QUANTITATIVE HERD-LEVEL EVALUATION OF A COMMERCIALLY AVAILABLE VACCINE FOR CONTROL OF *SALMONELLA* IN DAIRY CATTLE

A Dissertation

by

RUSSELL LEE FARROW

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2011

Major Subject: Biomedical Sciences

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Approved by:

Co-Chairs of Committee,	Guy H. Loneragan
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ABSTRACT

Quantitative Herd-level Evaluation of a Commercially Available Vaccine for Control of *Salmonella* in Dairy Cattle.

(December 2011)

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Salmonella continues to threaten public health as well as negatively impact dairy producers on multiple levels. Efficacious solutions to control *Salmonella* among dairy cattle have long been sought to alleviate these problems. A novel vaccine technology has been developed based on purified siderophore receptors and porin proteins (SRP®) derived from *Salmonella* Newport. When vaccinated with these SRP® cattle are stimulated to produce antibodies which act in concert with host defenses to disrupt iron acquisition of pathogenic bacteria. To evaluate the effectiveness of this technology, a prospective cohort study was designed utilizing herds (n = 11) that practiced whole herd vaccination with the SRP® vaccine (vaccinated cohort) and herds (n = 11) that had not used the SRP® vaccine. Samples were collected during four rounds at approximately six week intervals from June through October 2009. Samples were transported to the laboratory at West Texas A&M University and cultured for the prevalence of *Salmonella*

using selective enrichment methods. *Salmonella* isolates were evaluated for antimicrobial susceptibility and serotype. Data was analyzed using commercially available software to evaluate the herd-level effects of vaccination. *Salmonella* was ubiquitous throughout the Texas Panhandle and Eastern New Mexico, within-herd animal level estimates of prevalence ranged from 0.0 – 92%, over the length of the study period. Overall all rounds vaccinated herds had decreased (P = 0.012) *Salmonella* prevalence (15.3 vs. 27.5%). Vaccinated herds had numerically fewer *Salmonella* isolates belonging to the Newport serotype. *Salmonella* Typhimurium isolates were recovered approximately equally from vaccinated and non-vaccinated herds. Isolates from vaccinated herds were resistant to fewer antimicrobials throughout the study period. The ACSSuT(resistant to ampicillin, chloramphenicol, streptomycin, sulphisoxazole, and tetracycline) and MDR-AmpC (ACSSuT resistance plus resistance to ceftiofur and amoxicillin/clavulanate) resistant phenotypes were more frequently observed among non-vaccinated herds and none of the isolates from vaccinated or non-vaccinated herds were resistant to nalidixic acid, gentamicin, ciprofloxacin, or amikacin. These findings indicate vaccine efficacy for the reduction of *Salmonella* prevalence. Dairy operators along with herd veterinarians are encouraged to utilize this data with other herd specific factors in determining whether to use this specific vaccine.

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CHAPTER I

INTRODUCTION

Salmonella continues to be a critical area of concern amongst animal and public health officials alike. As a result, dairy producers have sought efficacious vaccines to control *Salmonella* in dairy cattle. Effective control of *Salmonella* in dairy cattle has the potential to decrease associated animal treatment cost, increase production parameters, and subsequently decrease the public health burden attributed to the consumption of *Salmonella*-contaminated beef products. Therefore, the authors designed a prospective cohort study to evaluate the efficacy of a commercially available *Salmonella* Newport siderophore receptors and porin proteins (SRP®) vaccine in dairy cattle (AgriLabs, St. Joseph, MO).

The following dissertation provides a literature review (Chapter II) describing issues pertinent to *Salmonella* control from animal and public health perspectives. Chapters III & IV are products of the aforementioned study comparing herds vaccinated with the *Salmonella* Newport SRP® vaccine and non-vaccinated herds and will be presented as standalone manuscripts. Previous studies have measured efficacy (comparisons of *Salmonella* prevalence) within individual herds and have yet to show a vaccine effect. To the author's knowledge, this will be the first study to evaluate this vaccine between vaccinated and non-vaccinated herds. Chapter V will present significant cross-chapter conclusions.

This dissertation follows the style of the *Journal of Food Protection*.

CHAPTER II

LITERATURE REVIEW

History of *Salmonella*

Salmonella is named after Dr. Daniel E. Salmon, an American veterinary pathologist and USDA administrator. Salmon was a graduate of Cornell University and was mentored by the French chemist Louis Pasteur (*30*). In 1885, the genus *Salmonella* was discovered by Salmon's research assistant Theobald Smith who had been investigating the cause of common hog cholera (*38*) and believed *Salmonella* to be the causal agent; however, this was later proven incorrect. Since its discovery, the *Salmonella* genus has grown to include over 2,500 different serotypes.

Salmonellosis is a worldwide public health concern, and wild and domestic animals and fowl have been shown to be reservoirs for *Salmonella*. Transmission via ingestion of food and water contaminated with feces is regarded as the most common source of human infection; however, infection via direct contact with infected animals and fowl is also frequently reported. Throughout many years of research and the discovery of new *Salmonella* serotypes, the nomenclature has become complex and considered by some as an ongoing area of debate. Currently, the genus *Salmonella* is divided into two recognized species – *Salmonella enterica* and *Salmonella bongori* – with six main subspecies of e*nterica* (I), s*alamae* (II), a*rizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and i*ndica* (VI), where *Salmonella bongori* was previously identified as subspecies V of *Salmonella enterica*. Several *Salmonella enterica* subspecies *enterica*

serotypes have traditionally been found in specific species and are generally regarded as host adapted, typically causing systemic disease in a related group of animals. For example, Typhi in humans, Cholerasuis in swine, Gallinarum in poultry, and Dublin in cattle, whereas other serotypes such as Enteritidis routinely produce systemic disease in a broad range of species, including cattle, swine, sheep, rodents, poultry, and humans (*35*).

Salmonella Typhi is believed to be the cause of death for multiple historical figures, including Alexander the Great in 323 B.C. as well as Prince Albert, husband of Queen Victoria, in 1861. Additionally, historical scholars believe that a *Salmonella* Typhi outbreak was responsible for the deaths of more than 6,000 settlers at Jamestown, VA between 1607 and 1624. Epidemics were also reported in the Spanish-American (1898) and South African (1899-1902) Wars (*37*).

Recent outbreaks of salmonellosis in humans have been linked to a variety of sources, including, but not limited to, dairy products, eggs, fruits, vegetables, meat, seafood, petting zoo animals, and reptiles. In 1985, a salmonellosis outbreak was linked to more than 16,000 confirmed cases in 6 states (*44*). This outbreak was determined to have been caused by the inadvertent mixing of raw and pasteurized milk at a Chicago dairy. During 2000 and 2001, infants were admitted to hospitals exhibiting symptoms of salmonellosis and were culture positive for *Salmonella* believed to be contracted from reptiles maintained as family pets (*37*). Salmonellosis in human populations is multi-faceted. Microbiological contamination, food preparation standards, and extrinsic factors such as cross-contamination of raw and cooked food products are potential

components that may result in human salmonellosis. Fortunately, many of these issues are amendable to preventive activities, including pre-harvest and post harvest interventions and programs to educate consumers in safe food-handling procedures (*39*). Symptoms of salmonellosis include nausea, diarrhea, fever, chills, and abdominal cramping. Symptoms usually persist between four and seven days with no sequelae. However, infections in immuno-compromised and immuno-suppressed individuals can develop into more serious complications; for example, children with sickle cell anemia can develop osteomyelitis.

Despite technological advances in the understanding of this bacteria as well as increased attention to prevent its transmission in the production of food products, *Salmonella* continues to cause significant morbidity and mortality in human and animal populations. The CDC estimates that more than 1 million people suffer from salmonellosis each year, and of these afflicted people, approximately 500 die (*29*).

Description of *Salmonella*

Salmonella is a genus composed of rod shaped, non-spore-forming, predominately motile, Gram-negative bacteria belonging to the *Enterobacteriaceae* family (*15*). Gram staining shows straight rods ranging in length from 2.0 – 5.0 μm and 0.7 - 1.5 μm in width with peritrichous flagella. Most species produce hydrogen sulfide and are identified as black colonies when using an agar containing ferrous sulfate (*4*) such as xylose lysine deoxycholate (XLD), xylose lysine tergitol-4 (XLT4), and brilliant green agar (BGA). As mentioned briefly above, two species are currently recognized in

the genus *Salmonella*: *Salmonella enterica* and *Salmonella bongori.* *Salmonella enterica* is comprised of six subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI) (*24*). *Salmonella enterica* subspecies *enterica* (I) are usually isolated from humans and other warm-blooded animals, while subspecies II-VI and *Salmonella bongori* are usually isolated from reptiles and environmental sources (*31*). Currently, significant resources have been directed towards and allocated to further understanding *Salmonella enterica* and how this subspecies infects cattle and subsequently humans via the consumption of contaminated meat and milk products. This research will focus on the evaluation of subspecies *Salmonella enterica* supspecies *enterica* that infect the gastro-intestinal tract of dairy cattle that could potentially result in human cases of salmonellosis via consumption of contaminated beef products derived from culled dairy cows.

Clinical Significance

In the United States, new cases of **s**almonellosis continues to be one of the top three bacterial foodborne illnesses (CDC estimated cases 1,400,000) along with *Campylobacter* (2,400,000) and *E. coli* O157:H7 (70,000). *Salmonella* infections commonly result in fever, diarrhea, and severe abdominal cramping (*29*). Among those that become infected, the young, elderly, and those with compromised immune systems are at the highest risk of severe infections as the organism can gain access to the blood stream resulting in life-threatening complications. These persons may be at an increased risk of human to human transmission when they are exposed to such environments as

child and adult day cares as well as nursing home facilities. Additionally, severe cases may develop reactive arthritis or Reiter's syndrome, a chronic, long-term illness characterized by joint pain, painful urination, and eye irritation. Children with sickle-cell anemia have an increased likelihood to develop osteomyelitis as a result of *Salmonella* infection. Healthy adults typically recover completely in 4-7 days and do not require treatment; however, those cases with severe diarrhea or systemic infection may require hydration with intravenous fluids and treatment with antimicrobial drugs (*3*). The two primary methods of transmission include the ingestion of food products with sufficient quantities of viable bacteria to cause infection and by direct contact and inadvertent consumption of feces from *Salmonella* contaminated livestock, fowl, and reptiles. It is believed that large quantities (100,000) of viable cells are required to cause infection; however, this would certainly depend on host susceptibility as well as pathogenicity of the *Salmonella* strain. The first method of transmission is generally responsible for larger, more widely distributed outbreaks resulting from national food distribution chains, especially in the U.S., and it is estimated to be responsible for 85% of cases (*17*). Moreover, many of these outbreaks, especially those caused by drug resistant phenotypes, have been linked to ground beef products. The second method of transmission via direct contact with contaminated feces and subsequent ingestion gives rise to substantially fewer and more sporadic *Salmonella* cases and or outbreaks.

Salmonella in Dairy Cattle

Dairy cattle are known reservoirs for S*almonella* and have been linked to human infections via consumption of contaminated food products (*2*, *45*). Most cattle infected with S*almonella* are asymptomatic carriers shedding fewer than 2.0 x 10^2 CFU per gram of feces (*12*); however, other asymptomatic carriers have been shown to shed *Salmonella* at concentrations greater than 1.0 x 10^6 (*12*). Asymptomatic (sub-clinical) carriers may develop into clinical cases typically as a result of environmental and management effects that combine to reduce host immunity. As a result of subclinical infection, other cattle may be subsequently exposed to sufficient quantities of bacteria to cause infection (*23*); however, exactly what factors combine to produce clinical outbreaks are largely unknown. Modern dairy farms house cattle at high densities resulting in accumulation of significant quantities of manure which represents a large reservoir of *Salmonella* on farm. Many dairies remove feces using pen scrapers and or flush systems to clean pens and ramps; however, these methods cannot completely remove all feces, and contamination may persist. Free stall housing systems are generally cleaned on a daily basis using specialized machines to clean bedding material as well as water to flush concrete ramps. Dairy cows maintained in dry lot housing are similarly maintained, but to the authors knowledge not at the frequency at which free stall barns are cleaned, therefore the possibility of increased contamination exist. Up to 50% of calves exposed to contaminated teats and pen floors during the first six hours of life may begin shedding *Salmonella* within 24 hours (*23*). While clinical infections among healthy adult cows are infrequent, clinical infections among calves born into contaminated environments are

common and especially costly to producers due to treatment cost and an increase in death loss. In many instances, dairy producers apply lime to pen floors to reduce bacterial growth especially in maternity and calving pens as a cost-effective means to prevent infections. After removal from the maternity pen, multiple sanitation strategies are generally employed to decrease contamination from individual feed buckets and bottles via chemical sanitizers as well as hot water rinses (*23*). Calves are also reared in individual hutches to decrease the potential for animal to animal transmission of pathogenic organisms. Feeding waste milk from the sick cows may serve to further increase bacterial exposure among calves; however, this may potentially be mitigated either by pasteurizing waste milk or feeding a milk replacer. Appropriate calf management including, the consumption of adequate amounts of high quality colostrum during the first 4-6 hours of life, are paramount in preventing a broad range of infections including *Salmonella* (*23*).

Many feed ingredients have been shown to be contaminated with S*almonella* and are a likely source of continuous exposure for cattle (*9*). Feed grown locally using wastewater produced from dairies has been shown to increase contamination versus crops grown using well water (*9*). Ensiling crops at a pH of less than 4.5 can effectively reduce contamination by providing unfavorable growth conditions for *Salmonella* (*23*); however, some crops may not reach a pH of 4.5 and can potentially serve as a growth medium for *Salmonella* as well as clostridial organisms.

Endemic *Salmonella* infections on commercial dairies provide a means of contamination of both milk and meat products; i.e., milk produced as well as cows being

sold for slaughter and entering the domestic fresh meat supply. Milk, by state laws, must be pasteurized prior to being sold for retail consumption thereby reducing its ability to deliver viable *Salmonella* to consumers. Meat products derived from infected, cull dairy cows that may have been inadvertently contaminated through the harvest and fabrication processes are subject to a variety of control measures including carcass pasteurization and the application of organic acids to reduce and or eliminate pathogens, however, these systems have not completely eliminated pathogens resulting in the potential delivery of S*almonella*-contaminated products to consumers. Consumers have thus far proved inadequate of fully protecting themselves from these pathogens, and outbreaks have occurred. Therefore, effective control of *Salmonella* on dairy farms may have a profound two-fold effect: 1) reduced incidence of salmonellosis on dairies, and 2) reduce the burden of *Salmonella* in ground beef and a proportionate reduction of salmonellosis in human populations.

Vaccine Technology

Vaccines by definition are suspensions of dead, attenuated, or otherwise modified microorganisms that when inoculated stimulate immunity in the recipient via antibody production (*43*). Historically, *Salmonella* vaccines have utilized live non-pathogenic strains or strains that have been stripped of their ability to produce illness in the host but are capable of stimulating the production of protective antibodies. Killed bacteria have also been used in an effort to obtain the same outcome. However, these methods have proved largely unsuccessful in their attempt to control *Salmonella* in

cattle. A novel subunit vaccine technology produced by purifying specific parts of pathogenic strains of *Salmonella* has been developed and enjoys broad acceptance among dairy producers as a result of increased milk production in vaccinated cows. The following is a brief description of this new technology, being that this is novel and proprietary details of its exact mode of action are limited.

All organisms require iron for cellular function (*5*). Vertebrates use multiple mechanisms to bind, transport, and make iron available to cells. Iron made available to vertebrates is actively acquired via host-produced proteins and subsequently acquired at the cellular level by transferrin and lactoferrin proteins. When iron-loaded ferrin proteins encounter receptors at the cell, the iron is transferred into cell cytoplasm and subsequently used for cellular function. Given that host cells require iron and have a strong affinity for binding available iron, this often results in an iron shortage for foreign cells (e.g. bacteria) within the host. However, during infection bacteria acquire iron from the host via the production of siderophores which are responsible for active iron transport to these bacteria. Within the host immune system, when pathogenic bacteria are encountered, host immune cells are stimulated to secrete lipocalin 2 (*27*). This compound acts to sequester the siderophores and limit the amount of iron available to invading bacteria. Where iron is freely available, bacteria use porin proteins, tube-shaped proteins located on the outer surface of bacteria contained in the cell wall to transport iron into the cell; however, when iron shortages exists, as in a vertebrate, these bacteria must have specialized mechanisms with greater iron affinity than the host to, in effect, steal iron from the host organism (*27*). This specialized mechanism involves the

release of siderophores which scavenge iron from host-specific bound iron; subsequently, siderophore receptors on the cell wall actively pass iron into the cell cytoplasm where it is utilized in intracellular functions by the bacteria (*26, 34*). Without iron or the ability to essentially obtain iron from the host, these foreign and often pathogenic bacteria would be starved of iron and die. Given the iron acquisition competition occurring within host animals, it is believed that by concentrating and inoculating host animals with siderophore receptor and porin proteins (SRP®), the host can be effectively immunized against these proteins thus providing an additional form of immunological resistance to pathogens that rely on these proteins for iron metabolism. Currently, a conditionally licensed *Salmonella* Newport SRP® vaccine consisting of purified siderophore receptors and porin proteins is available for use in dairy cattle as a means to reduce fecal shedding of *Salmonella* and clinical salmonellosis outbreaks on dairy farms. Field trials involving vaccinated and non-vaccinated herds have not been conducted to determine the ability of the vaccine to reduce herd-level prevalence of *Salmonella*.

Critical Review of *Salmonella* SRP® Vaccine Trials

Salmonellosis continues to be an area of concern across all regions and sizes of dairy farms as well as a significant public health concern. Given that this vaccine technology is quite new, there are currently no peer-reviewed published results regarding the efficacy of this vaccine in challenge models. However the vaccine maker reports significant reductions in the occurrence of diarrhea as well as a significant reduction in

colony forming units per gram of feces in a poorly described challenge study posted on their web site. Unfortunately, these results have not been subjected to peer review and published. To the credit of the vaccine manufacturer, the author is aware of multiple challenge studies that are currently in the planning stages to further investigate the efficacy of this vaccine. Additionally, to date, few large-scale field trials have been conducted to examine the efficacy of this *Salmonella* SRP® vaccine at the herd level. To the author's knowledge, a total of three peer-reviewed published studies are currently available, two dairy and one feedlot study examining the efficacy of this vaccine (*10, 19, 22*). Collectively these studies have largely shown little to no effect comparing *Salmonella* prevalence between vaccinates to non-vaccinates; regardless, each study will be described and reviewed to discuss potential shortfalls in study design and analysis.

In 2007, Hermesch et al. submitted a journal article describing a prospective cohort study consisting of 180 Holstein cows and heifers within a 1200-cow confinement dairy with a herd history of salmonellosis. Cattle were randomly assigned to treatments and were either injected with the *Salmonella* Newport SRP® vaccine or a control solution 45-60 days prior to parturition with a second vaccine administration 14-21 days prior to parturition. Fecal and blood samples were collected at multiple intervals for isolation of *Salmonella* and for antibodies against *Salmonella* Newport, respectively. Binary data (*Salmonella* prevalence) were analyzed using logistic regression techniques. Over the study period, vaccinated had cohorts significantly greater milk production (1.14kg/d). Cattle receiving the vaccine had significantly higher concentrations of circulating antibodies of *Salmonella* Newport; however, *Salmonella* Newport was not

recovered from fecal samples. Additionally, *Salmonella* Agona was recovered from 20.3% of cattle, but the likelihood of recovery was not significantly different between controls and vaccinates (*22*). The second dairy study was conducted on two dairies in Ohio with a history of *Salmonella enterica* in 2006 by Heider et al. Twenty-five percent of the mature cows from each herd were systematically randomized to serve as vaccinates while the remaining 75% of the herds served as non-vaccinated controls within 2 dairies. Cattle belonging to the vaccinated group were vaccinated twice during the study period, once at day 0 followed by a booster on day 14. It is important to note that the study on farm one was conducted in the fall of the year while on farm two the study was conducted during the summer months. Fecal samples were collected at day 0, 14, 28, and 70 for a total of four collections on each dairy. Samples were examined for the presence of *Salmonella* via standard culture methods. Mixed effect logistic regression models with a random effect to control for the effects of clustering within herd were employed. *Salmonella* was recovered at all collection periods and no differences in prevalence were observed between vaccinates and controls at any of the four collection periods, prevalence throughout the sampling period was 7.1%. Similarly, when analyzed across the four collection periods, no differences were observed in fecal *Salmonella* prevalence (*19*).

A third study conducted in 2008, by Dodd et al. at a commercial feedlot was designed to evaluate the SRP® vaccine in beef cattle (*10*). Upon arrival to the feedlot, feeder cattle (227 -250kg) were allocated in pairs (replicates) to study pens. Twenty pens of cattle (approximately 79 head per pen) were utilized with 10 pens per treatment

group. Cattle were vaccinated as per arrival protocol typical for feedlots in the region and animals in the vaccinated cohort were administered the *Salmonella* Newport SRP® vaccine while control animals were administered a placebo, vaccinates were maintained in separate pens. An additional dose or placebo was administered 21 days post enrollment. Fecal samples were collected from pen floors (25 per pen) at 0, 60, 120 days, and immediately prior to harvest for a total of four collections. Logistic regression models were developed to assess binary outcomes among vaccinates and controls. There were no significant differences between vaccinates and controls in respect to fecal prevalence of *Salmonella*. Overall prevalence of *Salmonella* was 10.2 and 10.9% in vaccinated and control cattle, respectively. Likewise, mortality and morbidity were not significantly different between vaccinates and controls (*10*).

In both of the dairy studies and the feedlot study, only a portion of the animals on each farm were vaccinated; while this may provide control animals for analysis, this design may not fully address all aspects in determining the efficacy of this vaccine. To eliminate the potential effects of herd immunity on determining vaccine efficacy, it may be necessary for larger studies to be conducted utilizing multiple dairies with whole herd vaccination and herds with no history of vaccinations with the *Salmonella* Newport SRP® in an effort to be able to determine what amount of variation is attributable to the herd level and to the individual cow level. By gaining an understanding of how variation is portioned between and within herds, researchers may be better equipped to measure the true measure of association of the *Salmonella* Newport SRP® vaccine. The author recognizes the difficulties that this type of design will present from logistical and

financial standpoints as well as the unique data analysis components that would be necessary to accurately determine the efficacy of this vaccine using a study design that would incorporate multiple herds. However, this scenario may well be one of the few ways in determining the real world efficacy of such a vaccine. Additionally, in the two dairy and one feedlot studies, *Salmonella* prevalence was relatively low (20.3, 7.1, and 10.5%, respectively) and may have contributed to the observed outcomes of no difference. Research has shown that prevalence and incidence rates can vary widely within and among dairies and have often sampled dairies in the summer months that have shown increased prevalence within herds, with the vast majority of cases being subclinical or asymptomatic (*12, 45*).

Furthermore, a phenomenon known as herd immunity may also be a plausible explanation for the observed outcomes in these studies. Herd immunity is a type of protection among the entire herd when a large proportion of the herd has been vaccinated, thereby in theory reducing the number of animals shedding pathogenic organisms such as *Salmonella* while also decreasing the number of animals that may be susceptible to infection. This phenomenon also has the ability to introduce bias into study outcomes potentially obscuring a vaccine effect. Additionally, questions have arisen as to whether this vaccine can be used as a means to prevent new cases or as a tool to help clear cases of salmonellosis (symptomatic or asymptomatic) in dairy cattle. As previously mentioned, the author is aware of two studies in the planning stages designed to address these questions. Answers to these questions will be beneficial to the producer

in an effort to reduce clinical salmonellosis as well as to downstream processors where food safety concerns are greatest.

In an additional study conducted in our laboratory under the supervision of Guy Loneragan, cull dairy cows were sampled at local auction markets originating from herds vaccinated with the *Salmonella* Newport SRP® and non-vaccinated herds (*28*). Cull cows are derived from the general herd and similar distributions are observed in the wider milking herd. The dairies using the conditionally licensed *Salmonella* vaccine exhibited the lowest burden of *Salmonella*. Substantial variation in shedding not attributable to use of the vaccine was noted (i.e., non-vaccinated herds prevalence varies from ~15% to greater than 80%). That said, however, analysis indicates vaccination is associated with a 76% reduction in *Salmonella* shedding (RR= 0.24; $P<0.01$). While caution should be used when making inferences because the study was not designed to evaluate the vaccine, it does appear that 1) the vaccine holds distinct promise; 2) a herd-level approach is most appropriate.

While the true measure of association between vaccinated and non-vaccinated cows and herds will be difficult to determine, these studies have produced results that will encourage their use on dairy farms as it relates to prevalence of *Salmonella* and the ability of this vaccine to increase milk production as shown in the Hermesch et al. (*22*), this study will undoubtedly pique the interest of dairy operators. With more widespread use of the vaccine, researchers may more easily conduct trials to observe the efficacy of the vaccine. However, determining proper study design and analysis to account for variables between dairies will be challenging. Perhaps an example of such a study

would be one that measures herd-level factors that may be associated with prevalence of *Salmonella* when measured at the cow level, rather than aggregated at the herd level. Measuring at the individual animal level may potentially be the best tool to determine vaccine efficacy as it is unlikely and generally recognized that *Salmonella* cannot be completely removed from herds.

Objectives

The objectives of the current research effort were to 1) quantitatively determine whether a commercially available vaccine can effectively control the *Salmonella* burden on dairies; and 2) partition unexplained variation in *Salmonella* shedding to within- and between- herd dynamics. These objectives will work to identify potential vaccine efficacy observed through wholly vaccinated herds whereas previous experiments utilizing different study designs have thus far remained unsuccessful. By portioning the unexplained variation observed in the model further research will be better focused on particular areas where the greatest change may potentially be effected.

CHAPTER III

EFFECTS OF A COMMERCIALLY AVAILABLE VACCINE AGAINST *SALMONELLA ENTERICA* SEROTYPE NEWPORT ON FECAL PREVALENCE OF *SALMONELLA* IN DAIRY COWS

Introduction

Salmonella continues to be a critical area of concern amongst animal and public health officials alike. Human *Salmonella* infections cause approximately 1.4 million illnesses resulting in economic losses greater than 2.5 billion dollars in the United States (*42*). Food-source attribution estimates indicate that approximately 10% of human salmonellosis cases are a result of consuming contaminated beef products (*1*). Additionally, multiple interstate *Salmonella* outbreaks have been attributed to the consumption of under-cooked ground beef contaminated with *Salmonella* (*6, 7, 40*).

Salmonellosis in dairy cattle can cause clinical and sub-clinical illness that can decrease herd production and increase herd health expenditures (*48*). With an increasing frequency, clinical illnesses that develop as a result of *Salmonella enterica* infections are often difficult to treat by herd veterinarians due to a decrease in the susceptibility of the organisms to commonly used veterinary antimicrobials. In an effort to mitigate these concerns and ultimately increase herd immunity to this pathogen, producers have sought efficacious vaccines to prevent salmonellosis within their herds. To date, few traditional vaccines have been shown to effectively reduce the burden of *Salmonella* in dairy cattle. This is due in large part to the inability of traditionally produced, autogenous vaccines to

target more than a single serotype or strain. Therefore, to control the vast array of *Salmonella* serotypes found in dairy cattle, new vaccine technology with the ability to act against multiple serotypes has been sought.

A novel vaccine was recently made available through a conditional license for the control of clinical salmonellosis caused by serotype *Salmonella* Newport. This vaccine currently enjoys broad acceptance among dairy producers due in part to a study by Hermesch et al. that has shown a significant increase in daily milk production (*22*). The vaccine contains purified extracts of siderophore receptors and porin proteins (SRP®), which are specialized proteins found in the cell wall of Gram-negative bacteria. These proteins play a critical role in the acquisition of elemental iron necessary for cell survival. The components of the vaccine work to induce an antibody response against SRP® to reduce the cells' ability to acquire iron, resulting in a competitive disadvantage. Early reports (*14*) indicated that a vaccine targeting siderophores may have decreased fecal shedding and rectal temperatures of Holstein bull calves challenged with *Salmonella* Newport. However, despite multiple investigations the efficacy of this vaccine technology to significantly reduce fecal prevalence of *Salmonella* in field trials has not been proven (*19, 22*).

Dairy farmers routinely cull cows as a means to recover salvage value for these animals. Multiple reports have identified that a majority of dairy farms have at least some level of *Salmonella* prevalence within herds (*16, 41*). Given the fact that, at some level of prevalence, culled dairy cattle are entering the abattoir with at least sub-clinical salmonellosis, pre-harvest intervention strategies aimed at reducing *Salmonella*

prevalence in dairy cattle are critically important to a systems-based, multi-faceted approach to food safety. The cyclical nature of beef as well as milk prices has at times resulted in large sell offs of dairy herds in an attempt to control production, these large influxes of known *Salmonella* reservoirs represents a unique stress upon current post harvest intervention strategies and potentially the opportunity to overwhelm the current interventions. Additionally, if dairy producers can reduce *Salmonella* prevalence in their herds, they will benefit from increased health and wellness within their herds.

Previous research to determine the efficacy of this *Salmonella* Newport SRP® vaccine has been conducted within single dairy herds and has yet to show statistical differences between vaccinated and non-vaccinated cows. These findings may be the result of a phenomenon called herd immunity, a type of protection among the entire herd when a proportion of the herd has been vaccinated, thereby in theory reducing the number of animals capable of transmitting or acquiring disease. A study conducted by Loneragan et al. (*28)* evaluating cull cows originating from herds vaccinated with *Salmonella* Newport SRP® reported deceased prevalence relative to non-vaccinated herds. A herd level approach including vaccinated and non-vaccinated herds will remove any potential effects related to herd immunity that may have occurred in previous research, while recognizing these results are not the product of an experiment but rather an observational study. Therefore, the objectives of the current research effort were to 1) quantitatively determine whether a commercially available vaccine can effectively control the *Salmonella* burden on dairies; and 2) partition unexplained variation in *Salmonella* shedding to within- and between- herd dynamics.

Materials and Methods

Cattle

To evaluate the effectiveness of this technology, a prospective cohort study was designed utilizing herds (n = 11) that practiced whole herd vaccination with the SRP® vaccine (vaccinated cohort) and herds (n = 11) that had not used the SRP® vaccine. Commercial dairy herds were voluntarily enrolled via consultations with herd veterinarians and herd managers in the Texas Panhandle and Eastern New Mexico. Herds were enrolled based on proximity, animal breed, and animal housing type. Eight herds were enrolled with a majority of cows within these herds being Jersey, housed in free stall barns, while the remaining 14 herds consisted of a majority of Holstein cows housed in dry-lot facilities. Dairies ranged in size from 500 to 6,000 head of milking cows. Care was taken to collect samples from animals at or near peak lactation, but not to collect from the same cows in subsequent periods, as the purpose of the study was not to examine cow-level effects but rather herd-level responses. Herds enrolled in this study were managed in accordance with typical practices and under typical conditions for dairies located in this region of the U.S. and were under the supervision of herd veterinarians.

Study Design

Using a prospective cohort design with repeated sampling, dairies in the Texas Panhandle and Eastern New Mexico were enrolled as either vaccinated (n = 11) or non-

vaccinated (n = 11) herds to test the efficacy of a conditionally licensed *Salmonella* Newport SRP® vaccine. To qualify as vaccinated, each herd must have employed a whole-herd approach to vaccination (two doses during dry period) and have used the vaccine continuously for at least the previous 12 months. Due to circumstances beyond the control of the investigators two herds began vaccination regimens during the study period and were incorporated into the vaccinated cohort upon the completion of herd wide vaccination and is described in depth below. Non-vaccinated herds qualified for study inclusion if the herds had not been vaccinated with the trial vaccine within the previous 12 months.

Each dairy was visited four times with approximately 6-week intervals between collections beginning June, 15 2009 and ending October, 27 2009. At each visit, 50 fecal samples (~ 50g) were obtained via rectal palpation with individual sleeves from cows in peak lactation that were locked in head restraints during the A.M. feeding period. Across the four sampling periods, a total of 4,400 fecal samples were collected.

Sample Size Determination

Sample sizes were calculated to detect a 55% reduction in the fecal prevalence of *Salmonella* attributable to the vaccine (α=0.05; β=0.20). Cows within a herd are considered to be clustered or that they share some common features and therefore are expected to have dependence between observations within a cluster (herd). When clustering is present in an experiment where one observes correlated binary outcomes of different cluster sizes, increasing the number of clusters is more critical than increasing

the number of observations within a single cluster. To control for clustering, plausible bounds of shedding in the non-vaccinated group were set from 12.5-80% with an expected prevalence of 35%. Using these constraints, 50 samples per herd would provide a precise estimate of the fecal prevalence of *Salmonella*. Additional sample size calculations indicate that a total of 22 clusters were necessary to achieve sufficient statistical power.

Vaccination Protocol

The vaccine under consideration in this study was a conditionally licensed, commercially available *Salmonella* Newport bacterial extract vaccine containing *Salmonella* SRP®. Label directions indicate that a vaccination of 2 mL should be administered subcutaneously ahead of the shoulder, and animals should be revaccinated in two to four weeks. Additionally, dry cows and bred heifers should be vaccinated twice prior to parturition with annual boosters. While the authors cannot verify that each cow on all 22 dairies were properly vaccinated, consultations with herd managers and herd veterinarians confirm that vaccination schedules had been followed for each of the vaccinated herds. Where protocol deviations did occur, the observations and resulting data were included in the analysis under the vaccine status at which point the fecal samples were collected.

Sample Collection

Fecal samples (~ 50 g) were collected as above from restrained cows in head stalls and subsequently placed in sterile collection cups. Appropriate cautions were taken to avoid contamination and maintain individual sample integrity. Individual animal identification numbers and breed were recorded at the time of sample collection. Samples were placed on wet ice and transported to a laboratory at West Texas A&M University for qualitative and quantitative bacterial culture.

Bacterial Culture Methods

All fecal samples were processed the day of collection for qualitative analysis of *Salmonella*. Five grams of feces were inoculated into 45 ml Rappaport Vassiliadis R-10 broth (RV) and 5g into 45 ml tetrathionate broth (TT) and incubated at 42 °C for 24 h. The remaining portion of the sample was refrigerated for subsequent quantitative determination. Following enrichment, 1 µl of each of the inoculants were streaked onto xylose lysine tergitol-4 (XLT4) agar and incubated for an additional 24 h at 37 °C. Post-incubation plates were examined for morphologically typical *Salmonella* colonies (black center); negative plates were maintained at room temperature for an additional 24 h and re-examined for morphological characteristics. Single colonies from *Salmonella* isolates were sent to the reference laboratory at the University of Pennsylvania for serotype determination. For quantification, fecal samples that yielded a positive outcome from either RV or TT were retrieved and 10 grams of each fecal sample was diluted in 90 ml of tryptic soy broth (TSB) with phosphate (30 g TSB, 2.31 g KH_2PO4, 12.54 g K_2HPO_4 l^{-1}, final pH 7.2) and spiral plated onto XLT4 using a commercially available spiral

plater (Spiral Biotech Autoplate 4000, Advanced Instruments, Inc., Norwood, MA). Plates were incubated at 42 °C for 24 h after incubation morphologically typical *Salmonella* colonies were counted and concentrations calculated.

Statistical Analysis

Data were electronically captured, examined for errors, and imported into commercially available software for analysis. Descriptive statistics were calculated and the results presented below. For model construction and evaluation, the comparison of *Salmonella* fecal prevalence (proportion of positive outcomes for each herd at each sampling period) was the outcome variable. Categorical responses (*Salmonella* positive or negative outcomes) were modeled using logistic regression. The fixed effects of vaccination status and collection round and the vaccination status by collection round interaction were included. To account for clustering of cows within herds and repeated measures, R- and G-side random effects for dairy and round were also included in the model. Appropriate model diagnostics were used to assess statistical assumptions and model fit. Estimations of variance within and between herds were calculated.

Results

It was the intent of the investigators to maintain each of the 11 dairies within their assigned group; however, due to circumstances beyond the investigators' control, two deviations to allocation occurred. Two herds which began the study as non-vaccinates began a *Salmonella* Newport SRP® vaccination program between rounds 2 &

3; therefore, each of the first two rounds from these dairies were included as non-vaccinates in the data analysis. Round 3 for one of these dairies was excluded from the analysis to allow a period of time for all animals within the herd to be vaccinated due to the large herd size; round 4 results from the newly vaccinated herds were included in the analysis as vaccinated herds.

A total of 4,400 fecal samples were collected and 4,399 cultured for *Salmonella* with one sample lost. A total of 4,249 observations were included in the analysis due to the aforementioned protocol deviations and the lost sample. Across all rounds, a total of 1,064 (24.2%) of samples were culture positive for *Salmonella*. For rounds 1to 4, the crude prevalence was 17.2, 28.4, 32.9, and 18.3%, respectively. Model adjusted (for study design and clustering) means for rounds 1to 4 were 13.7, 25.7, 31.5, and 14.6%, respectively (Table 3.1; note tables located in appendix B). Model adjusted means and their respective 95% confidence limits are displayed in Figure 3.1 (note; figures located in appendix A). Within-herd *Salmonella* prevalence among all dairies and across all sampling rounds ranged from 0.0-92.0%, *Salmonella* was recovered from all dairies during the study.

Analysis indicated significant ($P = 0.019$) differences in prevalence among vaccinated and non-vaccinated herds. Additionally, significant ($P < 0.001$) differences in prevalence were observed among sampling rounds as well as a significant ($P = 0.0001$) vaccination status by sampling round interaction. For rounds 1 and 3, prevalence was numerically lower among vaccinates (round 1, 10.7 vs. 16.6%; round 3, 29.8 vs. 33.7%) compared to non-vaccinates; however, these differences were not

statistically significant (round 1, $P = 0.14$; round 3, $P = 0.63$). Significant differences in mean prevalence between vaccinates and non-vaccinates were observed for rounds 2 and 4. For round 2, prevalence among vaccinates was significantly higher than non-vaccinated herds (16.3 vs. 38.3%, $P = 0.001$). Similarly, for round 4, a statistical difference (P = 0.001) was observed between the mean prevalence of vaccinated and non-vaccinated herds ((9.7 vs. 24.6%) Figure 3.2).

To explore the seasonal variations in *Salmonella* prevalence over time, the data were also analyzed by collection month. Prevalence was lowest (12.5%) during the first collection month (June) and increased until peaking in September at 33.1%. During the final collection month (October) prevalence decreased to 14.7%. Monthly mean values along with 95% confidence intervals are presented in Figure 3.3.

Mean concentration of direct plated quantifiable *Salmonella* was 2.1 x 10^2 CFU / gram of feces and ranged from below detectable limits (2.0 x 10^2 CFU/g) to 6.1 x 10^2 CFU/g. Unfortunately, too few samples yielded quantifiable results to be included for further analysis (n=85). To partition the variance to between- and within-herd dynamics the following random effects model was used to control for the effect of round and dairy ($Salm = \beta_0 + \beta_1 Round_2 + \beta_2 Round_3 + \beta_3 Round_4 + \gamma_{Dairy} + \alpha_{Round} + (\gamma_{Dairy} \times \alpha_{Round}) + \varepsilon_{ijk}$). Between-dairy variance accounted for 41.2% while within-herd variance accounted for 58.8% of the observed variation in the model. This was calculated by dividing the sum of the residual covariance (random intercept, covariance component, auto regressive and residual for the random effect) divided by the sum of the residual variance plus the logistic residual ($\pi^2/3$).

Discussion

To the author's knowledge, the study reported here provides the first significant evidence indicating the efficacy of the *Salmonella* Newport SRP® vaccine in reducing *Salmonella* fecal prevalence among vaccinated herds. While vaccinated herds differed significantly only during two sampling periods, all sampling periods showed numerical decreases in *Salmonella* prevalence amongst vaccinates. In addition, these data further confirm findings (*13*) that illustrate seasonal fluctuations in prevalence. Given these late summer and early fall spikes in prevalence, dairy operators may find it useful to employ more stringent sanitation practices during this period to limit the potential risks of a clinical outbreak during this period. Furthermore, in an effort to reduce the number of sub-clinically infected animals entering the food chain, dairy operators may find it useful to investigate potential avenues to decrease the number of culled animals during these peaks, however, it is unlikely that in the interest of food safety alone dairy producers would be willing to adopt such a strategy. Variance partitioning demonstrated that greater than half of the unexplained variation exists within dairies and that further research to understand herd-level factors that could be responsible for these findings is warranted. In future research efforts farm level variables may be incorporated into the model to better understand herd-level factors that are associated with *Salmonella* prevalence.

These results are in contrast to previous research (*10, 19, 22*) that did not report an effect of vaccine on *Salmonella* prevalence. The previous research, while well

designed had several factors that may have contributed to their outcomes of no differences. First, among these three studies *Salmonella* prevalence was relatively low, potentially decreasing the ability to determine if *Salmonella* prevalence differences attributable to vaccine use existed. Additionally, these studies utilized portions of the herds as cohorts and may have given rise to the phenomenon herd immunity, effectively reducing the number of cows that may become infected as well as the number of infected animals capable of transmitting disease. These results, however, confirm earlier findings by Loneragan et al. (*28*) indicating decreased *Salmonella* prevalence among herds practicing whole-herd vaccination using the *Salmonella* Newport SRP® vaccine. This study as well as the present research utilized a herd-level approach to determine vaccine efficacy. This study design, while not conducted as an experiment but rather an observational study, may serve as an example to others who are investigating the efficacy of vaccines as well as other interventions that are applied at the herd level. It should also be noted that behavior modification as a result of outcomes observed during the study period are possible such as those that occurred on the two dairies within this study are possible. In retrospect, to maintain study design integrity it may be necessary to maintain confidentiality of results, however many study participants were inclined to participate because they would receive in essence free *Salmonella* testing on a portion of their herds over a period of time.

The authors are encouraged by the results of this study illustrating the ability of the vaccine to effectively reduce fecal prevalence of *Salmonella* in vaccinated herds, as well as evidence illustrating increased milk production (*22*) among vaccinates. Given

these beneficial findings, we believe that careful consideration of the vaccine use by dairy operators and herd veterinarians is warranted as a cost-effective on-farm tool to reduce on-farm *Salmonella* burdens. Most importantly, further research should evaluate the direct impacts that vaccination of dairy herds with the *Salmonella* Newport SRP® vaccine has on the public health burden associated with *Salmonella* contaminated beef products.

CHAPTER IV

EVALUATION OF DIFFERENCES BETWEEN *SALMONELLA* SEROTYPES RECOVERED FROM HERDS VACCINATED WITH *SALMONELLA* NEWPORT SRP® VACCINE AND NON-VACCINATED HERDS

Introduction

Salmonella species are ubiquitous in the environment and can colonize and cause disease in a variety of food-producing animals and humans. *Salmonella* infections in humans and animals continue to be a driving force for concern among public and animal health officials alike. Confirmed clinical cases of salmonellosis in humans exceed 40,000, with an estimated 500 deaths annually (*29*). Concerns regarding specific serotypes routinely isolated in humans and livestock have frequently been described in popular press and of particular interest are serotypes Typhimurium, Enteriditis, and Newport. These three serotypes alone account for nearly 50% of the 2009 confirmed cases of human salmonellosis (*8*). Further challenging public health efforts to decrease the incidence of salmonellosis is that disease caused by drug resistant *Salmonella* is frequently more severe and results in greater mortality than drug-susceptible strains (*20, 21, 46, 47*). This concerns food animal producers and public health officials alike because a greater proportion of isolates recovered from clinically infected animals and humans are more drug resistant than that observed previously (*11, 18*). For example, ceftiofur resistance among *Salmonella* was essentially undetectable until the mid 1990s

and is now observed in ~20% of isolates recovered from cattle and 5% of isolates recovered from humans were resistant to ceftriaxone (*11, 18*). Despite ongoing arguments regarding specific factors that give rise to the increase of antimicrobial resistant bacteria, the problem persist as one of the greatest concerns to public health.

As public health officials struggle to control *Salmonella* outbreaks attributed to a variety of food products, dairy operators likewise direct considerable effort to control *Salmonella* within their herds to decrease associated healthcare cost, increase production parameters, and most importantly to ensure the delivery of pathogen free milk and meat products into commerce. To achieve these goals dairy producers have long sought efficacious vaccines for the control of *Salmonella*. Novel vaccine technology exploiting an animal's immune function to interfere with the ability of *Salmonella* to uptake iron, essential for survival, has recently been made commercially available. Although previous reports (*19, 22*) have not demonstrated significant differences in prevalence between vaccinated and non-vaccinated animals within herds; one of these reports has indicated a significant increase in milk production which consequently encouraged use among dairy operators. Given that an increasing number of dairy operators are practicing whole-herd vaccination using the *Salmonella* Newport SRP® vaccine, researchers now have the opportunity to evaluate the effectiveness of the vaccine at the herd-level which has previously not been feasible.

Therefore, the objective of this study was to determine if differences exist in *Salmonella* serotypes and their respective antimicrobial resistance patterns in isolates

recovered from herds vaccinated with a commercially available *Salmonella* Newport SRP® vaccine and non-vaccinated herds.

Materials and Methods

As detailed in the previous chapter a prospective cohort study was undertaken to evaluate the efficacy of a commercially available *Salmonella* Newport SRP® vaccine to reduce *Salmonella* prevalence comparing dairies that practiced either whole herd vaccination with the SRP® vaccine or dairies that did not employ any *Salmonella* vaccine programs. In short 22 dairies were enrolled and fecal samples were collected from cows at or near peak lactation four times at approximately six week intervals. Eleven herds were enrolled to each group, either vaccinated or non-vaccinated. It was the intent of the investigators to maintain each of the eleven dairies within their assigned group; however, due to circumstances beyond the investigators control, two deviations to allocation occurred. Two herds which began the study as non-vaccinates began a *Salmonella* Newport SRP® vaccination program between rounds 2 & 3; therefore, each of the first two rounds from these dairies were included as non-vaccinates in the data analysis. Round 3 for one of these dairies was excluded from the analysis to allow a period of time for all animals within the herd to be vaccinated due to the large herd size; round 4 results from the newly vaccinated herds were included in the analysis as vaccinated herds. Fecal samples were collected and transported to the laboratory at West Texas A&M University for bacterial culture.

Bacterial Culture and Serotyping

Five grams of feces were inoculated into 45 ml Rappaport Vassiliadis R-10 broth (RV) and 5g into 45 ml tetrathionate broth (TT) and incubated at 42 °C for 24 h. Following enrichment, 1 µl of each of the inoculants were streaked onto xylose lysine tergitol-4 (XLT4) agar and incubated for an additional 24 h at 37 °C. Post incubation plates were examined for morphologically typical *Salmonella* colonies (black center); negative plates were maintained at room temperature for an additional 24 h and re-examined. For *Salmonella* positive samples one distinct colony per sample was transferred onto tryptic soy agar (TSA) plates and incubated at 37 ^{o}C for 24 hours. Post incubation a single, isolated colony was selected and subcultured onto TSA slants and incubated as above. Incubated slants were then frozen (-80 ^{o}C) and forwarded to the *Salmonella* Reference Center at the University of Pennsylvania School of Veterinary Medicine for serotyping by standard techniques (*25*).

Antimicrobial Susceptibility

Susceptibility to a panel of antimicrobial drugs was determined for all *Salmonella* positive isolates from qualitative culture and isolation using broth dilution susceptibility testing using vendor supplied plates (Sensititre, TREK Diagnostics). Isolates were evaluated for resistance to 14 antimicrobials including ampicillin, amoxicillin/clavulanate, ceftriaxone, chloramphenicol, ciprofloxacin, trimethoprim/sulphamethoxazole, cefoxitin, gentamicin, kanamycin, nalidixic acid, sulphisoxazole, streptomycin, tetracycline, and ceftiofur. The minimum inhibitory

concentration (MIC) breakpoints of each antimicrobial agent were determined according to the breakpoints used by the National Antimicrobial Resistance Monitoring System (NARMS) and the CLSI (formerly NCCLS)-established guidelines for bacteria isolated from animals (*32, 33*).

Three to five colonies from TSA plates were placed into 5 ml of sterile, de-ionized water and adjusted to a 0.5 McFarland standard. A 10-µl portion of the suspension was transferred to 11ml Mueller-Hinton broth and vortexed. An automated inoculator dispensed 50 µl of the culture into wells of the 96-well plates. The plates were covered with the vendor-supplied covers and incubated at 35 ^{o}C for 18-24 h. Plates were manually read using the Sensititre manual viewer. The MIC was recorded at the lowest concentration that inhibited visible growth.

Data Analysis

Data were electronically captured, examined for errors, and imported into commercially available software for analysis. Descriptive statistics were calculated and the results presented below.

Results

A total of 1,012 *Salmonella* positive isolates were evaluated for antimicrobial susceptibility. A majority of isolates, 92.3% were resistant to 3 or fewer antibiotics. Consequently, 7.7% of these isolates were resistant to 4 or more antibiotics with no isolate resistant to more than 11 antibiotics (Table 4.1). Several phenotypes of concern

to public and animal health officials were observed in a small proportion of isolates. Isolates resistant to ampicillin, chloramphenicol, streptomycin, sulphisoxazole, and tetracycline (ACSSuT) accounted for 6.7% (n=68) of *Salmonella* isolates (Table 4.2) and the MDR-AmpC (ACSSuT resistance plus resistance to ceftiofur and amoxicillin/clavulanate) phenotype accounted for 4.6% (n=46, Table 4.2) of isolates recovered from all herds. Notably, within the ACSSuT phenotype when comparing vaccinated herds to non-vaccinated herds *Salmonella* Newport accounted for 16 versus 33 isolates whereas, *Salmonella* Typhimurium accounted for 9 versus 4 isolates, respectively. Within the MDR-AmpC resistance phenotype, similar outcomes were noted between vaccinated herds and herds not vaccinated with the *Salmonella* Newport SRP® vaccine; serotype Newport (n = 39) accounted for a numerically higher number of isolates (26 versus 13) among non-vaccinated herds relative to vaccinated herds. These two serotypes accounted for the majority of the MDR-AmpC phenotypes (Table 4.3).

No *Salmonella* positive isolates were resistant to nalidixic acid, gentamicin, ciprofloxacin, or amikacin. Additionally, only seven isolates from the non-vaccinated group were resistant to trimethoprim/sulphamethoxazole. *Salmonella* isolates displayed the greatest percentage of resistance to sulphisoxazole (32.7%). The antibiotics tetracycline, streptomycin, and ampicillin accounted for 12.0, 10.5, and 8.5%, respectively, of the observed resistance. Additional antibiotic data is contained in Table 4.6. For all antibiotics, excluding those where no resistance was observed (n=4), vaccinated herds had fewer isolates resistant to these antibiotics (n=11, Table 4.6).

A total of thirty nine named serotypes were observed across all sampling units (Table 4.4). The top five most prevalent serotypes observed were *Salmonella* Montevideo, Mbandaka, Cerro, Meleagridis, and Newport (21.8, 10.4, 9.9, 7.9 and 7.7%, respectively). Collectively the top 15 observed serotypes accounted for 90.1% of the *Salmonella* positive isolates recovered. Additionally, 2.6% of isolates were classified as other; these isolates include rough-strain, un-named, and un-typable *Salmonella* positive isolates. Within the top ten serotypes, vaccinated herds had numerically fewer isolates from serotypes Mbandaka, Meleagridis, Newport, Kentucky, and Anatum. Non-vaccinated herds had numerically fewer *Salmonella* positives from the following serotypes, Montevideo, Cerro, Newington, Uganda, and Muenster. Additional differences for all observed serotypes are presented in Table 4.5.

Discussion

While it was not within the scope of the objectives described in the original proposal, the investigators have described herein differences in serotypes and antibiotic resistance patterns that existed between herds vaccinated with the *Salmonella* Newport SRP® vaccine and herds that had not been vaccinated with this vaccine. The data presented here are to be interpreted as descriptive observations with no statistical comparisons due to the fact that the intent of the original study was not designed to evaluate statistical differences in serotypes and or associated antimicrobial resistance patterns. The authors however, believe these findings warrant consideration and further

research designed to evaluate the effects of the vaccine on observed serotypes and antimicrobial resistance.

Herds vaccinated with the *Salmonella* Newport SRP® vaccine had considerably fewer *Salmonella* Newport isolates. Additionally, markedly fewer isolates derived from vaccinated herds demonstrated ACSSuT or MDR-AmpC resistance phenotypes, largely a result of the decreased number of Newport isolates recovered from vaccinated herds. In addition to increased milk production (*22*); these findings represent added data that dairy operators may wish to consider when deciding to utilize this vaccine technology. By using this vaccine, dairy operators may likely recoup associated vaccination costs and therefore may be further inclined to utilize this resource in the interest of food safety. If these findings can be confirmed in future research, this approach to increasing herd health and food safety should be considered as a meaningful pre-harvest tool by all industry stakeholders.

CHAPTER V

CONCLUSIONS

The following are a list, highlighting the outcomes of the studies conducted examining the effects of a conditionally licensed *Salmonella* Newport SRP® vaccine.

- *Salmonella* is ubiquitous throughout dairies located in the Texas Panhandle and Eastern New Mexico
- Within dairy, animal level *Salmonella* prevalence ranged from 0-92% across all dairies
- *Salmonella* prevalence follows a seasonal pattern, increasing throughout early summer months and peaking in late summer and early fall
- Herds vaccinated with the *Salmonella* Newport SRP® vaccine had lower *Salmonella* prevalence compared to non-vaccinated herds (15.3 vs. 27.5%) throughout the study period
- Model explained variance within dairies accounted for 76.2% of the model, while 23.8% of the model explained variance existed between dairies
- Herds vaccinated with *Salmonella* Newport SRP® vaccine had numerically fewer *Salmonella* isolates of the serotype Newport
- Herds vaccinated with *Salmonella* Newport SRP® vaccine were resistant to fewer antimicrobials throughout the study period

- ACSSuT and MDR-AmpC resistance phenotypes were more frequently observed in non-vaccinated herds

Given these results the author is encouraged and recommends that careful consideration should be given to the implementation of a *Salmonella* Newport SRP® vaccine to effectively reduce on farm prevalence of *Salmonella* and to increase milk production.

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APPENDIX A

TABLE 3.1. *Raw and adjusted mean* Salmonella *prevalence (%) across sampling periods.*

Round	**1**	**2**	**3**	**4**
Raw	17.2	28.4	32.9	18.3
Model Adjusted	13.7	25.7	31.5	14.6

TABLE 4.1. *Quantity of antimicrobials to which resistance was observed in* Salmonella *isolates.*

Antimicrobials	**Frequency**	**Percent**
0	650	64.23%
1	257	25.40%
2	18	1.78%
3	9	0.89%
4	2	0.20%
5	2	0.20%
6	12	1.19%
7	9	0.89%
8	12	1.19%
9	36	3.56%
10	3	0.30%
11	2	0.20%
MDRAmpC	**46**	**4.55%**
ACSSuT	**68**	**6.72%**

TABLE 4.2. Salmonella *serotypes by ACSSuT (resistant to ampicillin, chloramphenicol, streptomycin, sulphisoxazole, and tetracycline) and MDR-AmpC (ACSSuT resistance plus resistance to ceftiofur and amoxicillin/clavulanate) resistance phenotypes.*

Serotype	**ACSSuT**	**MDR-AmpC**
S. Newport	49	39
S. Typhimurium	13	3
S. Agona	2	2
S. Meleagridis	1	0
S. Orion	1	0
Degraded monophasic	1	1
Rough strain	1	1
Total	**68**	**46**

TABLE 4.3. Salmonella *serotypes with ACSSuT (resistant to ampicillin, chloramphenicol, streptomycin, sulphisoxazole, and tetracycline) and MDR-AmpC (ACSSuT resistance plus resistance to ceftiofur and amoxicillin/clavulanate) phenotypes by herd vaccination status.*

Serotype	ACSSuT		MDR-AmpC	
Vaccination Status*	0	1	0	1
S. Newport	33	16	26	13
S. Typhimurium	9	4	1	2
S. Agona	2	0	2	0
S. Meleagridis	1	0	0	0
S. Orion	1	0	0	0
Degraded monophasic	0	1	0	1
Rough strain	1	0	1	0
Total	47	21	30	16

*where 0=non-vaccinated, 1=vaccinated with *Salmonella* Newport SRP® vaccine

TABLE 4.4. Salmonella *serotype diversity and rank across all sampling rounds and herds.*

Serotype	Frequency	%	Rank
Montevideo	206	21.78	1
Mbandaka	98	10.36	2
Cerro	94	9.94	3
Meleagridis	75	7.93	4
Newport	73	7.72	5
Kentucky	54	5.71	6
Newington	49	5.18	7
Uganda	39	4.12	8
Muenster	32	3.38	9
Anatum	29	3.07	10
Typhimurium	29	3.07	11
*Other	25	2.64	12
Infantis	17	1.80	13
Havana	16	1.69	14
Nottingham	16	1.69	15
Cubana	15	1.59	16
Muenchen	12	1.27	17
Senftenberg	12	1.27	18
Barranquilla	7	0.74	19
Agona	6	0.63	20
Give	5	0.53	21
Tennessee	5	0.53	22
Bredeney	4	0.42	23
Orion	3	0.32	24
Schwarzengrund	3	0.32	25
Alachua	2	0.21	26
Amager	2	0.21	27
Cambridge	2	0.21	28
Derby	2	0.21	29
Kinshasa	2	0.21	30
Lexington	2	0.21	31
Manila	2	0.21	32
Agama	1	0.11	33
Bietri	1	0.11	34
Fresno	1	0.11	35
Hato	1	0.11	36
Liverpool	1	0.11	37
Manhattan	1	0.11	38
Newbrunswick	1	0.11	39
Norwich	1	0.11	40

* Includes rough strain, un-named and un-typable

TABLE 4.5. Salmonella *serotypes by herd vaccination status.*

Serotype	Non-Vaccinated	Vaccinated	Non-Vaccinated, %	Vaccinated, %
Montevideo	92	114	20.18	23.27
Mbandaka	68	30	14.91	6.12
Cerro	35	59	7.68	12.04
Meleagridis	57	18	12.50	3.67
Newport	41	32	8.99	6.53
Kentucky	41	13	8.99	2.65
Newington	6	43	1.32	8.78
Uganda	0	39	0.00	7.96
Muenster	10	22	2.19	4.49
Anatum	16	13	3.51	2.65
Typhimurium	14	15	3.07	3.06
*Other	16	9	3.51	1.84
Infantis	7	10	1.54	2.04
Havana	7	9	1.54	1.84
Nottingham	0	16	0.00	3.27
Cubana	2	13	0.44	2.65
Muenchen	4	8	0.88	1.63
Senftenberg	9	3	1.97	0.61
Barranquilla	2	5	0.44	1.02
Agona	6	0	1.32	0.00
Give	3	2	0.66	0.41
Tennessee	2	3	0.44	0.61
Bredeney	4	0	0.88	0.00
Orion	2	1	0.44	0.20
Schwarzengrund	0	3	0.00	0.61
Alachua	1	1	0.22	0.20
Amager	2	0	0.44	0.00
Cambridge	0	2	0.00	0.41
Derby	2	0	0.44	0.00
Kinshasa	1	1	0.22	0.20
Lexington	2	0	0.44	0.00
Manila	0	2	0.00	0.41
Agama	1	0	0.22	0.00
Bietri	1	0	0.22	0.00
Fresno	0	1	0.00	0.20
Hato	0	1	0.00	0.20
Liverpool	1	0	0.22	0.00
Manhattan	0	1	0.00	0.20
Newbrunswick	1	0	0.22	0.00
Norwich	0	1	0.00	0.20

* Includes rough strain, un-named and un-typable

TABLE 4.6. *Percentage and total number of* Salmonella *isolates resistant to each of the evaluated antibiotics by herd vaccination status.*

Antimicrobials	***Salmonella* pos, %**		***Salmonella* pos, n**		**Total**
Vaccination status	**0**	**1**	**0**	**1**	
Sulphisoxazole	17.89	12.45	181	126	307
Tetracycline	6.52	4.64	66	47	113
Streptomycin	6.82	2.96	69	30	99
Ampicillin	5.34	2.57	54	26	80
Chloramphenicol	5.24	2.27	53	23	76
Augmentin	4.05	2.67	41	27	68
Ceftriaxone	3.95	2.37	40	24	64
Cefoxitin	2.96	2.27	30	23	53
Ceftiofur	3.26	1.68	33	17	50
Kanamycin	1.88	0.40	19	4	23
Tri/Sulph	0.69	0.00	7	0	7
Nalidixic acid,	0.00	0.00	0	0	0
Gentamicin	0.00	0.00	0	0	0
Ciprofloxacin	0.00	0.00	0	0	0
Amikacin	0.00	0.00	0	0	0

where 0=non-vaccinated, 1=vaccinated with *Salmonella* Newport SRP® vaccine

APPENDIX B

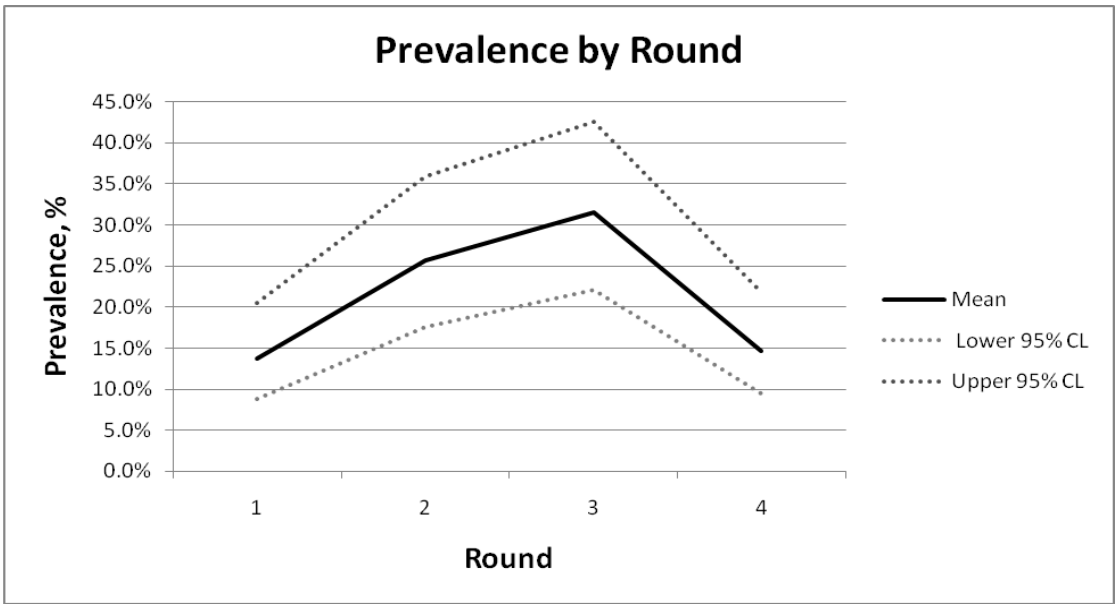

FIGURE 3.1. *Model adjusted* Salmonella *prevalence and 95% confidence intervals by sampling round.*

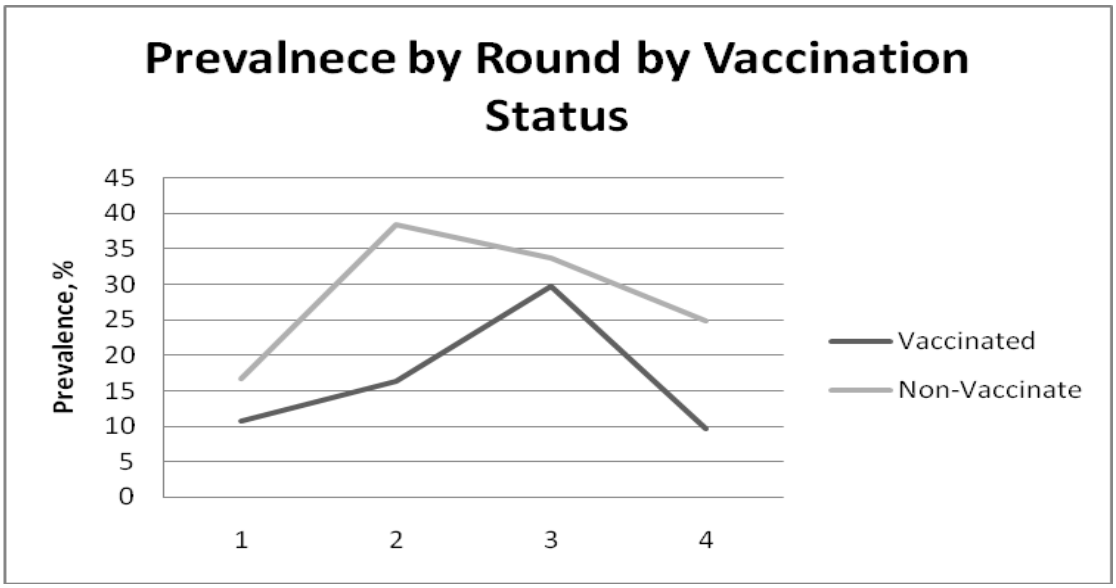

FIGURE 3.2. *Model adjusted* Salmonella *prevalence by sampling round and vaccination status.*

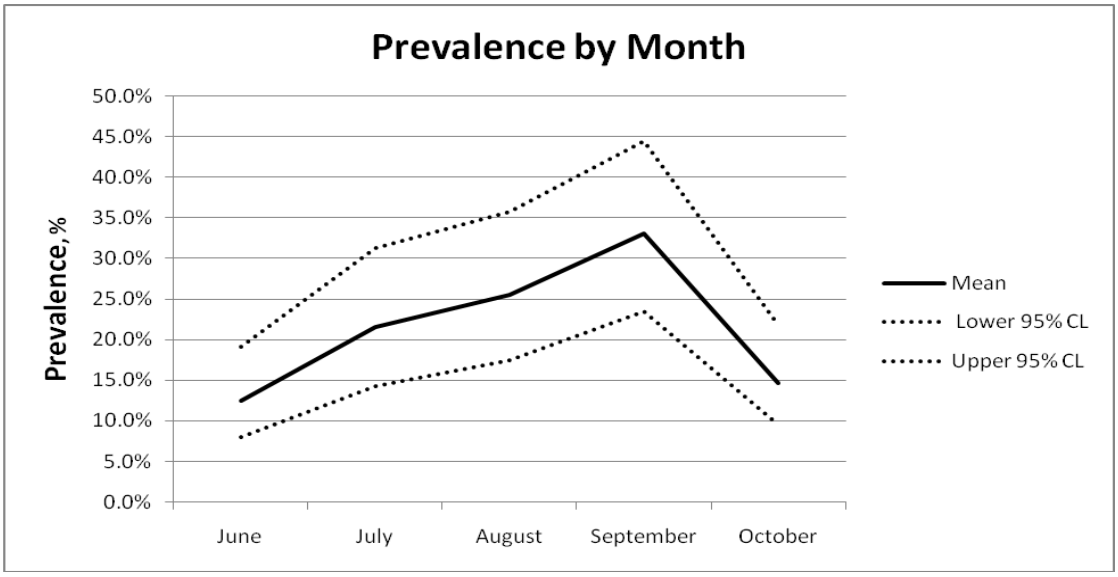

FIGURE 3.3. *Model adjusted* Salmonella *prevalence and 95% confidence intervals by sampling month.*

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