IDENTIFYING BACTERIOPHAGES CAPABLE OF INACTIVATING SALMONELLA ISOLATED FROM BEEF CATTLE FEEDLOTS AS A PRE-HARVEST ANTIMICROBIAL INTERVENTION

A Thesis

by

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ABSTRACT

Asymptomatic *Salmonella* carriage in beef cattle is a significant food safety concern and the beef feedlot environment is a reservoir of this pathogen. The goal of this study was to identify and isolate bacteriophages against *Salmonella* from beef cattle feedlots. Three feedlots in south Texas previously characterized for *Salmonella enterica* prevalence were visited and samples (n=108) collected from dropped feces, feed, drinking water, and soil in cattle pens. Pre-enrichment, selective enrichment and selective/differential isolation of *Salmonella* were performed on each sample. A representative subset of presumptive *Salmonella* isolates was prepared for biochemical identification and serotyping. Samples were pooled by site to create 36 samples and enriched to recover phage. Recovered phages were tested for host range against a diverse panel of 20 *Salmonella* strains representing 12 serotypes. *Salmonella* were identified in 20 of 108 samples (18.5%) by biochemical and/or serological testing. Comparative testing between biochemical and molecular methods yielded only one disagreement, where molecular detection failed to confirm *Salmonella* presence from pre-enrichment medium. Serovars including *S.* Anatum, Muenchen, Altona, Kralingen, Kentucky and Montevideo were identified in the samples; *S.* Anatum was the most frequently recovered serotype of *Salmonella*. Phage-positive samples were distributed evenly over the three sampled feedlot sites, suggesting that phage prevalence is not strongly correlated with the presence of culturable *Salmonella.* Phages were found more frequently in soil and feces compared to feed and water samples. Host ranges of the

phages were highly variable, with the broadest host range phage infecting 16 of the 20 *Salmonella* strains tested. Bacteriophages found in the *Salmonella*-free feedlot suggest that phages might play a role in suppressing the *Salmonella* population in a feedlot environment. This may also indicate that endogenous phages may contribute to false-negative results in culture-based *Salmonella* detection. Some phages were able to infect a broad range of *Salmonella* serovars, giving them possible utility as an intervention against *Salmonella* in feedlots.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Salmonella and Salmonellosis

Salmonella are facultative anaerobic, Gram-negative, non spore-forming rod-shape enteric bacteria belong to the family *Enterobacteriaceae* (Bell and Kyriakides, 2002). Although *Salmonella* are generally motile with peritrichous flagella, non-flagellated and non-motile strains with dysfunctional flagella do exist (Montville and Matthews, 2008). *Salmonella* are able to utilize a wide range of organic compounds and metabolize nutrients via respiratory and fermentative pathways (Montville and Matthews, 2008). Isolates generally catabolize glucose with production of acid and gas. Most strains are unable to ferment lactose, though several mutants that have ability to utilize lactose have been identified (Gonzalez, 1966). *Salmonella* are oxidase negative, catalase positive, indole negative, Voges-Proskauer negative, methyl red positive, and are able to grow on citrate as a sole carbon source (Bell and Kyriakides, 2002). They produce hydrogen sulfide, decarboxylate lysine and ornithine, and do not produce urease (Montville and Matthews, 2008).

Salmonella is divided into two species: *S. enterica* and *S. bongori*. *S. enterica* is divided into six subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*) (Sanderson and Nair, 2010). The majority of human *Salmonella* infections are caused by *S*. enterica subsp. *enterica* (Bell and Kyriakides, 2002). Subspecies are divided into more than 2600 serotypes based on their serological reaction to somatic

lipopolysaccharide (O), flagellar (H) and capsular antigens according to the Kauffmann-White Scheme (Sanderson and Nair, 2010). Ninety-nine percent of serotypes were found within the species *S. enterica*, of which 59% belong to subspecies *enterica*. The Kauffmann-White Scheme for *Salmonella* serotyping is maintained by World Health Organization (WHO) Collaborating Center for Reference and Research on *Salmonella* at Institut Pasteur, Paris, France (Grimont and Weill, 2007).

Salmonella are mesophilic and can grow within a temperature range of 5.2 to 46.2 °C, with the optimal temperatures for growth from 35 to 43 °C (ICMSF, 1996). Many factors may enhance heat resistance of *Salmonella*; low water activity, high fatty acid content, and sub-lethal stimulation could increase the heat resistance of this organism (Montville and Matthews, 2008). Freezing can be detrimental to *Salmonella,* but it does not guarantee destruction (Montville and Matthews, 2008). Research showed that 0.2$\log_{10}$ CFU/ml reduction of *S.* Typhimurium was observed on chicken carcass during crust freezing (Chaves et al., 2011). However, *Salmonella* are able to survive longer at frozen condition compared to ambient and refrigeration temperature (ICMSF, 1996). The optimal water activity (a_w) for the growth of *Salmonella* is 0.99 and the minimal a_w limit is 0.93. *Salmonella* are capable to survive for months in foods with low a_w such as nuts and spice (ICMSF, 1996). *Salmonella* can growth in a broad pH range from 3.8 to 9.5, with an optimum pH range for growth of 7.0 to 7.5. However, the capacity for growth in extreme pH condition is dependent on other factors such as the presence of chemicals, temperature and a_w (Montville and Matthews, 2008).

Salmonellosis is a human gastrointestinal (GI) illness caused by exposure to non-typhoidal *Salmonella* spp. Outcomes of non-typhoidal *Salmonella* infection can range from asymptomatic colonization of the GI tract to acute gastroenteritis, septicemia, bacteremia or chronic conditions such as arthritis (Hohmann, 2001). After the organism is ingested via a cross-contaminated food, the organism multiplies in the small intestine, colonizing and invading the intestinal tissue, producing an heat-stable enterotoxin that causes an inflammatory reaction (Bell and Kyriakides, 2002). The inflammatory reaction of human body causes symptoms typical of gastroenteritis including watery diarrhea, persistent and spiking fever, abdominal pain, headache, nausea, prostration, and a rash of rose-colored spots on the shoulders, thorax, or abdomen (D'aoust, 1991). Approximately 5% of individuals with gastroenteritis caused by non-typhoidal *Salmonella* infection develop bacteremia, which occurs when *Salmonella* spp. enter the bloodstream (Hohmann, 2001). Bacteremia is associated with high fever, lethargy, abdominal and chest pain, chills and anorexia (Bell and Kyriakides, 2002). Reactive arthritis, a chronic sequela, may occur after *Salmonella* infection and it was estimated to happen in a prevalence of 12 cases per 1,000 Salmonella infections(Ajene et al., 2013). The incubation period for typical *Salmonella* infection is 6 to 72 hours and illness generally lasts 2 to 7 days (2002; Hohmann, 2001). The infectious dose was reported large (10^5 – 10^{10}) in volunteer study, but data from outbreak indicated that the infectious dose could be as low as a few cells (Bell and Kyriakides, 2002; Todd et al., 2008). Factors that affect the determination of infectious dose include immunological heterogeneity within human populations, virulence of infecting strains and chemical composition of

contaminated food (Montville and Matthews, 2008). Infants, the elderly, and immunocompromised individuals are more susceptible to *Salmonella* infections than (Montville and Matthews, 2008). Foods with high fat content are more likely to be associated with low dose *Salmonella* infection. Organisms may be protected against the bactericidal action of gastric acidity by being encapsulated within hydrophobic lipid micelles (Montville and Matthews, 2008).

Epidemiological and Economic Impacts of *Salmonella*

Salmonella has been reported a leading bacterial cause of foodborne illnesses in the United States. Foodborne illnesses caused by *Salmonella* are estimated to be more than 1.2 million each year in the United States, with more than 23,000 hospitalizations and 450 deaths (Scallan et al., 2011). In 2013, among 19,056 foodborne illness cases identified by the FoodNet sentinel network, *Salmonella* was the most common cause of bacterial illness, accounting for 7,277 cases (38.2%) (Crim et al., 2014); 2003 hospitalizations (28% of *Salmonella* caused infections) and 27 (0.4% of *Salmonella* caused infections) deaths were identified through FoodNet (Crim et al., 2014). Although incidence of salmonellosis was lower in 2013 compared to 2010-2012, it was similar to 2006-2008 and has remained stable over the long term (Figure 1) (CDC, 2013; Crim et al., 2014; Scallan et al., 2011).

In 2013, 157 *Salmonella* outbreaks (34% of total outbreaks reported in US) were identified to occur in the United States, causing 3553 illness (38% of total illnesses associated with outbreaks) and 623 hospitalizations (65% of total hospitalizations

associated with outbreaks) (CDC, 2015; Gould et al., 2013a). Outbreaks caused by *Salmonella* increased 39% from 2012 (113) to 2013 (157) and hospitalizations associated with *Salmonella* outbreak increased 38% from 2012 (454) to 2013 (628) (CDC, 2015). From 1998 to 2008, 128 multistate foodborne illness outbreaks were reported in the United States, of which 68 (53%) were caused by *Salmonella* (Gould et al., 2013b).

Figure 1. Incidence rate of Salmonella infections in the U.S. Incidence rate of laboratory-confirmed human non-typhoidal *Salmonella* infection in the U.S. has remained stable from 1970-2011 (CDC, 2011).

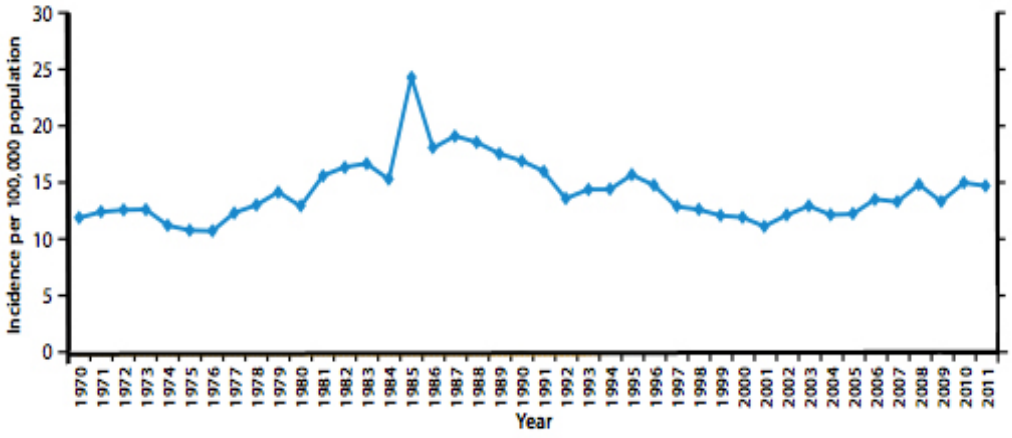


Figure 2. Annual cost and percentage cost estimates in 2010 U.S. dollars based on disease incidence estimates published 2010. *Salmonella* imposes the greatest cost of 14 major foodborne pathogens investigated in the United States (Hoffmann and Anekwe, 2013).

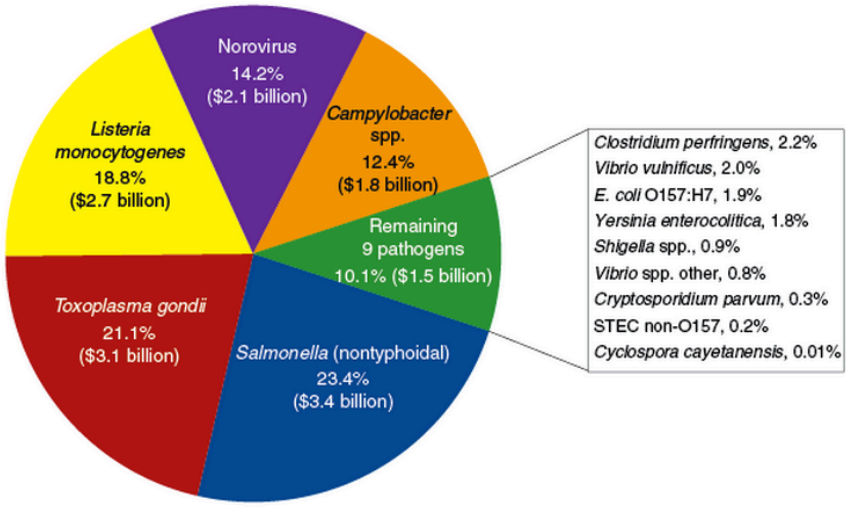


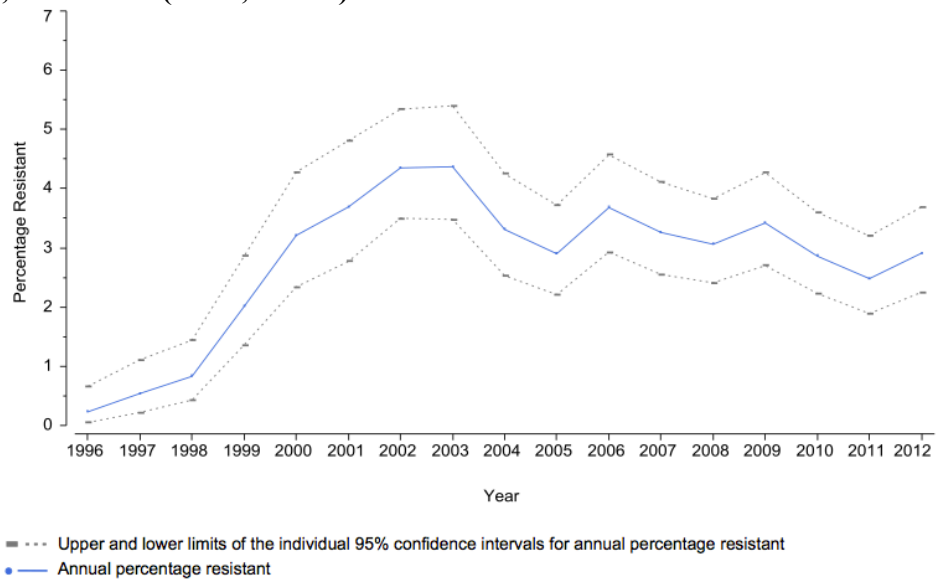
Figure 2 from the United States Department of Agriculture (USDA) Economic Research Service (ERS) indicates that *Salmonella* imposes the greatest cost of 14 major foodborne pathogens investigated in the United States (Hoffmann and Anekwe, 2013; Hoffmann et al., 2012). In 2010, the estimated annual economic cost of illness caused by *Salmonella* was $3.4 billion. This estimate is for all cases of salmonellosis and included medical costs due to illness, the cost of time lost from work due to nonfatal illness, and the cost of premature deaths (Hoffmann and Anekwe, 2013).

Antimicrobial Resistance of *Salmonella*

In the United States, severe non-typhoidal salmonellosis is commonly treated with fluoroquinolone and third-generation cephalosporin antibiotics (CDC, 2014b).

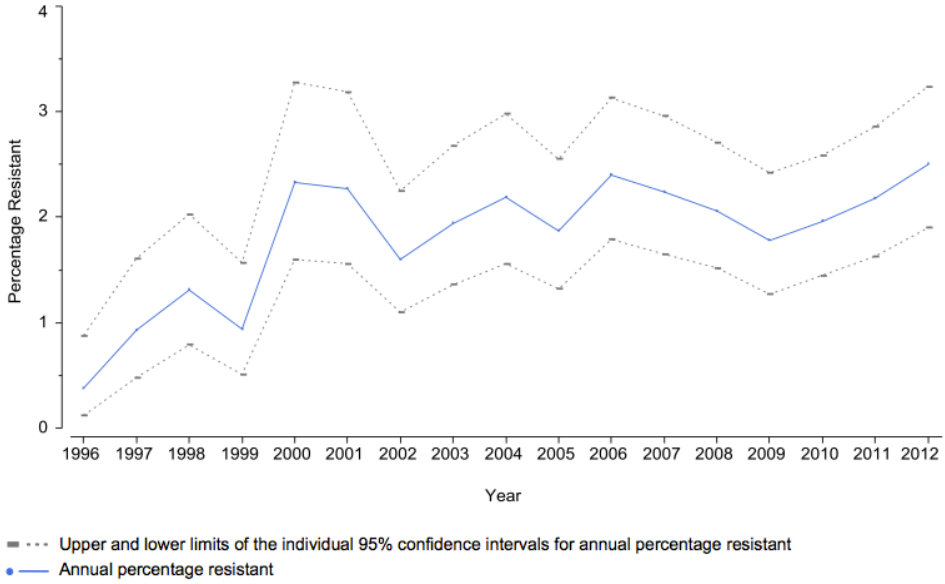
Recently recognized decreases in susceptibility to ceftriaxone indicates possible increasing resistance pattern to 3rd generation cephalosporins (Chen et al., 2007). In the *Enterobacteriaceae*, resistance to nalidixic acid, an elementary quinolone, correlates with decreased susceptibility to ciprofloxacin and may result in fluoroquinolone treatment failure (CDC, 2014b; Piddock, 2002). Other Clinical and Laboratory Standards Institute (CLSI) drugs such as macrolides, penicillins and trimethoprim-sulfamethoxazole are also of clinical importance (CDC, 2014b). The Centers for Disease Control and Prevention (CDC; Atlanta, GA) National Antimicrobial Resistance Monitoring System (NARMS) reported 3% of tested clinical non-typhoidal *Salmonella* isolates were resistant to ceftriaxone and nalidixic acid (CDC, 2014b) in 2012. Nine percent of non-typhoidal *Salmonella* isolates tested were resistant to three or more CLSI classes (CDC, 2014b). Compared to NARMS-reported data from 2003-2007, *Salmonella* isolates resistant to three or more CLSI classes lower in 2012 than in 2003–2007 (9% vs. 12%). However, the differences in resistance to nalidixic acid and ceftriaxone between 2003-2007 and 2012 are not significant (Figure 3, 4) (CDC, 2014b). Crump *et al*. (2011) summarized antimicrobial resistance among invasive non-typhoidal *Salmonella* submitted to NARMS from 1996 to 2007 and found that 19.8% were resistant to ampicillin, 11.1% to chloramphenicol and 2.5% to trimethoprim-sulfamethoxazole; 2.7% isolates were resistant to nalidixic acid and 2.5% to ceftriaxone. Previous research has shown that patients with antimicrobial resistant non-typhoidal *Salmonella* infection are more likely to develop bloodstream infection (bacteremia) compared to patients with pan-susceptible *Salmonella* infection (Varma et al., 2005b).

Figure 3. Percentage of non-typhoidal *Salmonella* isolates resistant to ceftriaxone, by year, 1996–2012(CDC, 2014b).



In the United States, within the 1.2 million estimated *Salmonella* annual infections, 100,000 cases and 40 deaths are estimated to be caused by drug-resistant *Salmonella* (CDC, 2014a). From 1984 to 2002, among non-typhoidal *Salmonella* outbreaks in which antimicrobial resistance information is available, 28% of outbreaks were caused by antimicrobial-resistant strains (Varma et al., 2005a). Outbreaks caused by antimicrobial-resistant non-typhoidal *Salmonella* resulted in greater hospitalization rates and a greater portion of deaths (Varma et al., 2005a; Varma et al., 2005b).

Figure 4. Percentage of non-typhoidal Salmonella isolates resistant to nalidixic acid, by year, 1996–2012(CDC, 2014b).



Antibiotic resistant bacteria can be spread from person to person, and from non-human sources in the environment such as food (CDC, 2014a). Antibiotics are commonly used in food animals to prevent, control, and treat disease, and to promote the growth of food-producing animals (FDA, 2013). Higher quantities of antibiotics were sold in the United States for food-producing animals than for people (FDA, 2013). The over-prescription of antibiotics in food animals may contribute to the emergence of antibiotic-resistant bacteria, which is concerning because people can develop infections by consuming food contaminated with antibiotic-resistant bacteria from these animals, resulting in reduced efficacy of antimicrobials for disease therapy (CDC, 2014a). Therefore, antibiotics must be cautiously used in animal food production and antimicrobial substitutes have to be developed to prevent the emergence and spread of antibiotic-resistant bacteria.

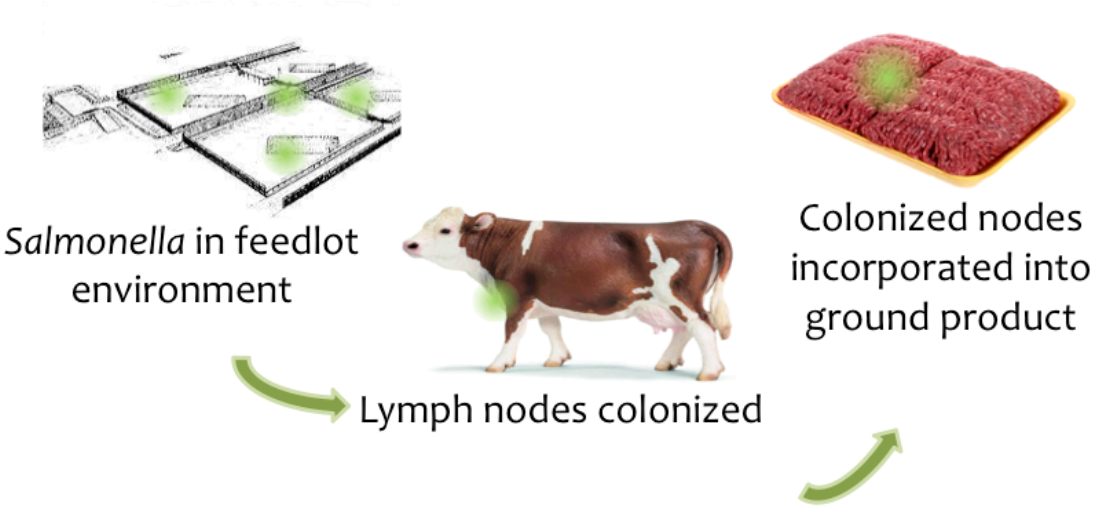
Beef Cattle as a *Salmonella* Carrier

In the U.S., commercial ground beef has been reported to be contaminated with *Salmonella* at a rate of 4.2% (Bosilevac et al., 2009), despite the adoption of antimicrobial interventions on the carcass such as sodium hydroxide, sodium sulfide, chlorine, lactic acid and acetic acid solutions (Carlson et al., 2008). Antimicrobial interventions have been demonstrated to be able to reduce *Salmonella* prevalence on beef carcasses from 50.2% to 0.8%, but have little effect on ground beef made from edible beef trimmings (Brichta-Harhay et al., 2011). The carriage of *Salmonella* in the lymph nodes of beef cattle may contribute to the transmission of the pathogen to consumers via ground beef. Gragg *et al.* (2013) reported that *Salmonella enterica* was found in asymptomatic cattle lymph nodes at slaughter, which means in a low dose infection, beef cattle could serve as *Salmonella* carriers without being detected. Without showing any clinical symptoms of *Salmonella* infection or carriage, detection of beef cattle carrying *Salmonella* is difficult. Therefore, there risks of releasing *Salmonella*-contaminated ground beef to consumers.

Major lymph nodes are reported to have relatively high *Salmonella* prevalence in beef cattle. Mandibular, mesenteric, mediastinal, and sub-iliac nodes, which may be removed during evisceration, reportedly may harbor *Salmonella* at rates of 55.9% (95% CL: 43.7 to 67.4%), 91.2% (81.6 to 96.0%), 7.4% (3.1 to 16.2%), and 76.5% (64.8 to 85.2%), respectively (Gragg et al., 2013b). Flank- and chuck-associated nodes, which have been reported to bear *Salmonella* at 3.86% and 0.35%, respectively, are not removed during carcass dressing and may be present in trimmings destined for use in

ground beef (Arthur et al., 2008). Brichta-Harhay et al. (2012) indicated that several *Salmonella* serovars, including *S.* Anatum, Dublin, Cubana, Typhimurium, and Montevideo could be recovered from lymph nodes and node-surrounding adipose tissue. A similar study showed that among serotypes found in lymph nodes, *S.* Montevideo (44.0%) and *S.* Anatum (24.8%) made up the majority of recovered *Salmonella* (Gragg et al., 2013b). This same research team also conducted research on *Salmonella* related to lymph nodes and environmental samples and found that *S.* Kentucky (32.2%), *S.* Anatum (29.9%) and *S.* Reading (17.2%) made up the majority of recovered *Salmonella* serovars (Gragg et al., 2013b). In the same study, antimicrobial resistance of *Salmonella* isolates recovered from lymph nodes (n = 56), feces (n = 18), and hide samples (n = 17) was characterized. Thirty (53.6%), 3 (16.7%), and 4 (23.5%) of *Salmonella* from these sample types, respectively, exhibited resistance to one or more drugs (Gragg et al., 2013b). In the meantime, the differences of *Salmonella* presence and serotypes across multiple lymph nodes within the same animal indicate that multiple routes of entry into the lymphoidal system for *Salmonella* may exist (Gragg et al., 2013b). Although hypothesized *Salmonella* transmission routes have not yet been comprehensively described in the published literature, exposure to *Salmonella* within the feedlot environment seems to serve as the origin despite the various transmission routes (Figure 5).

Figure 5. A model of *Salmonella* transmission from feedlot to ground beef. *Salmonella* in the feedlot environment colonizes lymph nodes. Lymph nodes contaminated with *Salmonella* that are not removed during fabrication end up in fat trim and eventually become part of ground beef .



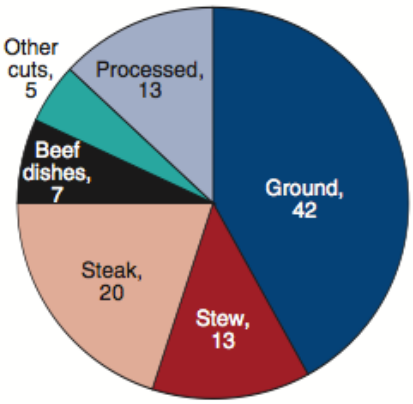
Beef cattle hides and feces have been found to have a *Salmonella* prevalence of 100% and 94.1%, respectively (Gragg et al., 2013b). Other findings indicate variable *Salmonella* prevalence in cattle feedlots within the same geographic region could also differ, possibly due to animal management practices or animal origin (Haneklaus et al., 2012) . Being an important factor affecting *Salmonella* carriage in beef cattle lymph nodes, studies on factors affecting *Salmonella* prevalence in beef cattle feedlots have not been conducted systemically. Further studies exploring possible antimicrobial interventions on multiple drug-resistant *Salmonella* in cattle lymph nodes and post-harvest environments could also be conducted to reduce *Salmonella* issues in beef industry.

Ground Beef Consumption in the U.S. and *Salmonella* Problem in Ground Beef

Figure 6 from USDA-ERS indicates that ground beef held the largest market share for all identifiable beef products (Davis and Lin, 2005). An industry statistics report in 2012 from National Cattlemen's Beef Association (NCBA) showed that although the market share of ground beef consumption has decreased in recent decades, it still accounts for 42% of total beef consumption in the United States (NCBA, 2012). This translates into the average American consuming 28 pounds per year (Davis and Lin, 2005; NCBA, 2012)

Consuming raw or undercooked ground beef is a risk factor for contracting a foodborne illness, including *Salmonella* infection. In one survey, 18% of persons who consumed ground beef in the home indicated that they consumed pink/undercooked ground beef (Taylor et al., 2012). Among all age groups, children younger than 18 years old consumed ground beef product most frequently (82% of total respondents), in which 7.9% reported consuming undercooked ground beef. Senior age group (>65 years of age) consumed ground beef least frequently; however, this age group reported higher rate of consumption of undercooked ground beef (18.5% of total respondents) (Taylor et al., 2012). Since younger children are more susceptible to *Salmonella* (CDC, 2011), and children are consuming ground beef more frequently than other age groups (Taylor et al., 2012), public education of safe food handling practice and antimicrobial interventions usage on pre- and post-harvest for beef are required to prevent *Salmonella* transmission.

Figure 6. Share of beef consumption by cuts in the U.S. USDA-ERS indicated that ground beef held the largest market share for all identifiable beef cut (Davis and Lin, 2005).



In the United States, despite efforts to prevent *Salmonella* contamination in foods, *Salmonella* incidence rates remain stable and outbreaks caused by *Salmonella* have continued to occur (CDC, 2011; Scallan et al., 2011). From 1973 to 2011, of 1965 *Salmonella* foodborne outbreaks reported to CDC, beef was the implicated food vehicle in 96 outbreaks (Laufer et al., 2014). These 96 outbreaks caused 3,684 illnesses, 318 hospitalizations and five deaths (Laufer et al., 2014). Ground beef accounts for 23% of total beef related outbreaks with median size of 36 illnesses per outbreak (Laufer et al., 2014). Fifty-nine percent of ground beef attributed outbreaks were caused by serotypes Typhimurium and Newport. Ground beef emerged as the predominant transmission vehicle to beef related outbreak in 2002. From 2002 to 2011, ground beef was reported responsible for 45% of beef related outbreaks in the United States. Among 11 multistate beef related outbreaks, ground beef contributed to 11 multistate outbreaks (Laufer et al., 2014). Among 14 beef-transmitted outbreaks where antimicrobial resistance data were

available, ground beef accounted for 3 outbreaks (Laufer et al., 2014). Although *Salmonella* prevalence in commercial ground beef is low, a survey showed that about 67% *Salmonella*-positive commercial ground beef samples were contaminated with MDR *Salmonella* (Talbot et al., 2006).

There were factors contribute to ground beef attributed Salmonella outbreaks such improper food handling, environmental contamination, cross-contamination, worker contamination where environmental contamination was reported in over half (59%) ground beef attributed outbreaks (Laufer et al., 2014), suggesting *Salmonella* contamination might occur mainly in the pre-harvest level prior food processing. Reducing use of antibiotics for agricultural production efficiency and developing antimicrobial intervention targeting MDR *Salmonella* are becoming urging needs to prevent ground beef attributed *Salmonella* outbreaks, particularly MDR *Salmonella* outbreaks.

Bacteriophage Characteristics and Infection Cycle

Bacteriophages (phage) are viruses that infect bacteria, and are the most abundant form of life on earth, estimated to number some 10^{31} to 10^{32} organisms in total (Barr et al., 2013; Brüssow, 2005). Phage are ubiquitous in natural environment as such soil, fresh water, open ocean, and are present in plants and animals as a part of their normal flora. Phages are non-pathogenic to humans and are normal residents of the human microbiome (Barr et al., 2013; Brüssow, 2005).

Figure 7. Life cycle of virulent phages. Lytic phage adsorbs to its host and ejects its DNA (blue) into the cell. The host metabolism is redirected to the replication of new virion components. New phage virions are assembled and released upon the lysing the host.

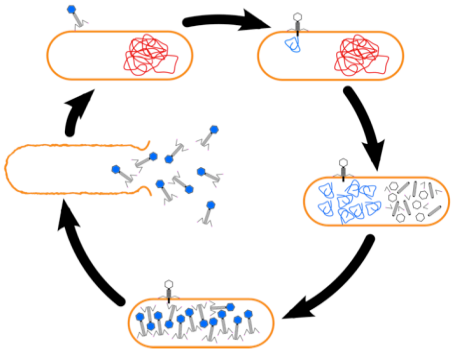
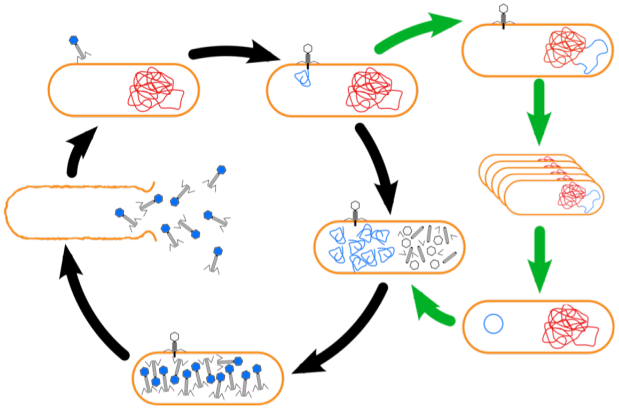


Figure 8. Life cycle of temperate phages. A decision between the lytic cycle and lysogenic cycle is determined by the phage shortly after infecting the cell. In the lytic cycle (black arrows), new phage particles are produced and released upon lysis of the host cell. In the lysogenic cycle (green arrows), the phage DNA (blue) is incorporated into the host chromosome (red) and replicated together with the cell chromosome when cell division occurs.



A phage virion consists a single-stranded or double stranded DNA or RNA genome and a protein or lipoprotein coat (Ceyssens and Lavigne, 2010). Most phages

associated with food-borne pathogens belong to the order of the *Caudovirales* or tailed dsDNA phages (Ceyssens and Lavigne, 2010). *Caudovirales* consists three major families with distinct tail morphologies: *Myoviridae* with contractile tails, *Siphoviridae* with long flexible tails and *Podoviridae* with short stubby tails (Abedon, 2012).

Phage infection starts with adsorption of the phage to its host, where specialized adsorption structures, such as tail fibers and spikes, bind to specific surface receptor on the target bacteria such as proteins, oligosaccharides, and liposaccharides (Ceyssens and Lavigne, 2010). After adsorption, phage DNA is injected into the host bacterium. Based on their infection cycles, *Caudovirales* are divided into two major groups: virulent and temperate (Ceyssens and Lavigne, 2010). Virulent phages obligately undergo a lytic life cycle upon DNA injection while temperate phages, also known as lysogenic phages, are capable of incorporating their genomes into that of the host cell and coexisting with the host in a quiescent state (Abedon, 2012). After infection, virulent phages direct the host cell to produce more copies of the phage genome and capsid components (Figure 7). After phage genome replication and protein synthesis, phage particles are assembled to new virions. New virions are released upon the lysing the cell wall of the host (Rabinovitch et al., 1999). In contrast, when temperate phages infect the cell a lysis-lysogeny decision is made. If it follows the lysogenic pathway, the phage DNA is incorporated into the host chromosome or exists separately as an episomal element and replicates together with the host chromosome as the host divides (Figure 8) (Ceyssens and Lavigne, 2010). When a temperate phage undergoes its lytic cycle, the phage replicates within the host cell to produce new progeny and lyse the cell upon

release of new virions in the same manner as virulent phage (Figure 8) (Ceyssens and Lavigne, 2010).

Bacteriophage as an Antimicrobial Intervention

The antimicrobial activity of bacteriophage was discovered independently by Frederick Twort in 1915 and Felix d'Herelle in 1917 (Sulakvelidze et al., 2001). The therapeutic application for treating human bacterial infections was hypothesized by Felix d'Herelle during an outbreak of severe *Shigella* hemorrhagic dysentery among French troops stationed at Maisons-Laffitte (Sulakvelidze et al., 2001). He filtered out the bacterium from patients' fecal samples and combined the filtrates with *Shigella* strains isolated from the patients. After incubation, a clear area was observed on the agar culture, which were later called plaques (Sulakvelidze et al., 2001). D'Herelle also ran a small clinical trial treating young children suffering from bacterial dysentery with phage preparations and observed recovery within 24 hours (Sulakvelidze, 2011b). Phage therapy attracted interest from pharmaceutical companies, including Eli Lilly and Company, E.R. Squibb & Sons, and Swan-Myers/Abbott Laboratories, who produced phage preparations for clinical applications (Sulakvelidze, 2011b). However, in the 1940s and 1950s, due to the discovery and later use of antibiotics, along with doubts on efficiency of phage therapy, bacteriophage therapy research was not continued in the West (Garcia et al., 2008). Nevertheless, phage therapy continued to develop in Eastern Europe and the former Soviet Union, including work at several institutions such as the Eliava Institute of Bacteriophage, Microbiology and Virology (Tbilisi, Georgia) and the

Hirszfeld Institute of Immunology and Experimental Therapy (Wroclaw, Poland) (Garcia et al., 2008).

The spread of antibiotic resistant bacteria has become a worldwide threat, which has renewed the interest in exploring bacteriophage as a potential alternative to control pathogenic bacteria in Western countries (Sulakvelidze, 2011a). Garcia et al. (2008) suggested that phage can be addressed to all stage of food production from "farm to fork" to prevent foodborne infections. Several phage-based commercial products have gained approval for use by the U.S. Department of Agriculture (USDA), Food and Drug Agency (FDA) and Environmental Protection Agency (EPA) regulations and are now available for food producers as an antimicrobial intervention. EcoShield™, a bacteriophage preparation produced by Intralytix Inc., obtained regulatory approval from the FDA through a "Food Contact Notification" (FCN No. 1018) for use on raw meat cuts and trim to control *E. coli* O157:H7 prior to grinding (Goodridge and Bisha, 2011). The Food and Drug Administration (FDA) has also approved ListShield™, another phage-based product from Intralytix Inc., for application on ready-to-eat (RTE) meat and poultry products for *Listeria monocytogenes* reduction (Sulakvelidze, 2011a). AgriPhage™, a phage cocktail produced by OmniLytics Inc., gained approval from the Environmental Protection Agency for application on growing produce in the field to reduce plant pathogenic bacteria (Garcia et al., 2008). Finalyse®, produced by Elanco Food Solutions (Indianapolis, IN), is approved for application on the hides of beef cattle to reduce *E. coli* O157:H7 prior to slaughter (Sillankorva et al., 2012).

Prevalence and antimicrobial activities of phages in the pre-harvest environment has been studied. Callaway et al. has shown that phages targeting *E. coli* O157:H7 were widely distributed in beef cattle feedlots (2006). This research group also conducted studies on utilizing phage antimicrobial interventions against *E. coli* O157:H7 and demonstrated their efficacy for eliminating *E. coli* O157:H7 in the ceca and rectums of sheep (Callaway et al., 2008; Sheng et al., 2006). Phage have also been shown to be effective in controlling a wide variety of pathogens in other animal models, including *Salmonella* in poultry (Atterbury et al., 2007) and *E. coli* O157:H7 in cattle (Sheng et al., 2006). Due to their ubiquity within the environment, research on bacteriophage roles in ecological system regulation that potentially affect *Salmonella* prevalence in cattle feedlot environments should be conducted. The presence of phages in samples may lead to a false negative result for the detection of *Salmonella* within a feedlot sample, should virulent bacteriophages infective of *Salmonella* be found in corresponding samples (Muniesa et al., 2005). On the other hand, bacteriophages against *Salmonella* could be possibly isolated from feedlots, identified and utilized as an antimicrobial intervention to reduce *Salmonella* carriage in high *Salmonella*-positive feedlots.

CHAPTER II

MATERIALS AND METHODS

Sampling Procedure

Three feedlots located in south Texas were selected for sample collection. Feedlots are located in Gonzales, TX, Floville, TX and Rio Grande, TX and were coded for the sake of sample labeling/recording. Previous studies by Haneklaus et al. (2012) identified these feedlots to be consistently positive or negative for *Salmonella* carriage in lymph nodes of cattle presented for harvest. In each feedlot, three pens were selected for sampling. Triplicate subsamples each were taken from dropped feces, animal pen soils, feed from feed bunks, and water from drinking troughs; a total of 108 samples were collected. During sampling, approximately 250 g was aseptically collected into a sterile Whirl-Pak bag (Nasco, Inc., Fort Atkinson, WI). Sample containers were labeled, sealed and transported under refrigerated conditions to the Food Microbiology Laboratory in the Department of Animal Science on the campus of Texas A&M University (College Station, TX) for *Salmonella* recovery and subsequent phage isolation.

Salmonella Recovery and Selective Plating

Salmonella isolation methods were adapted from selected components of relevant procedures from the USDA Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) (Lattuada et al., 1998), the U.S. Environmental Protection Agency (EPA) Sewage Sludge (Biosolids) *Salmonella* isolation method (Meckes and

Oshiro, 2006), and the FDA Bacteriological Analytical Manual (BAM) (Andrews et al., 2014). Twenty five gram of sample was added to a Fisherbrand™ Lab Blender Bag (Thermo Fisher Scientific, Inc., Waltham, MA), loaded with 225 ml sterilized lactose broth (Becton, Dickinson and Company, Franklin Lakes, NJ), and then pulverized for 1 min in a stomacher to homogenize the sample in the pre-enrichment medium. Bags were closed and allowed to stand at 25 °C for 60 min, and then transferred to an incubator and held at 35 °C without shaking for 24 h. After *Salmonella* pre-enrichment was completed, 0.1 ml of lactose broth was aseptically collected and inoculated into sterilized Rappaport-Vassiliadis (RV) broth (Thermo Scientific™ Remel™) and vortexed to mix. Selective enrichment of *Salmonella* in RV-containing tubes were completed in an incubator held at 42±0.2 °C for 24 hr.

Following selective enrichment, RV tubes were removed from the incubator. Selective plating was performed by aseptically using a sterile loop to streak 10 µl of RV enrichment onto Xylose Lysine Desoxycholate (XLD) (Becton, Dickinson and Company, Franklin Lakes, NJ) agar-containing Petri plates. Streaked plates were lidded and incubated inverted at 35 °C for 24±2 hr. Following incubation, colonies possessing typical or atypical *Salmonella* appearance/morphology were picked from each sample-derived Petri plate. Typical *Salmonella* colonies appear pink and may produce blackened centers due to the reduction of H_2S to form a black precipitate in the presence of available iron (Andrews et al., 2014). Atypical *Salmonella* colonies may appear yellow due to pH reduction caused by fermentation of lactose and sucrose (Gonzalez, 1966). Colonies were picked and streaked onto tryptic soy agar (TSA) (Becton, Dickinson and

Company, Franklin Lakes, NJ) slants, and incubated at 35°C for 24±2 hr. Slants then were preserved by layering of sterilized mineral oil and held at 4°C for further identification.

Presumptive Identification Using Biochemical Methods

Presumptive identification of *Salmonella* from XLD-picked colonies was completed at the Texas A&M Center for Food Safety (College Station, TX) using the Vitek® 2 system (bioMérieux N.A., Durham, NC). *Salmonella* isolates on TSA slants were streaked onto TSA plates and incubated at 35°C for 24±2 hr. Plates then were packaged and transferred to the Texas A&M Center for Food Safety. Isolated colonies were picked and suspended in saline solution to an optical density (OD) 0.5-0.63 according to the manufacturer's instructions. The saline solution then was transferred to cards designed for identification of organisms within the taxonomic family *Enterobacteriaceae* and placed into the Vitek® 2 system for identification.

Rapid Detection of *Salmonella*

In addition to fermentation- and biochemistry-based presumptive identification of *Salmonella* from samples, detection of *Salmonella* in feedlot samples was also completed by utilizing a rapid nucleic acid amplification-based detection method via the Roka Bioscience, Inc. (San Diego, CA) Atlas® System. Lactose broth pre-enrichment containers, stored under refrigeration following completion of pre-enrichment as described above, were retrieved and used in this test. Following the lactose broth pre-

enrichment, 400 µl and 40 µl of each enriched lactose broth were transferred to a Roka Transfer Tube and transported to the Center for Food Safety. Roka Transfer Tubes were placed into the Roka Bioscience, Inc. (San Diego, CA) Atlas® System for analysis and processed according to manufacturer instructions.

Serotyping of *Salmonella*

A representative subset of presumptive *Salmonella* isolates was selected based on morphology difference to represent sample types in *Salmonella*-positive feedlots. Isolates were prepared on TSA slants and shipped to the Molecular Laboratory at the Ryan Veterinary Hospital (University of Pennsylvania, Philadelphia, PA) for serotyping via the xMAP *Salmonella* Serotyping Assay (SSA) (Luminex Corp., Austin, TX). Isolates were also serotyped by the USDA Animal and Plant Health Inspection Service (APHIS) (Ames, IA) via independent agglutination assay and Kauffmann-White Scheme destination (CDC, 2011).

Antimicrobial Resistance Determination

Antimicrobial resistance of serotyped isolates was determined at the Texas A&M Veterinary Medical Diagnostic Laboratory (College Station, TX) using the Sensititre® diagnostic system (Trek Diagnostic Systems, Thermo-Scientific, Waltham, MA). Frozen permanent stocks of *Salmonella* isolates were streaked onto sheep-blood agar (Becton, Dickinson and Company, Franklin Lakes, NJ) plates and incubated at 35°C for 24±2 hr. Plates then were packaged and transferred to the Texas A&M Veterinary Medical

Diagnostic Laboratory. Isolated colonies were picked and suspended in demineralized water to an optical density (OD) ~ 0.5 according to manufacturer's instructions and diluted 1,000-fold into 10 ml of Mueller-Hinton Broth (MHB) (Thermo Fisher Scientific Inc. Waltham, MA). Inoculated MHB was dispensed into Gram-negative National Antimicrobial Resistance Monitoring System (NARMS) Plates and incubated at 35°C for 24±2 h. Growth on the highest concentration of each antibiotic tested was observed to determine resistance.

Bacteriophage Enrichment

Triplicate subsamples were pooled to produce one composite sample per pen per sample type; a total of 36 pooled subsamples were prepared. For solid samples, the pooling process was performed by combining ~8 g of each subsample into a 50 ml Falcon tube, adding sterilized TSB to a final volume of 45 ml and vortexing to homogenize the mixture. Following homogenization, samples were centrifuged at 10,000 x *g* for 10 min at 4°C; supernatants then were transferred to new tubes and sterilized by adding 0.1 ml chloroform. For water samples, 15 ml of each subsample was taken and mixed in 50 ml Falcon tubes, followed by centrifuging as above and filter-sterilization (0.2 μm).

Sterilized samples were kept at 4°C for future phage detection and isolation. Two rounds of bacteriophage enrichment were conducted by using two sets of 5 *Salmonella* strains as phage host microbes (Gill et al., 2003). First round enrichments used five known strains: *S.* Typhimurium LT2 (ATCC 19585), *S.* Typhimurium USDA1,

S. Newport USDA2, *S.* Montevideo USDA3 and *S.* Anatum USDA4 (obtained from the collection of Dr. Tom Edrington, USDA-ARS, College Station, TX). Second round enrichment used one known *S.* Newport strain (obtained from the collection of Dr. Sara Lawhon, Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX) and four *Salmonella* strains isolated from feedlot samples as described above including *S.* Anatum, *S.* Montevideo, *S* Kentucky and *S.* Muenchen. The selection of feedlot isolates represented the diversity of serotypes among different locations and sample types. Bacteriophage enrichment was conducted by combining 10 ml of each sterilized sample supernatant, 100 µl fresh overnight culture of each of the five *Salmonella* strains and 40 ml sterile TSB in a 250 ml flask. Flasks were placed at 37°C with aeration and incubated overnight. Following incubation, 5 ml of each enrichment was taken and placed into a 15 ml Falcon tube and centrifuged at 8,000 x *g* for 10 min to pellet the remaining bacteria. Supernatant then was filter-sterilized through a 0.2 µm filter and stored at 4°C for future use.

Bacteriophage Detection

Bacteriophage plating was conducted on TSA bottom plates with a 0.5% top agar overlay supplemented with 5 mM each of $MgSO_4$ and $CaCl_2$ (Lech and Brent, 2001). For detecting bacteriophage prevalence, 10 µl of each bacteriophage enrichment was spotted on the surface of 0.5% top agar inoculated with 100 µl culture of each *Salmonella* culture prepared to a OD_{550} ~ 0.5 determined by McFarland standard. Plates

was then incubated at 37°C overnight. Observing a turbid or clear zone on the lawn was considered positive for the presence of bacteriophage.

Bacteriophage Isolation

All sample types (feed, water, feces, soil) in each pen of each feedlot were pooled to produce 9 composite samples for isolating individual bacteriophages. Pooled bacteriophage enrichments, containing 100 µl enrichment of each sample type by pen, were added to 4 ml sterilized TSB inoculated with 20 µl culture of each ten *Salmonella* strains. The mixture was incubated at 37°C overnight and then centrifuged and filter-sterilized as described above. Each enrichment was serially diluted and separately plated to soft agar lawns inoculated with hosts that were used for the enrichment, followed by incubating overnight at 37ºC. Individual plaques shows different morphology in each pen was picked and sub-cultured three times. Plaques picked from the third sub-culture of each bacteriophage were used to produce high-titer phage stocks by the confluent plate lysate method (Lysenko et al., 1974).

Bacteriophage Restriction Enzyme Digestion

Since one bacteriophage could have multiple hosts, restriction enzyme digestion was performed to distinguish possible duplicates during bacteriophage isolation. After harvesting 20ml high-titer lysates (>1x10^8 PFU/ml) of all isolated bacteriophages, phage DNA was prepared by using a modified Wizard® DNA Clean-Up System (Promega, Madison, WI) (Summer, 2009). To degrade bacterial host nucleic acid, 1µl DNase I

(New England Biolabs, Inc. Ipswich, MA) and RNase A (New England Biolabs, Inc. Ipswich, MA) were added to 20 ml of each phage lysate. Phage lysates were incubated at 37ºC for 30 minutes and 10 ml of polyethylene glycol (PEG)-based precipitant was added to each phage lysate. Phage lysate was mixed by inversion and stored at 4ºC overnight for phage precipitation. Phage particles were pelleted by centrifugation at 8,000 x *g* for 10 min. Phage pellets were re-suspended in 1.5 ml microcentrifuge tubes with 50µl SM buffer (100 mM NaCl, 8 mM MgSO4 • 7H2O, 50 mM Tris, pH 7.5) and centrifuged at ~14,000 x g for 10 s to remove insoluble materials. One ml of Wizard DNA Clean Up Resin was added to each phage solution and mixed by inversion. Each mixture was transferred to a 3 ml syringe with the column provided in the kit attached. The syringe plunger was used to push the mixture through the column. Each column was washed with 2 ml 80% isopropanol and centrifuges . Each column was dried by centrifugation at ~14,000 x g, 1 min and DNA eluted by addition of 100µl water heated to 80 ºC followed by centrifugation at ~14,000 x g for 1 min. Phage DNA was stored at 4ºC. Two rounds of restriction digestion were conducted by the same procedure with two restriction enzymes: EcoRI-HF® (New England BioLabs® Inc.) and DraI (New England BioLabs® Inc.). Following DNA preparation, 0.5 µg of bacteriophage DNA was added to ten unit of restriction enzyme, 2.0 µl of 10X reaction buffer and sterile Milli-Q water to a volume of 20 µl, followed by incubation at 37°C overnight. Following overnight digestion, DNA was mixed with 6X gel loading dye into a 1% agarose gel, followed by electrophoresis at 90 V for 2 hours in Tris/Borate/EDTA (TBE) buffer. The DNA was visualized with Gel Red stain (Biotium, Inc. Hayward, CA) and images were captures in

a Gel Doc™ XR+ System (Bio-Rad Laboratories, Inc. Beckley, CA). Bacteriophages with digested DNA that exhibited identical restriction patterns were grouped and considered to be the same bacteriophage strain.

Bacteriophage Phage Host Range Testing

The ten *Salmonella* strains that were used in bacteriophage enrichment and detection were used in the host range testing. In addition to the original host strains, another ten *Salmonella* strains, including *S.* Dublin, *S*. Anatum, *S*. Reading (obtained from the collection of Dr. Sara Lawhon (Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX), *S*. Cerro, *S*. Bergen (isolates of cattle feedlot environment from previous study (Haneklaus, et al), *S*. Enteritidis, *S*. Typhimurium (obtained from the collection of Dr. Matthew Taylor, Texas A&M University, College Station, TX), *S*. Enteritidis, *S*. Heidelberg (obtained from SGSC, University of Calgary, Calgary, Alberta, CA) and *S*. Anatum isolated from feedlot samples. The Routine Test Dilution (RTD), defined as the first tenfold serial dilution to produce countable plaques when 10 µl were spotted to a lawn of the phage's normal host strain, was determined for all unique phages. RTD and 100 x RTD of each phage were tested againsted to the 20 selected *Salmonella* strains to determine the host range by spotting on the surface of 0.5% top agar inoculated with 100 µl culture of each *Salmonella* culture prepared to a OD_{550} ~ 0.5 determined by McFarland standard. Plates was then incubated at 37°C overnight. A scoring system was developed to determine the capacity of each phage to infect selected *Salmonella* strains using its own host as a reference:

Score 4: On selected strain, a phage at RTD forms > 50% of the number of plaques formed on its host strain.

Score 3: On selected strain, a phage at RTD forms 5% to 50% of the number of plaques formed on its host strain

Score 2: On selected strain, a phage at 100 x RTD forms a confluent zone of lysis, but no plaques evident at RTD.

Score 1: On selected strain, a phage at 100 x RTD forms countable plaques.

Score 0: No plaques observed at 100 x RTD or RTD.

Minimum Inhibitory Concentration (MIC) Testing

In the MIC test, each of 18 unique phages was tested against their own host strain in Falcon® 96-Well Culture Plate via a modified MIC Broth Microdilution Method (CLSI, 2012).

Each of 18 unique phages was serially diluted and 100 µl of each dilution was mixed with 100 µl culture of each *Salmonella* culture host prepared to a OD_{550} ~ 0.5 determined by McFarland standard. Molten 0.5% top agar was inoculated with each mixture and plated onto TSA plates. The titer of each phage was determined via plate count and phage lysates were standardized to $10^3 – 10^9$ PFU/ml via dilution. Fresh overnight cultures of each host strain were sub-cultured to prepared to an OD_{550} ~ 0.5 and diluted 1000 fold. One hundred and eighty µl of each sub-culture was then combined with 20µl of each standardized phage dilution in Falcon® 96 well culture plates, aiming to test capacity of phage to clear culture from $10^2 – 10^8$ PFU/ml. Plates

were incubated at 37°C overnight. A direct observation of the lowest concentration of each phage that inhibited the visible growth of its own host was performed. Absorbance reading was also performed on all Falcon® 96 well culture plates in a Tecan Infinite® M200 PRO plate reader.

Bacteriophage Transmission Electron Microscopy Imagining

Phage isolates were prepared for TEM imaging by negative stain using a modification of the Valentine method. Fifty µl of phage lysate and 2% uranyl acetate were pipetted onto Parafilm. A small piece of carbon-coated mica was inserted into the phage lysate at a shallow angle for one minute and placed onto a drop of 2% uranyl acetate for staining. An isopropanol-cleaned copper grid was used to pick up the carbon film and excess stain was wicked off with a small piece of filter paper. Grids were then placed onto a grid mat in a Petri dish to dry. The dish containing grids was wrapped in foil and placed in a desiccator.

Grids were sent to Microscopy and Imaging Center at Texas A&M University to obtain image via JEOL 1200EX TEM at 100 keV.

CHAPTER III

RESULTS AND DISCUSSION

Salmonella Prevalence and Serotypes in Beef Cattle Feedlot

Detailed *Salmonella* prevalence information is showed in Table 1. The majority of *Salmonella* isolates were recovered from feedlot 1000, where *Salmonella* isolates were recovered from all sample types (drinking water, soils, feces, feed), though feces and soils samples yielded the greatest variety of *Salmonella* serovars. Previous research showed that 42.9% of lymph nodes from harvested cattle from this yard tested carried *Salmonella* (Haneklaus et al., 2012). No *Salmonella*-positive samples were recovered from feedlot 3000 (Table 1). This corresponds to feedlot A from Haneklaus et al. in which cattle from this feedlot was repeatedly negative for *Salmonella* in lymph node samples from cattle sent for slaughter. Beef cattle from feedlot 2000 in previous research was reported to be sporadically positive throughout researchers' visits, with 8.0% of *Salmonella* positive lymph nodes from cattle harvested from this yard at a first collection and 0% positive nodes from cattle from this yard taken from a subsequent visit. In our study, approximately 5.6% of feedlot 2000 samples were positive for *Salmonella*, all from soil samples. The apparently low prevalence of *Salmonella* in feedlot 2000, given the absence of *Salmonella*-positive feces samples, may point to a potential complex ecological function that inhibits *Salmonella* proliferation in the feedlot environment.

Table 1. Sampling, recovery, comparative identification and serotyping of *Salmonella* from south Texas beef cattle feedlots.

Type	Feedlot[a]	Atlas® System ID[b]	Vitek® 2 ID[c]	*Salmonella* Serotype ID[d]
Feed	1000	+	1001A1[e], 1001A2, 1001A4	Anatum
Feed	1000	+	1002A9	Anatum
Feed	1000			
Soil	1000	+	1004A2, 1004A3,	Anatum
Soil	1000	+	1005A1, 1005A3, 1005A4	Anatum (1005A1,1005A4); Montevideo (1005A3)
Soil	1000			
Feces	1000			
Feces	1000			
Feces	1000	+	1009A1, 1009A4, 1009A5	Anatum (1009A1,1009A4); Montevideo (1009A5)
Water	1000			
Water	1000			
Water	1000	+	1012A2,	Anatum
Feed	1000			
Feed	1000			
Feed	1000			
Soil	1000	+	1016B1	Anatum
Soil	1000	+	1017B2	Anatum
Soil	1000	+	1018B3	Altona
Feces	1000			
Feces	1000			
Feces	1000			
Water	1000			
Water	1000	+		
Water	1000			
Feed	1000			
Feed	1000			
Feed	1000			
Soil	1000	+	1028C1, 1028C3	Altona (1028C1); Anatum (1028C3)

Table 1. Continued

Type	Feedlot[a]	Atlas® System ID[b]	Vitek® 2 ID[c]	Salmonella Serotype ID[d]
Soil	1000	+	1029C1, 1029C2, 1029C3	Kralingen (1029C1); Anatum (1029C2, 1029C3)
Soil	1000	+	1030C1, 1030C2, 1030C3	Anatum (1030C1); Muenchen (1030C2, 1030C3)
Feces	1000			
Feces	1000			
Feces	1000	+	1033C2, 1033C3, 1033C5	Montevideo (1033C2); Kentuckey (1033C3); Anatum (1033C5)
Water	1000	+	1034C1, 1034C6	Anatum
Water	1000			
Water	1000	+		
Feed	2000			
Feed	2000			
Feed	2000			
Soil	2000			
Soil	2000			
Soil	2000			
Feces	2000			
Feces	2000			
Feces	2000			
Water	2000			
Water	2000			
Water	2000			
Feed	2000			
Feed	2000			
Feed	2000			
Soil	2000			
Soil	2000			
Soil	2000			
Feces	2000			

Table 1. Continued

Type	Feedlot[a]	Atlas® System ID[b]	Vitek® 2 ID[c]	Salmonella Serotype ID[d]
Feces	2000			
Feces	2000			
Water	2000			
Water	2000			
Water	2000			
Feed	2000			
Feed	2000			
Feed	2000			
Soil	2000	+	2028C1, 2028C2, 2028C3	Muechen (2028C1, 2028C2); Anatum (2028C3)
Soil	2000		2029C1, 2029C2, 2029C3	Anatum (2029C1, 2029C3); Montevideo (2029C2)
Soil	2000	+	2030C1, 2030C2, 2030C3	Anatum
Feces	2000			
Feces	2000			
Feces	2000			
Water	2000			
Water	2000			
Water	2000			

[a]Sample collection procedures and sample types were completed in identical fashion for feedlots 1000, 2000, and 3000. No *Salmonella*-containing samples were collected from feedlot 3000.

[b]*Salmonella* isolates recovered from feedlot samples were identified by molecular identification by Atlas® System (Roka Bioscience, Inc., San Diego, CA). Samples were prepared and subjected to analysis according to manufacturer's instructions at the Texas A&M University Center for Food Safety.

[c]Vitek® 2 (bioMérieux N.A., Durham, NC) analysis and biochemical identification was completed via manufacturer's instructions at the Texas A&M University Center for Food Safety.

[d]*Salmonella* submitted for serotyping (n=31) were typed by the Luminex xMAP *Salmonella* Serotyping Assay (SSA) according to manufacturer instructions at the Ryan Veterinary Hospital, University of Pennsylvania, Philadelphia, PA. Samples not differentiating serotypes with specific samples contained the same serotype for all submitted samples. *Salmonella* submitted for serotyping (n=7) were typed by the Ames Iowa USDA/APHIS lab via independent agglutination assays and Kauffmann-White Scheme destination.

[e]Sample ID codes indicate feedlot and sample number within feedlot (feedlot 1000 plus sample 1 = 1001), pen identifier (A, B, C) and *Salmonella* plate isolated colony (1, 2, 3, et al.): 1001A1.

Of a set of 31 isolates submitted for serotyping from *Salmonella*-positive samples, 26 were typed as *S.* Anatum, 4 were *S.* Montevideo, 4 were *S.* Muenchen, 2 were *S.*

Altona, and there one isolate each of *S.* Kentucky and Kralingen (Table 1). Previous research has reported that *S.* Anatum and *S.* Montevideo are commonly recovered from beef cattle in feedlots (Dodd et al., 2011; Gragg et al., 2013a). Gragg et al. (2013b; 2013) reported that *S.* Anatum was recovered from both the mesenteric as well as subiliac nodes, and from the hide of cattle at slaughter. Given previous reports describing the isolation and characterization of *S.* Anatum from beef and dairy cattle hide or fecal samples, results from our study are not surprising, with *S.* Anatum being the most frequently detected serotype among *Salmonella*-positive samples.

The presence of *Salmonella* in feed and water samples suggested a potential cross-contamination of these organisms via human practice (feed bunk filling) or other routes of transmission (e.g., transmission by winged insects or vermin bearing the pathogen on outer bodily surfaces) (Lanzas et al., 2011). Results obtained from pens A and C within feedlot 1000, where serovars of *Salmonella* were detected not only in soil and feces samples, but also in feed and water (pen A) or water only (pen C), would suggest some form of water and feed cross-contamination is taking place. Cross-contamination may put animals in these pens at elevated risk for acquiring and carrying/shedding these serovars versus other pens on feedlot 1000, though the risk for subsequent carriage in lymph nodes cannot be assessed.

Antibiotic Resistance of Feedlot Salmonella Isolates

Antimicrobial resistance was variable but generally low amongst *Salmonella* isolates in this study; 78.9% of isolates were resistant to streptomycin (8.0 μg/ml), while eight isolates bore intermediate resistance to the drug at 4.0 μg/ml. Seven point nine percent (7.9%) of *Salmonella* isolates were resistant to >256 μg/ml sulfisoxazole; one isolate (2.6%) bore intermediate resistance at 8.0 μg/ml gentamicin (Table 1). All recovered isolates demonstrated susceptibility to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, nalidixic acid, tetracycline and trimethoprim/sulfamethoxazole. Among 29 *Salmonella* strains from feedlot 1000, 21 (72.4%) isolates were resistant to 8.0 μg/ml streptomycin, while the remaining eight isolates bore intermediate resistance to the drug at 4.0 μg/ml. All *Salmonella* isolates from feedlot 2000 were resistant to 8.0 μg/ml streptomycin. Three *Salmonella* isolates from feedlot 1000 were resistant to >256 μg/ml sulfisoxazole while sulfisoxazole resistance was found in *Salmonella* isolates from feedlot 2000.

Gragg et al. (2013b) reported that 59% *Salmonella* isolates from lymph nodes, feces and hides of cattle were susceptible to all antibiotics while tetracycline resistance was most frequently observed in 22% of *Salmonella* strains. The difference in antibiotic resistance observed between other studies and our study may point to diverse antibiotic treatment used on beef cattle from feedlot to feedlot. Although the drug resistance data in this study is not dissimilar from Gragg et al. (2013b), *S.* Anatum strains previously isolated were resistant to six antimicrobial classes while no such multiple class drug resistance was found in this study. The NARMS report indicated that multiple drug

resistance was commonly found in serovars Typhimurium, Enteritidis and Newport (CDC, 2014b). Since these serovars were not identified in this study, it is not necessarily surprising that multi-drug resistance was not observed (CDC, 2014b). In our study, *Salmonella* strains resistant to nalidixic acid and ceftriaxone were not observed. Thus, the risk to public health from antibiotic resistance in these strains is relatively mild at the present time.

Bacteriophage Prevalence in Beef Cattle Feedlots

Detailed *Salmonella* phage prevalence information upon first enrichment and second enrichment are showed in Table 2 and Table 3, respectively.

Table 2. Detection of *Salmonella*-infecting phage recovered from beef cattle feedlots and host ranges of isolated phage following enrichment using laboratory *Salmonella* serovars by feedlot, pen and sample type.

Sample				Host *Salmonella* Serovar[a,b]				
Feedlot	Pen	Type	*Salmonella* confirmed	Typhimurium LT2	Typhimurium USDA1	Newport USDA2	Montevideo USDA3	Anatum USDA4
1000	A	Feces	+	+	+	+	-	+
	B	Feces		+	+	+	+	+
	C	Feces	+	+	+	+	+	-
2000	A	Feces		+	+	+	+	+
	B	Feces		+	+	+	+	-
	C	Feces		+	+	+	+	+
3000	A	Feces		+	+	+	+	-
	B	Feces		+	+	+	+	-
	C	Feces		+	+	+	+	-
1000	A	Feed	+	+	-	+	-	-

Table 2. Continued

Sample				Host *Salmonella* Serovar[a,b]				
Feedlot	Pen	Type	*Salmonella* confirmed	Typhimurium LT2	Typhimurium USDA1	Newport USDA2	Montevideo USDA3	Anatum USDA4
		B	Feed		+	-	+	-	-
2000		A	Feed		+	-	+	-	-
		B	Feed		+	-	-	-	-
		C	Feed		+	-	-	-	-
3000		A	Feed		+	-	+	-	-
		B	Feed		+	-	+	-	-
		C	Feed		+	-	-	-	-
1000		A	Soil	+	+	+	+	+	+
		B	Soil	+	+	+	+	+	+
		C	Soil	+	+	+	+	-	+
2000		A	Soil		+	+	+	+	+
		B	Soil		+	+	+	+	-
		C	Soil	+	+	+	+	+	+
3000		A	Soil		+	+	+	+	+
		B	Soil		+	+	+	+	-
		C	Soil		+	+	+	+	-
1000		A	Water	+	+	+	+	-	-
		B	Water		+	+	+	-	-
		C	Water	+	+	+	+	-	-
2000		A	Water		+	+	+	-	-
		B	Water		+	+	+	-	-
		C	Water		+	+	+	-	-
3000		A	Water		+	+	+	-	-
		B	Water		+	+	+	-	-
		C	Water		+	+	+	-	-
Total positive					**36**	**27**	**34**	**16**	**10**

[a] *Salmonella* host serovars were provided by the Food Microbiology Laboratory, Department of Animal Science, Texas A&M University (*S.* Typhimurium LT2), or by Tom Edrington, USDA-ARS, College Station, TX (*S.* Typhimurium USDA1, *S.* Newport USDA2, *S.* Montevideo USDA3, *S.* Anatum USDA4).

[b] Phage enrichments were conducted by adding 10 ml sample supernatant to 40 ml sterile TSB in 250 ml flask, and inoculation of each flask with 25 µl of overnight culture of each of the five enrichment *Salmonella* strains. Enrichments were incubated

overnight at 37°C with aeration, 5 ml of culture was removed, centrifuged at 8,000 x *g*, 10 min, to pellet the remaining bacteria, and the phage-containing supernatant filter-sterilized through a 0.2 µm syringe filter (EMD Millipore, Billerica, MA). Sample enrichments were stored in the dark at 4°C until further use.

Table 3. Detection of *Salmonella*-infecting phage recovered from beef cattle feedlots and host ranges of isolated phage following enrichment using feedlot-recovered *Salmonella* isolates by feedlot, pen and sample type.

Sample				Host *Salmonella* Serovar[a,b]				
Feedlot	Pen	Type	*Salmonella* confirmed	Muechen 2028C1	Newport 10-014	Montevideo 2029C2	Anatum 1001A1	Kentucky 1033C3
1000	A	Feces	+	+	-	+	-	+
	B	Feces		+	-	+	-	+
	C	Feces	+	-	-	-	-	-
2000	A	Feces		-	-	-	-	-
	B	Feces		-	-	-	-	-
	C	Feces		+	+	+	-	-
3000	A	Feces		-	-	-	-	-
	B	Feces		-	-	-	-	-
	C	Feces		-	-	-	-	-
1000	A	Feed	+	-	-	-	-	-
	B	Feed		-	-	-	-	-
	C	Feed		-	-	-	-	-
2000	A	Feed		-	-	-	-	-
	B	Feed		-	-	-	-	-
	C	Feed		-	-	-	-	-
3000	A	Feed		-	-	-	-	-
	B	Feed		-	-	-	-	-
	C	Feed		-	-	-	-	-
1000	A	Soil	+	+	+	+	-	+
	B	Soil	+	-	-	-	-	-
	C	Soil	+	-	-	-	-	-
2000	A	Soil		+	+	+	-	-
	B	Soil		-	+	+	-	-
	C	Soil	+	-	-	-	-	-

Table 3. Continued

Sample				Host *Salmonella* Serovar[a,b]				
Feedlot	Pen	Type	*Salmonella* confirmed	Muechen 2028C1	Newport 10-014	Montevideo 2029C2	Anatum 1001A1	Kentucky 1033C3
3000	A	Soil		-	-	-	-	-
	B	Soil		-	-	-	-	-
	C	Soil		-	-	-	-	-
1000	A	Water	+	-	-	-	-	-
	B	Water		-	-	-	+	-
	C	Water	+	-	-	-	-	-
2000	A	Water		-	-	-	+	-
	B	Water		-	-	-	+	-
	C	Water		-	-	-	+	-
3000	A	Water		-	-	-	-	-
	B	Water		-	-	-	+	-
	C	Water		-	-	-	+	-
Total phage positive				**5**	**4**	**6**	**6**	**3**

[a]*Salmonella* host serovars were obtained by selective enrichment and plating as described elsewhere (Andrews et al., 1998), with *Salmonella* confirmation by Atlas® System (Roka Bioscience, Inc., San Diego, CA) and serotyping by the xMAP *Salmonella* Serotyping Assay (SSA) (Luminex Corp., Austin, TX) according to manufacturers' instructions.

[b]Phage enrichments were conducted by adding 10 ml sample supernatant to 40 ml sterile TSB in 250 ml flask, and inoculation of each flask with 25 µl of overnight culture of each of the five enrichment *Salmonella* strains. Enrichments were incubated overnight at 37°C with aeration, 5 ml of culture was removed, centrifuged at 8,000 x *g*, 10 min, to pellet the remaining bacteria, and the phage-containing supernatant filter-sterilized through a 0.2 µm syringe filter (EMD Millipore, Billerica, MA). Sample enrichments were stored in the dark at 4°C until further use.

All 36 samples contained phage active against at least one of the *Salmonella* hosts tested (Table 2), though only 9 of 36 samples contained material that yielded confirmed culturable *Salmonella* bacteria (Table 2). Due to the logistical issues of enriching, isolating and confirming *Salmonella* strains from the feedlot environmental samples, detection of phage from these samples was conducted in two rounds: the first

using a panel of *Salmonella* isolates obtained from unrelated sources, and the second using *Salmonella* isolates obtained directly from the surveyed feedlots. In the first round of enrichment, all 36 samples contained phage active against the well-characterized laboratory strain *S.* Typhimurium LT2, and 34 of 36 (94%) contained phage active against the MDR *S.* Newport USDA2. Phages against *S.* Typhimurium USDA were found in 27 of 36 (75%) feedlot samples. *S.* Montevideo USDA3 and *S.* Anatum USDA4 were least sensitive to phage in the feedlot samples (16 and 10 samples, respectively). All feces, soil and water samples contained phages active against *S.* Typhimurium USDA1, but none were detected in feed samples. Phages against *S.* Montevideo and *S.* Anatum were found in soil and fecal samples, but none were found in feed or water (Table 2). Over 90% of soil and feces samples in all feedlots were phage-positive to at least one *Salmonella* strain tested, where less phage prevalence against *Salmonella* panel tested was found in water and feed samples (35% and 60% respectively). Phage-positive samples were distributed evenly over the three samples feedlot sites, suggesting that phage prevalence is not strongly correlated with culturable *Salmonella.*

In the second round of phage detection using *Salmonella* isolates obtained from feedlot samples, phages were detected far less frequently, with only 12 of 36 samples yielding detectable phage upon enrichment (Table 3). Phage against *S.* Montevideo 2029C2 and *S.* Anatum 1001A1 were found most frequently (six samples each), followed by phage against *S.* Muenchen 2028C1 (five samples), *S.* Newport 10-014 (four samples) and *S.* Kentucky 1033C3 (three samples). Similar to the first round of phage detection, most of the positive samples were either feces or soil. The reason fewer

phages were detected in second enrichment round is uncertain. It is possible that some degradation of the phage occurred between the two enrichment rounds, which were conducted some four weeks apart due to the time required to process, culture and serotype the *Salmonella* isolates used. Another possibility is that the strains used in the second round were simply not sensitive to the phages in the samples, which would suggest that strain diversity is high in the feedlot environment or that phage host range is narrow.

Phage prevalence in these feedlot samples appeared to correlate with both strain and sample type. Phages against *S.* Anatum and *S*. Montevideo were not detected in feed samples in both detection rounds. Phage detection in water showed similarity to the detection in feed in the first round detection, where no phage against *S.* Anatum and *S*. Montevideo were observed. However, phage against a different *S.* Anatum isolate, 1001A1, were found in six of nine water samples in second round of detection. Feces and soil yielded the most positive samples across all strains; this is possibly not surprising because low-frequency fecal shedding of *Salmonella* is well-documented in bovines (Beach et al., 2002) and feedlot soil is the common deposition point for fecal material.

Phage Isolation and Molecular Characterization

In phage isolation, three sub-cultures were performed on each isolate to ensure the clonality of each phage. Twenty-nine phages survived through the sub-culturing process. Information of plaque morphology and host strain is shown in Table 4.

Table 4. Phages with source, host strain and plaque morphology information and grouping according to restriction digestion pattern.

Phage ID	Feedlot	Pen	Host	Plaque Morphology[a]	Group[b]
1	1000	A	*S*. Newport (Vet)	Small turbid plaques, hard to see	1
2	1000	A	*S*. Muenchen (feedlot)	Big clear plaques, smooth edge	2
3	1000	A	*S*. Kentucy (feedlot)	Turbid plaques with halo	2 sub1
4	1000	A	*S*. Montevideo (Feedlot)	Big clear plaques with halo	2 sub1
5	1000	A	*S*. Montevideo (Feedlot)	Big clear plaques with halo	2
6	1000	B	*S*. Typhimurium LT2	Small turbid plaques, smooth edge	3
7	1000	B	*S*. Muenchen (Feedlot)	Big clear plaques, smooth edge	2
8	1000	B	*S*. Anatum (feedlot)	Small turbid plaques, hard to see	18
9	1000	B	*S*. Kentucy (feedlot)	Turbid plaques with halo	2 sub2
10	1000	B	*S*. Montevideo (feedlot)	Clear plaques, smooth edge	2
11	1000	C	*S*. Montevideo (feedlot)	Turbid plaques, smooth edge	5
12	1000	C	*S*. Newport (USDA)	Small clear plaques, fuzzy edge	6
13	2000	A	*S*. Typhimurium LT2	Small turbid plaques, fuzzy edge	7
14	2000	A	*S*. Typhimurium LT2	Large turbid plaques with halo	7
15	2000	A	*S*. Montevideo (USDA)	Clear plaques, fuzzy edge	
16	2000	A	*S*. Newport (USDA)	Small turbid plaques, smooth edge	5
17	2000	A	*S*. Muenchen (feedlot)	Clear plaques, fuzzy edge	5
18	2000	B	*S*. Montevideo (USDA)	Small turbid plaques, fuzzy edge	8
19	2000	B	*S*. Newport (USDA)	Small turbid plaques, fuzzy edge	7
20	2000	B	*S*. Muenchen (feedlot)	Small turbid plaques, fuzzy edge	5
21	2000	B	*S*. Anatum (feedlot)	Small turbid plaques, fuzzy edge	5 sub
22	2000	B	*S*. Kentucy (feedlot)	Small turbid plaques, hard to see	9
23	2000	B	*S*. Newport (feedlot)	Small turbid plaques, smooth edge	7
24	2000	C	*S*. Anatum(USDA)	Small turbid plaques, fuzzy edge	10
25	2000	C	*S*. Muenchen (feedlot)	Turbid plaques, fuzzy edge	11
26	3000	A	*S*. Anatum (feedlot)	Small turbid plaques, hard to see	9 sub

Table 4. Continued

Phage ID	Feedlot	Pen	Host	Plaque Morphology[a]	Group[b]
27	3000	B	*S*. Kentucy (feedlot)	Clear plaques, smooth edge	12
28	3000	B	*S*. Newport (Vet)	Turbid plaques, smooth edge	13
29	3000	C	*S*. Newport (Vet)	Turbid plaques, smooth edge	13

[a] Phage isolation was conducted by picking plaques with different plaque morphology within each pooled samples each host strain. A phage with broad host range might be picked multiple time in thie step. Therefore, a restriction digestion was perform to identify duplicates of each phage..

[b] Phage grouping was performed by comparing the pattern restriction digestion and determine the similarity of a group of patterns. Restriction digest pattern image is shown in Figure 9.

Some phages could potentially infect several strains within a species, resulting in being picked multiple times during phage isolation. Thus, a restriction enzyme digestion was performed to remove duplicates isolates in the 29 phage collection. Images of restriction enzyme DraI digestion patterns of 29 phages are shown in Figure 9. Phage 2, 5, 7 and 10 were sorted as a group for showing identical patterns; Phage 3 and 4 were considered to be a sub-group because the identical patterns of Phage 3 showed similarity to Phage 2, 5, 7 and 10. Phage 11, 16, 17, and 20 were sorted as group with Phage 21 being a sub-group. Phage 13, 14, 19, 23 were sorted as a group. Phage 22 and Phage 26 are sub-groups to each other. Phage 28 and 29 were sorted as a group. The remaining phages produced unique patterns. Duplicates found within phage type suggest that some phages are capable of attacking more than one *Salmonella* strain used in phage isolation. Representatives of each phage group and sub-group were chosen to perform further characterization.

Host Range of Unique Phages

Host range testing was carried out to identify the capacity of each phage to infect various *Salmonella* strains. Scores of phages capable infecting each *Salmonella* strain tested in three replicate experiments were averaged and recorded in Table 5. Host ranges of the phages were highly variable, with the broadest host range phage infecting 16 of the 20 *Salmonella* strains tested and narrowest host range phage infecting 4 *Salmonella* strains. Phage 15, with broadest host range, was able to form plaques on beef cattle feedlot-related *Salmonella* strains *S.* Anatum, *S*. Muenchen and *S.* Montevideo as well as human disease-related serotypes including *S*. Heidelberg, *S.* Dublin, *S*. Typhimurium, *S*. Kentucky, *S*. Enteritidis and *S*. Newport on its RTD or 100×RTD. No single phage was able to infect all 20 *Salmonella* strains that were used in this host range experiment.

Figure 9. Restriction Digestion Patterns of 29 Phages. Phages with same restriction digestion patterns were grouped as phage type; phages with similar patterns were group as sub-types.

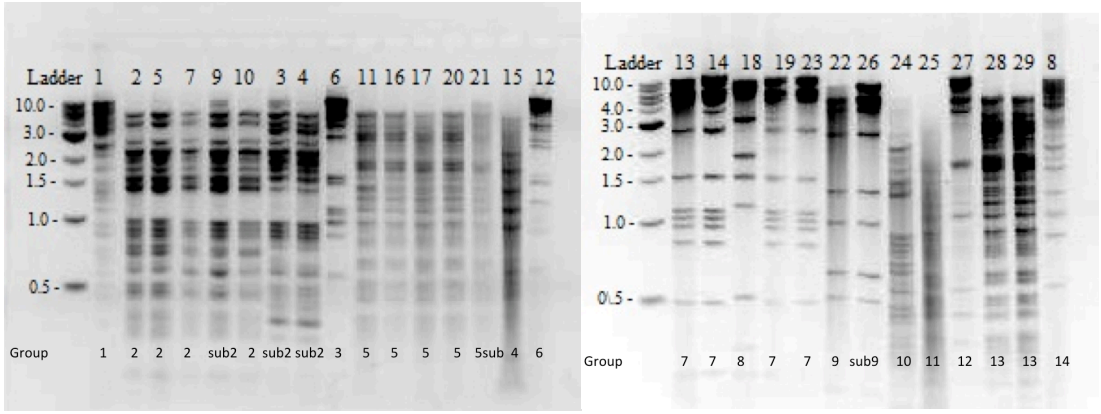


Table 5. Host Range Results of 18 Unique Phages

Phage ID	Anatum	Anatum	Anatum	Anatum	Bergen	Cerro	Dublin	Enteritidis	Enteritidis	Heidelberg	Kentucky	Montevideo	Montevideo	Muenchen	Newport	Newport	Reading	Typhimurium	Typhimurium	LT2
1	1.3[a]	1.3	0	0	0	1.3	0	2.7	1.3	0	0	0	4	0	4	4	1.33	4	4	4
3	4	0	0	4	0	0	0	0	0	0	4	0	4	4	0	0	0	0.7	0	0
5	2.7	0	0	4	0	0	0	0	0	0	0	0	4	0	0	0	0	0.7	0	0
6	0	0	0	0	0	0	0	2.3	0	0	4	0	0	4	2.3	3.7	0	1	0	4
8	1.7	0	4	0	0	0	0.7	0	0	0	1	0	0.7	0.7	0	0	0	0.3	0	0.7
9	2.3	0	0	3	0	0	0	0	0	0	4	0	3.7	3.3	0	0	0	0.7	0	0
12	0	0	0	0	0	0	1	1.3	0	0	3	0	0	3.3	4	3.7	0	1.3	0	3.3
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	3	0	4
15	3.3	2.7	0	3.3	0	0	4	4	2.3	1.7	3	4	4	3	0.7	3.3	2.7	3	0	1
17	4	0.7	0	4	0	0	0	4	1.4	0.3	2.7	3.7	4	4	1.3	4	0.7	0.3	0	1.3
18	1.7	2	1.3	1.3	0	0	4	4	2.7	4	0	4	1.3	0	3	1.3	1.3	4	0	0.7
21	1	0	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0.3
22	4	0.7	0.3	4	0	0	0	4	0	0.3	4	4	4	4	0	4	0.7	1.7	0	2.3
24	4	0	0	4	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0.3
25	4	1	0	3	0	0.7	0	4	0	0	4	4	4	4	0	4	0	0.3	0	1.3
26	0	0	4	0	0	0.7	1	0.3	0.7	0.3	0	0	0	0	1	0	0	0	0	0.3
27	3.7	0.7	0	4	0	1.3	0	4	0	0.3	4	3.7	3.7	4	2.7	4	0	0.3	1.3	4
28	3	0.7	0	3	0	0.7	0	3.7	0	0	2.3	2.7	2.7	2.7	2	4	0.7	0.3	0.7	3

[a] Phage host range scores were given according to standard mentioned in Chapter II. Score showed in this table is average of three replication experiments.

Score 4: On selected strain, a phage at RTD forms > 50% of the number of plaques formed on its host strain.
Score 3: On selected strain, a phage at RTD forms 5% to 50% of the number of plaques formed on its host strain
Score 2: On selected strain, a phage at 100 x RTD forms a confluent zone of lysis, but no plaques evident at RTD.
Score 1: On selected strain, a phage at 100 x RTD forms countable plaques.
Score 0: No plaques observed at 100 x RTD or RTD.

Phage sensitivity of *Salmonella* strains tested was found highly diverse as well. *S.* Typhimurium LT2 and *S.* Typhimurium USDA were sensitive to 15 out of 18 phages

tested, while *S*. Bergen showed no sensitivity to any phage in this collection. Although the host range study was not designed for phage typing, patterns of sensitivity to a panel of phages observed in this study can vary within a species (Hickman-Brenner et al., 1991). Two *S*. Newport strains used in the host range study have similar sensitivity to the panel of 18 phages while *S*. Typhimurium USDA and *S*. Typhimurium LT2 yielded different result of phage sensitivity compared to *S*. Typhimurium obtained from Dr. Taylor's collection. The two *S*. Montevideo strains and four *S*. Anatum were also observed to be different on a phage type standpoint.

In our host range result, Phage 15 has the broadest host range, infecting 80% of *Salmonella* strains tested. Phage 17, isolated from same feedlot and pen as Phage 15, has similar host range result as Phage 15 in despite of having completely different restriction patterns as Phage 15. Phage 5, which has narrowest host range infecting only 4 of 20 *Salmonella* strains tested, has similar host range result as Phage 3 that isolated from the same site and has similar restriction pattern as Phage 5. Phage 15 and Phage 17 are generally preferred to be used in a phage therapy or phage based antimicrobial intervention as they are able to cover more diverse *Salmonella* strains. However, annotation of the phage genomic sequence has to be conducted to remove out temperate phages in our collection before utilizing them as antimicrobial intervention.

Switt et al. (2013) conducted phage isolation in dairy farms in the U.S. that has different level of *Salmonella* prevalence and performed host range testing on recovered phages. A year later, Wongsuntornpoj et al. (2014) conducted phage isolations in Thailand utilizing the same hosts used in the Switt et al. (2013) study and found that

phages isolated in Thailand have broader host ranges compared to phages isolated by Switt et al., suggesting that in different locations, phages could have different host range performance even when isolated utilizing the same hosts. This phenomenon was also observed in our study. Isolated utilizing same set of *Salmonella* strains, phages found in Feedlot 2000 had a broader host range compared to phages found in other feedlots.

Minimum Inhibitory Concentration (MIC) of Phages

Inhibition of bacterial growth was only found in five out of 18 phages in this study. At concentrations of 10^7 PFU/ml, Phages 9, 12, 24, and 17 were able inhibit growth of *S.* Kentucky, *S.* Newport USDA, *S.* Anatum USDA, and *S.* Muenchen respectively. Phages 12 and 5 were observed to inhibit growth of *S.* Kentucky and *S.* Typhimurium respectively at concentration of 10^6 PFU/ml. Based on culture turbidity, Phages 18, 22, 25 and 27 reduced the OD of their host culture from ~1 to ~0.5 at concentration as low as 10^1 pfu/ml. Given the bacterial mutation rate being of the order of 1 in 100,000 (Yadav, 2003), it is possible that at the initial inoculum level used for the experiment (~100,000 cfu/ml) used for MIC test, the *Salmonella* phage-resistant mutants arose early enough in the experiment that they could successfully overgrow the culture. Given the host receptor likely differs from phage to phage (Shin et al., 2012), a cocktail of several phages may be used to slow the development of phage resistance.

Phage Morphology Identification via TEM Imaging

TEM imaging of eight unique phages (Phages 6, 12, 13, 15, 17, 22, 24, 27) was completed and shown in Figure 10. In eight phages where TEM images are available,

three phages belong to *Myoviridae* and five phages belong to *Siphoviridae*. Phage morphology and dimension information were determined and recorded in Table 6. Similar to our findings, a previous study that conducted isolation and characterization of *Salmonella* targeting phage also identified two different morphologies, *Myoviridae* and *Siphoviridae* in their isolated phages collection. However, they identified that *Siphoviridae* they isolated in their collection have broader host range while *Myoviridae* found to have broader host range among all isolated phages in our study (Akhtar et al., 2014).

Figure 10. Minimum Inhibitory Concentration (MIC) of Phages. Culture with an OD less than 0.1 was considered clear via direct observation. In this figure, a lighter color in the column indicated that the phage was able to reduce the OD to a lower level.

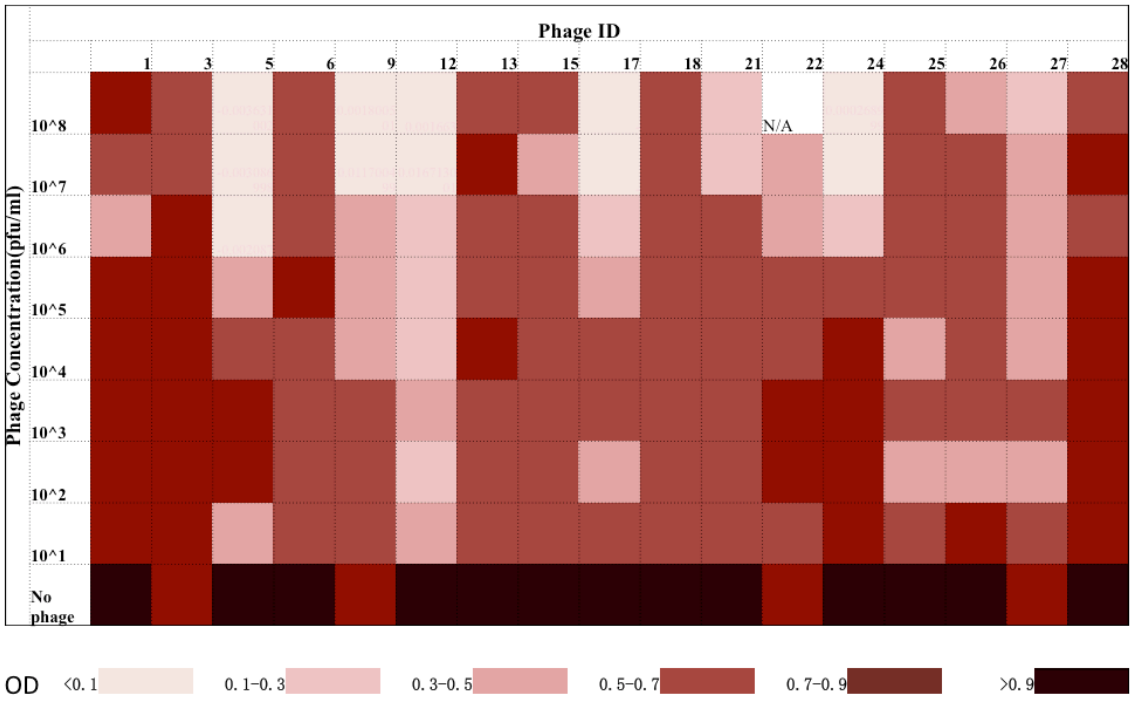
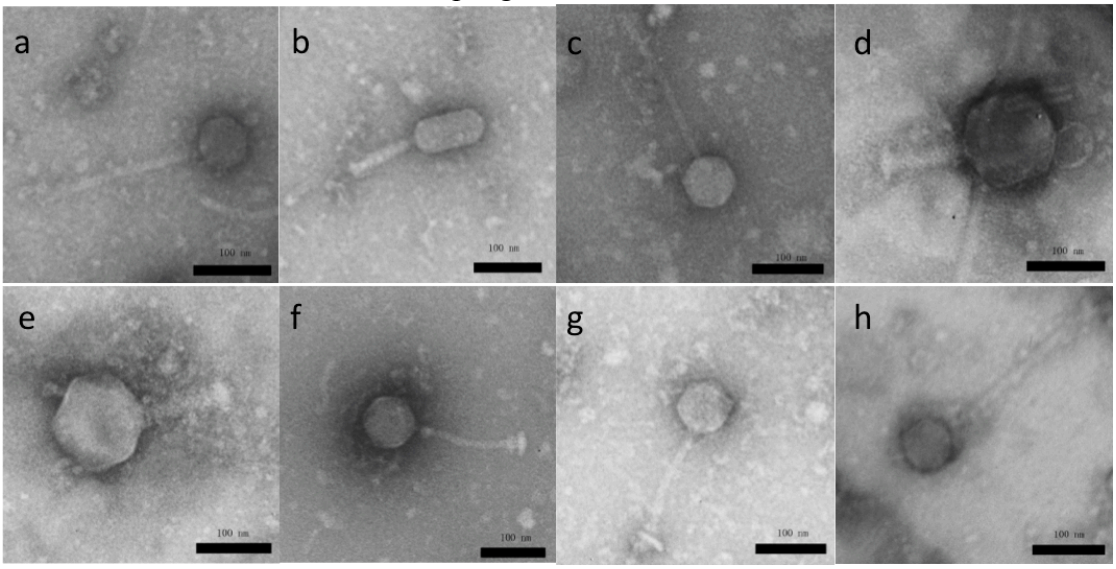


Table 6. Morphology Observation and Dimensions of Eight Phages Taken from TEM Images

Phage ID	Phage Morphology	Capsid Diameter	Capsid Length	Tail Length	TEM Image
6	Sipho	65nm	65nm	207nm	Figure 11a
12	Myo	45nm	103nm	90nm	Figure 11b
13	Sipho	65nm	65nm	212nm	Figure 11c
15	Myo	125nm	125nm	102nm	Figure 11d
17	Myo	120nm	120nm	125nm	Figure 11e
22	Sipho	63nm	63nm	125nm	Figure 11f
24	Sipho	65nm	65nm	165nm	Figure 11g
27	Sipho	70nm	70nm	182nm	Figure 11h

Figure 11. Phage TEM Image. Image a, b, c, d, e, f, g and correspond to Phage 6, 12, 13, 15, 17, 22, 24 and 7. Additional phage dimension information is available in Table 5.



CHAPTER IV

CONCLUSION

While the overall recovery and identification of *Salmonella* serovars was relatively low across the three sampled feedlots, an elevated rate of *Salmonella*-positive samples (feed, water, feces, soils) was identified from feedlot 1000. No *Salmonella*-positive samples were recovered from feedlot 3000 and only a small number from feedlot 2000. Prevalence of salmonellae found in feces and soil sample was higher compared to feed and water samples. Although approximately 79% *Salmonella* isolates were resistant to streptomycin, significant multi-drug resistance of *Salmonella* strains from beef cattle feedlot environment was not observed in this study.

The majority of *Salmonella* serotypes identified in this study were serovars Anatum and Montevideo; these serovars were frequently recorded by other researchers on beef and dairy cattle feedlot environments (Brichta-Harhay et al., 2011; Gragg et al., 2013b). Conversely, the presence of phages from amongst all sample types and across all feedlots indicates a potential host/pathogen ecology is at work to limit the numbers of *Salmonella* in the beef feedlot environment. Research has shown that phage can reduce the population of live *Salmonella* in standard enrichment cultures, which could lead to false-negative culture results when testing *Salmonella*-containing environmental samples heavily contaminated by phage. This phenomenon would be expected to affect both the traditional culture-based and molecular approaches used in this study, as both rely on an

initial non-selective pre-enrichment step that could allow endogenous *Salmonella* phage to infect and lyse their hosts (Muniesa et al., 2005).

The broadest phage host range infecting 80% *Salmonella* strains tested indicates potential phage therapy candidates found in beef cattle feedlot environment. However, genomic DNA sequencing and annotation is required to more fully understand the biology of these phages and make inferences on their lifestyle (temperate or virulent). Phages enable the clearing of *Salmonella* cultures at low concentration, or reduce the OD of *Salmonella* cultures to less than 0.1, suggesting that phage resistance might occur readily among *Salmonella* strains tested in this study. Using multiple phages against one *Salmonella* strain might reduce the development of phage resistance and increase their antibacterial activity in culture.

Efforts in this study are currently focused on further characterization of phage genomic DNA analysis and mechanisms of host infection and further elaboration of the antimicrobial resistance of *Salmonella* isolates, in efforts to determine the potential for MDR pathogen control via phages. Applying phages on beef cattle environment will help determine the stability of efficacy of phage working in realistic scenario. Further, we are working to determine the levels of genetic relatedness of *Salmonella* from within feedlots to assist the determination of the potential transmission of serovars throughout a feedlot through fecal shedding.

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