discussed
previously\textsuperscript{9,14,32}.

Comparable to what we found, other researchers have observed that although strains were
isolated from farms in the same geographical area, there was a low similarity between
strains from different production systems\textsuperscript{31,134}. In our study, only clusters H, J, M, and N
were found on more than one farm. Otherwise, the other 14 clusters were unique to the
farm they were found on. Based on these findings, it may be of interest to conduct further
testing in various production phases in Kenya to observe if there is clonality between the
isolates during production and slaughter. Additionally, it may be of interest to examine if
these strains are persisting in the environment, which may explain why the clusters we
found are fairly conserved on each farm despite farms being in the same geographical
area.

In conclusion, based on these findings, a study comparing the strains isolated in this study
with strains isolated from humans should be considered. Because humans and animals
live in such close contact with one another, we hypothesize that we would find
genotypically similar *Salmonella* serotypes between humans and animals. Additionally,
sampling chickens and other animals that are in close contact with the swine may provide
some insight on whether the strains isolated in this study are infecting multiple hosts.
Examining these relationships may explain the amount of diversity found within and
across farms, especially in regards to the 18 clusters we observed. We also suggest
expanding the sample size to provide more statistical power in order to draw conclusions
on what farm management practices might influence a farm testing positive for
*Salmonella.*
References


87. Smith JL. The Role of Gastric Acid in Preventing Foodborne Disease and How Bacteria Overcome Acid Conditions. *J Food Prot.* 66:1292–1303.


89. Mikkelsen LL, Naughton PJ, Hedemann MS, Jensen BB. Effects of Physical Properties of Feed on Microbial Ecology and Survival of Salmonella enterica


Appendix A: Sample MagPi Questionnaire

Form: MDR_Salmonella
89 Questions

====================================================
1. We would like to ask you some questions about the hogs and pigs on the land you operate. To understand important issues in the hog industry, we need to obtain information about the health status of your hogs and swine, as well as about your management practices. The facts about your operation and your identity will be kept confidential and not revealed to anyone outside of the study. Response is voluntary
2. Date
3. Site Number
4. GPS
5. Farm Name?
6. Contact Person: address, telephone, email:
7. What kinds of livestock do you rear?
Choose all that apply
- poultry
- cattle
- swine
8. What is your main source of income from the farm?
Choose one response
- crops/grains
- animal production
9. What is the main source of irrigation for your crops?
10. How would you classify your production system?
Choose one response
- extensive
- semi-intensive
- fully-intensive
11. Number of sows on the farm:
12. Number of piglets on the farm:
13. Number of nursery animals on site:
14. Number of grower animals on site:
15. Number of finisher animals on site:
16. Which type of facility is used for most pigs on this farm?
Choose one response
- total confinement with mechanical ventilation
- open building with natural ventilation and no outside access
- open building with outside access for hogs and pigs
- lot with hut or no building
- pasture with hut or no building

17. **What is the source of your feed?**
Choose one response
- Purchased commercial
- Mainly garbage/scavenging
- Mixed Commercial and Garbage

18. **What type of waste management is most used in your facility?**
Choose one response
- none
- pit-holding
- mechanical scraper or tractor
- hand cleaned
- flush under slats
- flush-open gutter
- other: please specify

19. **Other Waste Management**

20. **Biosecurity?**

21. **How often do you visit other farms?**
Choose one response
- at least once per day
- less than once per day, but greater than once per week
- less than once per week, but greater than once per month
- less than once per month
- never

22. **How many days ago from today’s date did you visit another farm?**

23. **How often do your workers visit other farms?**
Choose one response
- at least once per day
- less than once per day, but greater than once per week
- less than once per week, but greater than once per month
- less than once per month
- never

24. **Do your animals co-mingle with other livestock species on the farm? If so what animals?**

25. **Do your animals co-mingle with wildlife that may enter the farm? If so what animals?**

26. **How often are you bringing new animals onto the farm?**
Choose one response
- at least once per month
- less than once per month, but more often than every six months
- less than once every six months, but greater than once per year
- less than once per year
- never

27. How many days from today’s date where new animals introduced onto the farm?
28. How many animals were introduced onto the farm in the last 90 days? What species?
29. How many animals were introduced onto the farm in the last year? What species?
30. Are these new animals kept apart from the herd?
Choose one response
- Always
- Not Always
- Never

31. If introduced animals were kept apart, how many days were new animals kept apart from the rest of the herd?
32. Do workers shower before entering the farm on site?
Choose one response
- Always
- Not Always
- Never

33. Do visitors shower before entering the farm on site?
Choose one response
- Always
- Not Always
- Never

34. Are workers required to put on clean boots and coveralls when entering the farm?
Choose one response
- Always
- Not Always
- Never

35. Are visitors required to put on clean boots and coveralls when entering the farm?
Choose one response
- Always
- Not Always
- Never

36. Is it required that workers wash their hands before entering the farm?
Choose one response
- Always
- Not Always
- Never

37. Is it required that visitors wash their hands before entering the farm?
Choose one response
- Always
38. Is there on-site toilet access?
Choose one response
- Yes
- No

39. To the nearest km, how many kilometers is it from this site to the nearest site with any swine?

40. Other than this site, how many sites with swine are within 5 kilometers of this site? (include each individual site, regardless of ownership)

41. Any other procedures to prevent the spread of disease?

42. General hygiene comments:

43. Insect and Rodent Control Used In or Around Barns Where Pigs are Housed:

44. Are any of the following methods of insect control routinely used on this operation?
Choose all that apply
- none
- tape
- mist
- professional extermination
- other: please specify

45. Other methods of insect control routinely used on this operation:

46. Are any of the following methods of rodent control routinely used on this operation?
Choose all that apply
- none
- rodent traps
- professional extermination
- baits/chemicals
- cats
- dogs
- other: please specify

47. 1. Rodent trap types:?
2. Bait/chemical types:?
3. Other:

48. Concentration and Form of Other Additives:

49. Do you use heavy metal feed additives?
Choose one response
- Always
- Not Always
- Never

50. What is the route of administration of the metal feed additives?
Choose all that apply
- Delivered mixed with feed
- Mineral salt block
- Top Dressed
- Other: specify

51. Other route of administration of the metal feed additives:

52. What kind of copper do you use?
Choose all that apply
- Sulfate
- Chloride
- Oxide

53. What is the dose of the copper in feed?
Choose one response
- 0-100 ppm
- 100-250 ppm
- >250 ppm

54. What kind of zinc do you use?
Choose all that apply
- Sulfate
- Chloride
- Oxide

55. What is the dose of zinc in feed?
Choose one response
- 0-100 ppm
- 100-250 ppm
- >250 ppm

56. Do you use any other heavy metal feed additives? Please list:

57. How often do you add heavy metals in the feed?
Choose one response
- At least once per day
- less than once per day, but greater than once per week
- less than once per week, but greater than once per month
- less than once per month
- never

58. For which class of pigs do you add heavy metal to their feed?
Choose all that apply
- Nursery
- Grower
- Finisher

59. Vaccination Records

60. Is a specific Salmonella/Campylobacter Vaccine Used?
Choose one response
- Yes
- No

61. What is the name of the Salmonella/Campylobacter vaccine used?

62. What is the method of administration of the Salmonella/Campylobacter Vaccine?
Choose one response
- Injectable
- Water

63. What is the stage of production at which the vaccine was administered?
Choose all that apply
- Nursery
- Grower
- Finisher

64. What is the name or type of other vaccines used? Please list:

65. Types of Disinfectants & Methods of Application Used:

66. What types of disinfectants do you use? Select all that apply:
Choose all that apply
- Potassium Peroxymonosulfate/Sodium Chloride (Virkon-S)
- Quaterinary glutaraldehyde (Snynergize)
- Chlorhexidine 2% Solution
- Quaternary ammonium (Parvosol)
- Hot water only
- Other: please describe
- None

67. Other types of disinfectants used:

68. How often are the disinfectants used?
Choose one response
- At least once per day
- less than once per day, but greater than once per week
- less than once per week, but greater than once per moth
- less than once per month
- never

69. What is the method of application?
Choose all that apply
- Pressure washer
- Backpack
- Other (please describe)

70. Other methods of application:

71. Where are the disinfectants used? mark all that apply:
Choose all that apply
- around housing quarters/barns
- feed and water bins
- other: please specify

72. Other places where disinfectants are used:

73. Record Use of Antimicrobials Used:

74. Do you use antimicrobials in your pig's water?
Choose one response
- Yes
- No
75. Please list any antimicrobials you use in water, reason for use, how many times per day, dosage concentration, and duration of administration:

76. Do you use antimicrobials in your pig's feed?
Choose one response
- Yes
- No

77. Please list any antimicrobials you use in your pig's feed, reason for use, how many times per day, dosage concentration, and duration of administration:

78. Do you administer any antimicrobials by injection to your pigs?
Choose one response
- Yes
- No

79. Please list any antimicrobials you administer to your pigs, reason for use, how many times per day, dosage concentration, and duration of administration:

80. Animal Health

81. What is the most common health problem with your pigs?

82. How often do you see diarrheal outbreaks in your pigs?

83. What age group are the diarrheal issues most common?
Choose one response
- Nursery
- Grower
- Finisher

84. How long do these bouts of diarrhea last?

85. Any other comments about the animal's overall health?

86. Wildlife

87. How often do you see any wildlife on your property?
Choose one response
- At least once per day
- less than once per day, but greater than once per week
- less than once per week, but greater than once per month
- less than once per month
- never

88. Name any wildlife you see:

89. Describe any additional remarks