PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* SPP. IN SHEEP FECAL AND HIDE SAMPLES BEFORE AND AFTER TRANSPORTATION

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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* SPP. IN SHEEP FECAL AND HIDE SAMPLES BEFORE AND AFTER TRANSPORTATION

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ABSTRACT

Two hundred feedlot hair sheep (100/trial) were surveyed to determine the incidence of *Salmonella* spp. in fecal and hide samples before transportation and the incidence in hide samples after transportation. Microbroth dilution plates were used to establish antibiotic resistant profiles on the samples. Twenty-seven percent of all samples (n=600) tested positive for *Salmonella* using several selective and enrichment media. Of those, 40% were classified as serogroup B and 48% were from serogroup C. Other serogroup values were 10% or less of the total. Thirteen percent of 158 isolates were susceptible to all antimicrobial drugs tested, 26% were resistant to only one antimicrobial, and 38% were resistant to eight or more. The most common resistance was to Sulfisoxazole (77% of isolates). Sulfisoxazole MIC levels were the only ones impacted by an interaction between trial and sample type (P=0.02).Ten antimicrobials saw an effect due to trial ($P \leq 0.05$) but not type ($P > 0.05$).

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INTRODUCTION

Foodborne diseases caused by microorganisms are the number one food safety concern among consumers and regulatory agencies (Garvani, 1987). Illnesses attributed to foodborne microorganisms often cause severe symptoms affecting the digestive tract as well as other bodily functions, and in some cases, these illnesses may result in death if untreated. *Salmonella* is a common foodborne pathogen that often affects humans who consume contaminated meat and other food products. The Centers for Disease Control and Prevention (CDC) found that *Salmonella* was the most common infection (1.2 million U.S. illnesses annually) and the most common cause of hospitalization and death of all foodborne illnesses (2010). Meat and other common food products have been under constant scrutiny over the years after several *Salmonella* outbreaks occurred. Contamination of these products can often be traced back to the slaughtering and processing of these meats (Hjartardóttir, Gunnarsson, and Sigvaldadóttir, 2002), but prevention mechanisms can come into play before the animal arrives at the slaughtering plant. Several studies have reported on the prevalence of *Salmonella* and its many serotypes in cattle, pigs, and poultry, but little research has been performed on the prevalence in sheep. As the consumption of lamb products rise and the possible risk of *Salmonella* infections in the United States increases, it is important to research and understand the prevalence of this pathogen in sheep before and after shipment. The objective of this study was to determine the prevalence of *Salmonella* spp. and serogroups within sheep feces and examine the effect of animal transport on distribution of the bacteria on the hide of the animal. A secondary objective is to establish baseline

antibiotic resistant profiles on *Salmonella* spp. isolates obtained from the feces and hide samples.

LITERATURE REVIEW

Prevalence of Salmonella

Out of all of the foodborne infections that affect Americans each year, *Salmonella* was the most common cause of hospitalization and death in 2010 (CDC, 2010). With 1.2 million U.S. illnesses a year and the numbers steadily increasing from 2006, control and prevention of this pathogen is certainly a priority. The genus *Salmonella* comprises approximately 2,600 serotypes with infection by *Salmonella* typhimurium *and Salmonella* enteritidis as the most common causes of foodborne illnesses in America (Gilberts and Roberts, 1990). *Salmonella* is a gram-negative, rod-shaped bacterium that lives in the intestinal tracts of infected animals and humans (Jay, Loessner, and Golden, 2005). They are microscopic pathogens that pass from the feces of people or animals to other people or other animals (USDA, 2012). When contaminated food and water are ingested by humans or animals, the bacteria are once again passed through the fecal-oral route perpetuating the cycle. The international spread of *Salmonella* and its illness is often facilitated through the importation and exportation of contaminated goods (Jay, Loessner, and Golden, 2005). Symptoms for salmonellosis include diarrhea, abdominal cramps, and fever within 8 to 72 hours after the contaminated food was eaten. Additional symptoms may include chills, headache, nausea, and vomiting. These symptoms usually last 4-7 days and many infected individuals recover without seeking medical attention. The problem arises when infections affect infants and young children, the elderly, and people with compromised immune systems. If this occurs, the infection could become life threatening (USDA, 2012). Treatment for these individuals includes rehydration and possible intravenous fluids. Some antibiotics

can be administered if the infection spreads. As for livestock, infection by *Salmonella* can be either asymptomatic (showing no signs of infection or illness) or pathogenic. Within pathogenic infections, symptoms of Salmonellosis can range in severity from diarrhea and muscle weakness to more critical or acute symptoms such as convulsions or abortion seen in livestock (WHO, 1988). Antibiotics that are commonly used include ampicillin, trimethoprim-sulfamethoxazole, and ciprofloxacin.

Salmonella bacteria are heterogenous group found in the family *Enterobacteriaceae* that are divided into two species (*S. enterica* and *S. bongori*) and seven subspecies between the two. Within these subspecies, the bacteria are further categorized into serogroups or serotypes based on somatic or lipopolysaccharide (O) or flagellar (H) antigens (Iwen, 2013). Most of the *Salmonella* serotypes belong to *S. enterica*, with serogroups A, B (*S.* Typhimurium and *S.* Heidelberg), C1 (*S.* Braenderup), C2 (*S.* Newport), D (*S.* Enteritidis and *S.* Gallinarum), and E (*S.* Anatum) strains being the most common, contributing to 99% of reported Salmonellosis infections (Popoff and Le Minor, 1997). Serogroups in other subspecies of *S. enterica,* as well as the majority of serogroups in *S. bongori* are not commonly found in humans (Popoff and Le Minor, 1997). To identify *Salmonella* serogroups, scientists use agglutination kits based on the Kauffman-White method containing antisera and control antigens to distinguish groups, A, B, C1, C2, D, E, and the virulence (Vi) antigen that is used to select specifically for group D (Iwen, 2013). Identifying the serogroup becomes important in that it aids clinicians in providing proper health care to those infected. Serotypying also allows for scientists, health officials, and the public understand Salmonellosis trends and how or where an outbreak or contamination occurs.

Meat/Lamb Consumption

Meat is an important protein source in the human diet. As a whole, the food group helps provide a complete and balanced diet for many Americans. Meat consumption in the United States has increased significantly over the decades. In the U.S. and other developed countries, meat composes a significant portion of the diet, contributing more than 15% to daily energy intake, 40% to daily protein intake, and 20% to daily fat intake (USDA, 2005). The demand for meat continues to grow as the production and consumption of meat increases with available income (Speedy, 2003). Along with rising incomes, increased demand for meat fuels an increased production and availability, as well as lowered prices and costs of production.

Relative to the beef, pork, and poultry sectors, the U.S. lamb industry is quite small. The demand for lamb has declined in previous years with the U.S. per capita lamb consumption decreasing from 1.6 pounds in 1990 to 0.88 pounds in 2011 (Brester, 2012). Particularly starting in the 1950s, lamb consumption took a downward spiral that lasted for several decades. During that time, lamb comprised less than 1% of overall red meat consumption since 1975 (USDA/ERS, 2011). Prior to the 1950s, lamb meat was consumed due to it being a byproduct of the wool industry. As more wool was needed for military uniforms during both World Wars, more lamb meat was able to be consumed. As older lambs became unable to produce wool efficiently, they were harvested. Because of this, the meat tasted differently as do most other meat products as the animal gets older. Mutton is termed

as meat from older animals, typically older than twelve months, whereas lamb is classified as meat coming from animals harvested younger than twelve months of age. Consequently, the increased mutton consumption during the wars decreased when the wars were over. Servicemen who had to constantly eat canned mutton had no desire to eat it when they returned from the war and passed on that distaste to family members, and ultimately, later generations (Apple, 2006). Although some are still apprehensive to consuming sheep meat products (lamb or mutton) again, the quality and safety of the meat has improved tremendously.

Recently the amount of lamb meat consumed has begun to increase due to the correlating increase in ethnic communities. Ethnic populations are the basis for substantial consumption on the east and west coasts of the United States (Jones, 2004). Those of Greek, Middle Eastern, Hispanic, African, and Native American descents account for the majority of lamb consumers with lamb consumption at its highest during spring and fall holidays. Their concern for the products they consume, including food safety, has also developed. As these populations increase, lamb consumption also increases.

The agriculture industry, especially processing or harvesting plants, are highly regulated by government agencies in developed countries as a means of intervention of contamination from farm to table (CDC, 1997; Mead et al., 2009). These interventions allocate the reduction of contamination from bacteria at different steps of processing in order to follow specific critical control points set through HACCP plans regulated by the Food Safety Inspection Service (FSIS) and United States Department of Agriculture (USDA). Such steps include temperature, pressure, and time combinations, irradiation, vacuum packaging

depleting the oxygen supply, rinses containing specific chemicals at different levels, etc. (Romans et al., 1994). In 2010, Foodnet reported a decrease in foodborne illnesses attributing it to cleaner slaughter methods, better inspection and microbial testing, increased knowledge and awareness, and improvements in regulatory agencies (CDC, 2011). Surveillance efforts have been put in place through government agencies, but reports from these surveillances come with some complications. First of which, many people who become infected never seek medical attention. They may never see symptoms, or they have a milder case in which they are able to get over within several hours. Another complication is that it becomes difficult to follow the dissemination route. Transmission can not only be through food contamination, but also water contamination and person to person. Lastly, problems arise when illness occurs from pathogens not yet identified (Mead et al., 2009). These limitations have an effect on the accurate number of *Salmonellosis* illnesses each year (Mead et al., 2009; CDC, 2011).

Antimicrobial Resistance

Antimicrobial resistant organisms are able to combat drugs rendering them ineffective, and subsequent illnesses from these organisms are prolonged and may spread to others (WHO, 2012). Resistance can be naturally found in an organism or developed by the use and/or misuse of antimicrobial drugs. Salmonella's resistance to antimicrobial medicines is becoming increasingly common in the United States and can often be traced back to food producing animals. This creates a zoonotic issue that passes through the human food chain at a rapid rate (Threlfall, 2002). In 1992, D'Aoust et al., found the extensive use of antimicrobial drugs in food producing animals in the forms of subtheraputic, prophylactic,

and feed additives for growth promotion in Canada has increased the organism's prevalence and its ability to build up resistance to these drugs. This increase can ultimately compromise the human health system with the association of consuming contaminated meat (D'Aoust et al., 1992). Recently, Edrington et al. found in 2009 that most *Salmonella* isolates from sheep fecal and hide samples were susceptible to specific antimicrobial drugs used in their experiment. The bacteria showed the highest resistance to ceftiofur, enrofloxacin, and trimethoprim-sulfamethoxazol. As the experiment continued, the researchers found an increase in the number of antibiotics that *Salmonella* isolates were resistant to. For example, fecal isolates ranged in resistance from three to eight antibiotics, while wool samples varied in resistance from one to nine antibiotics. The study also observed the prevalence of *Salmonella* in fecal, wool, and carcass samples. Results included low levels of about 7% in fecal samples, and higher amounts around 50%, in wool samples (Edrington et al., 2009).

Examining antibiotic susceptibility of *Salmonella* isolates allows for future research in understanding increased resistance of pathogens as well as presenting the ability for clinicians to evaluate factors that should be taken into consideration when choosing an antibiotic for use. Antimicrobials are classified based on their bacteriostatic/bactericidal activity, spectrum level, mechanism of action, or according to their species. Bacteriostatic and bactericidal differ in that bacteriostatic only inhibit the growth of the target organism, while bactericidal agents actually kill the susceptible microorganism (Pankey and Sabath, 2004). Common bacteriostatic drugs include tetracycline, erythromycin, and chloramphenicol. Bactericidal drugs include β-lactamases (Penicillin), aminoglycosides (Gentamicin, Streptomycin), and Quinolones. Classification of an antimicrobial's spectrum is

separated into two categories as well. Narrow Spectrum antimicrobials act against a specific group, such as gram negative or gram positive bacteria. Broad Spectrum antimicrobials act against all groups, gram negative and gram positive, and other microorganisms giving it a broader range of microorganisms to attack. There are three main categories that antimicrobials fall under based on their mode of action: inhibition of bacterial cell wall synthesis, inhibition of protein synthesis, or inhibition of nucleic acid synthesis (Walsh, 2000). Inhibition of the bacterial cell wall works by interfering with the synthesis of peptidoglycan, which is an integral part of the bacterial cell wall and thus, the bacteria's survival (Tomasz, 1979). Certain antimicrobials prevent the formation of the peptidoglycan complex of the cell wall, resulting in cell rupture and death (Reynolds, 1989). Inhibition of protein synthesis works by binding to ribosomal subunits preventing proper formation of growing peptide chains as well as inserting incorrect amino acids into these peptide chains. The third group of antimicrobial drugs affects either the synthesis of nucleic acids or their required precursors such as folic acid, nalidixic acid, and specific enzymes required for the formation of the bacteria's DNA. Other antimicrobials in this group prevent replication and transcription of the bacteria's DNA resulting in death (Drlica and Zhao, 1997). These modes of action are very extensive and specific, but bacteria are finding ways to combat these drugs and survive thus leading to an increase in resistance resulting in prolonged illnesses and death.

MATERIALS AND METHODS

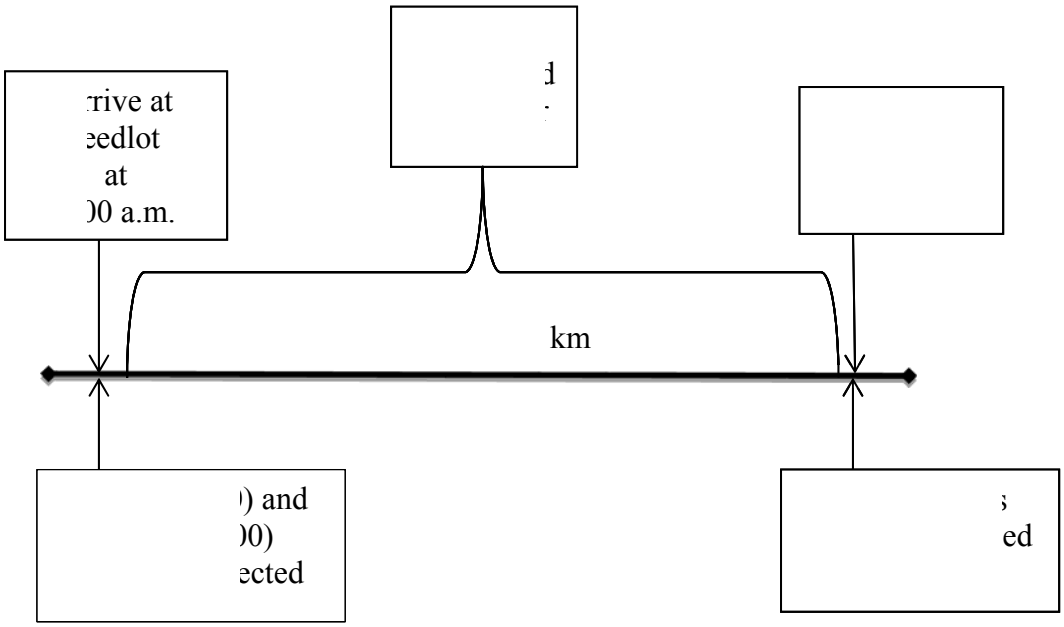
Sample Collection

Over the course of 4 weeks during late winter and early spring, 200 feedlot hair sheep (*Ovis aries*) were studied for the prevalence of *Salmonella* spp. Both fecal and hide samples were taken from flocks of sheep destined for harvest at a packing plant in north Texas on two separate occasions. At the feedlot located outside of Sterling City, TX, fecal samples (approximately 10-20 g) were collected aseptically from the rectum or anus by rectal palpation. New latex exam gloves were utilized to collect each sample to ensure sample integrity. Jointly, hide swab samples were taken from a 300 cm^2 area on the right side of the animal's abdomen, dorsal to the belly midline using prehydrated with Buffered Peptone Water Speci-sponges in individual Whirl-pak bags (Whirl-Pak. Nasco, Modesto, CA). Animals were then loaded into a traditional double-deck, gooseneck stock trailer and transported approximately 418 kilometers to a commercial harvest facility. Additional hide swab samples were collected from each animal upon arrival at the harvest facility. This post-transport sample was taken on the animal's left side, directly dorsal to the belly midline. A timeline of the collection is shown in Figure 1. For each set of samples, flock identity and individual-animal number were recorded, and the samples were processed using various enrichments and selective media for accurate bacterial identification. All samples were kept at refrigerated temperatures (4°C) and transported to the Angelo State University Food Microbiology Laboratory for processing within 36 hours of collection.

Identification

One gram of feces was suspended into 9 ml of Tetrathionate (TT) broth and incubated at 37˚C for 24 hr. Additionally, one gram of feces was suspended into 9 ml of Rappaport-Vassiliadis (RV) broth and incubated at 37˚C for 24 hr. Selective plating was performed by streaking the two selective enrichment broths onto Xylose-Lysine-Tergitol 4 (XLT4) agar, half

Figure 1. Timeline of Collection



of the plate from TT and half from RV, and then incubated at 37° C for 48 hr. Plates were observed for typical *Salmonella* spp. colonies presenting as red to yellow with black centers.

Hide samples were pre-enriched in a total of 20ml of Buffered Peptone Water (BPW) and incubated at 37° C for 24 hrs. to allow for bacterial repair. One ml aliquots of the pre-enrichment was suspended in 9 ml of TT broth and 9 ml RV broth and incubated at 37° C for 24 hr. Selective plating was performed by streaking the two selective enrichment broths onto XLT4 Agar, half of the plate from TT and the other half from RV and incubated at 37° C for 48 hr. Plates were observed for typical *Salmonella* spp. colonies presenting as red to yellow with black centers.

All presumptive positive samples were confirmed using a Salmonella Latex Agglutination Test Kit (Remel Europe Ltd., Dartford, Kent, UK). Agglutination consisted of using two different reagents given in the kit and mixing each reagent with a drop of isolate/water mixture and swirling to stimulate agglutination or clumping around the edges. Positives were confirmed by the clumping, as well as a color change of the background mixture. Two isolates from each positive sample were frozen for later antimicrobial testing. For cryopreservation, Brain Heart Infusion (BHI) broth was inoculated with a well isolated colony was and incubated at 37° C for 24 hr. Three microtubes of culture (from BHI) per colony were frozen in 0.5 ml microtubes (100µl of sample and 100µl of sterile glycerol solution (80% glycerol and 20% water)) and placed in frozen storage at -80° C.

Antimicrobial Resistance Testing

One isolate from each positive culture was regrown in a sterile BHI broth and incubated for 24 h at 37°C. A 0.1 mL of the solution was streaked onto a Tryptic Soy Agar petri-plate and incubated at 37°C for 24h. Colonies were isolated and transferred to 5 mL sterile water and compared with a 0.5 McFarland standard turbidity. After analysis of turbidity of the tubes, 10 microliters of culture was transferred to a 10 mL Mueller-Hinton broth suspension. Fifteen commonly used antimicrobials were verified using a microbroth dilution 96-well gram-negative Sensititre® (Trek Diagnostic Systems, Cleveland, OH) plate for each isolate. The wells were inoculated with 50 microliters in each well, sealed, and incubated for 18-24 hours at 36°C.

The antibiotics used in the experiment include Ampicillin, Amoxicillin/Clavulanic Acid, Azithromycin, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Trimethoprim/Sulfamethoxazole, Cefoxitin, Gentamicin, Kanamycin, Nalidixic Acid, Sulfisoxazole, Streptomycin, Tetracycline, and Ceftiofur. Minimum inhibitory concentrations (MICs) were determined for each antimicrobial by recording the lowest concentration of the antimicrobial contained on the plate which inhibited the visible growth of the *Salmonella* isolate, and isolates were classified as resistant or susceptible using predetermined breakpoints from the National Antimicrobial Resistance Monitoring System (NARMS)(Table 1).

Statistical Analysis

Descriptive statistics were generated using various procedures of SAS (Cory, NC; Version 9.3.1), and significant differences between populations and minimum inhibitory concentrations were evaluated using a predetermined alpha of less than or equal to 0.05 using several functions of SAS including: PROC FREQ for the descriptive statistics, PROC MIXED for the MIC data, and PROC GLM for the prevalence data. Our experimental unit for this study was the individual animal.

Table 1. Minimum Inhibitory Concentrations (MICs) and NARMS[a] Breakpoints Used for Susceptibility Testing of *Salmonella*

Antimicrobial Class	Antimicrobial Agent	MIC Resistant Breakpoints* (µg/ml)	MIC Plate Ranges (µg/ml)
Aminoglycosides	Gentamicin	≥ 16	0.25-32
	Kanamycin	≥ 64	8-128
	Streptomycin	≥ 64	32-128
Aminopenicillins	Ampicillin	≥ 32	1-64
B-Lactam/β-Lactamase Inhibitor Combinations	Amoxicillin/Clavulanic Acid	≥ 31/16	1/0.5-64/32
Cephalosporins	Ceftiofur	≥ 8	0.12-16
	Ceftriaxone	≥ 64	0.25-128
Cephamycins	Cefoxitin	≥ 32	0.5-64
Folate Pathway Inhibitors	Sulfisoxazole	≥ 512	16-512
	Trimethoprim/ Sulfamethoxazole	≥ 4/76	0.12/2.38-8/152
Phenicols	Chloramphenicol	≥ 32	2-64
Quinolones	Ciprofloxacin	≥4	0.015-4
	Nalidixic Acid	≥32	0.5-32
Tetracyclines	Tetracycline	≥16	4-32
Macrolides	Azithromycin	≥ 8	0.12-16

[a]NARMS-National Antimicrobial Monitoring System

*MIC Breakpoint=Minimum Inhibitory Concentration Breakpoint, obtained from National Antimicrobial Monitoring System

RESULTS

Prevalence

A total of six hundred samples were tested for *Salmonella*, 100 samples per type (fecal at the feedlot, hide at the feedlot, and hide at the plant) for each of two trials. Five percent of fecal samples from trial one tested positive (Table 2), while no *Salmonella* spp. was isolated from fecal samples of trial two tested positive using the methods detectable limits (P=0.02). When comparing feedlot hide samples taken from the two trials, 10% of hide samples from trial one tested positive, while five percent of trial two tested positive for the bacteria (P=0.18). No difference in prevalence between the two trials was found in hide samples taken at the plant (P=0.75). Sixty-nine percent of trial one hides taken at the plant and 71% from trial two were positive for the bacteria. In total (n=160), 26% of all samples (n=600) were positive. In both trials, there was a significant increase in prevalence of *Salmonella* spp. on hides pre- to post-transport (P<0.001).

Table 2. Percentage (Frequency) of Samples Testing Positive for Salmonella spp. for Two Trials (n=200)

Trial	Sample Type		
	Fecal % (Fr.)	**Hide-Feedlot % (Fr.)**	**Hide-Plant % (Fr.)**
1 (n=100)	5.0 (5)	10.0 (10)	69.0 (69)
2 (n=100)	0.0 (0)	5.0 (5)	71.0 (71)
Total	2.5 (5)	7.5 (15)	70.0 (140)

Serogroup

One isolate from each positive sample was tested using a Latex Agglutination kit and isolates were classified into one of six serogroups. Sixty-four (40%) of the total 160 isolates tested were classified as serogroup B and 77 (48.13%) were from serogroup C (Table 3). An additional 16 isolates (10%) fell into serogroups E or G, and one isolate was identified as containing the Vi Antigen. Interestingly, the isolates from trial one were predominantly serogroup C (88.10%); while within trial two, serogroup B was predominant (76.32%) (Table 3). Within the five fecal isolates of Trial 1, three (60%) were found to be in serogroup E or G. Both types of hide samples from Trial 1 were predominately serogroup C. Both also contained a few isolates from serogroup B, however, there was one isolate obtained from a feedlot hide sample that identified with serogroup E or G (Table 4). In Trial 2, all feedlot hide samples were identified as E or G serogroup. Hide samples from the plant ranged within all serogroup types although the majority was from serogroup B (Table 5).

Table 3. Percentage (Frequency) of *Salmonella* spp. within various Serogroups for Two Trials (n=160)

Trial	Serogroup				
	B	**B+C**	**C**	**E/G**	**Vi Antigen**
1 (n=84)	7.14 (6)	0.00 (0)	88.10 (74)	4.76 (4)	0.00 (0)
2 (n=76)	76.32 (58)	2.63 (2)	3.95 (3)	15.79 (12)	1.32 (1)
Total (n=160)	40.00 (64)	1.25 (2)	48.13 (77)	10.00 (16)	0.63 (1)

Table 4. Percentage (Frequency) of *Salmonella* spp. within a sample type based on serogroup for Trial 1 (n=160)

Type	Serogroup					
	B	**B+C***	**C**	**E/G**	**Vi Antigen***	**Total**
Fecal	20.0 (1)	-	20.0 (1)	60.0 (3)	-	5.95 (5)
Hide-Feedlot	10.0 (1)	-	80.0 (8)	10.0 (1)	-	11.90 (10)
Hide-Plant	5.80 (4)	-	94.2 (65)	-	-	82.14 (69)
Total	7.14 (6)	-	88.10 (74)	4.76 (4)	-	100.00 (84)

*(-) indicates no positive samples for this type of serogroup

Table 5. Percentage (Frequency) of *Salmonella* spp. within a sample type based on serogroup for Trial 2 (n=160)

Type	Serogroup					
	B	**B+C**	**C**	**E/G**	**Vi Antigen**	**Total**
Fecal	-	-	-	-	-	-
Hide-Feedlot	-	-	-	100.00 (5)	-	6.58 (5)
Hide-Plant	81.69 (58)	2.82 (2)	4.23 (3)	9.86 (7)	1.41 (1)	93.42 (71)
Total	76.32 (58)	2.63 (2)	3.95 (3)	15.79 (12)	1.32 (1)	100.00 (76)

*(-) indicates no positive samples for this type of serogroup

Antimicrobial Resistance

One hundred and fifty-eight *Salmonella* isolates (two were unable to be regrown) were examined to determine their levels of resistance to 15 antimicrobials of importance to the human and veterinarian health. Of these, 13% (21 of 158) were susceptible to all antimicrobial drugs tested, 26% (42 of 158) were resistant to only one antimicrobial, and 38% (61of 158) were resistant to between eight and twelve antimicrobial drugs (Figure 2). The most common resistance was to Sulfisoxazole, Cefoxitin, Amoxicillin/Clavulanic acid, Ampicillin, and Ceftiofur with values of 77.2%, 52.5%, 50.63%, 50.63%, and 44.9% respectively (Figure 3). Patterns of resistance can be seen in Table 5 with nine different isolates resistant to nine different antimicrobials. The complete *Salmonella* spp. resistance profile can be seen in Table 7.

When analyzing Minimum Inhibitory Concentrations (MICs) of various antimicrobials of hide samples, there was consistently no effect from sample type (feedlot/plant) on the antimicrobial MICs with the exception of Sulfisoxazole (Table 8). Sulfisoxazole MIC levels were impacted by an interaction between trial and sample type (P=0.02). All others showed no dependency on trial or type (P>0.05). All antimicrobials had higher MICs for trial 2 except for Gentamicin and Sulfisoxazole (P≤0.05) (Table 8).

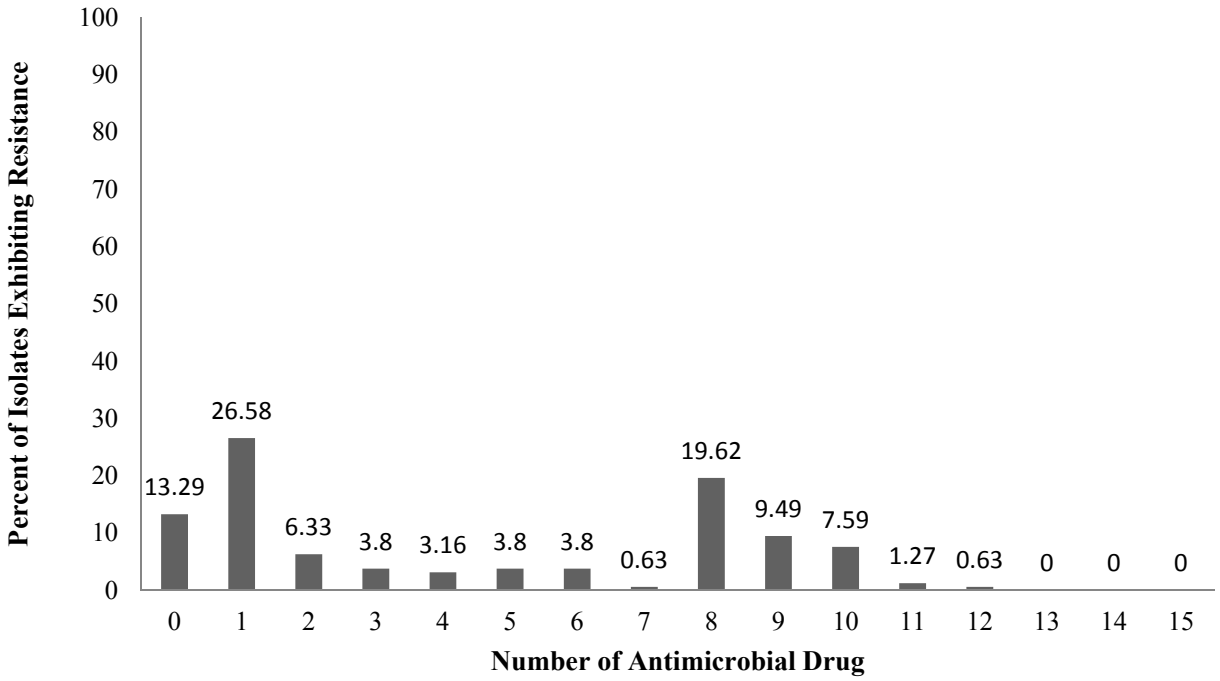


Figure 2. Percentage of total *Salmonella* spp. isolates based on number of antimicrobial drugs to which they showed resistance (n=158)

Table 6. Most frequent antimicrobial drug resistance patterns of *Salmonella* spp. isolates obtained from sheep samples (n=158)

% Isolates Resistant (Frequency)	# of Drugs Resistant	Antimicrobials to Which Isolates Were Resistant
24.68 (39)	1	Sulfisoxazole
19.62 (31)	7	Tetracycline, Streptomycin, Sulfisoxazole, Cefoxitin, Chloramphenicol, Amoxicillin/Clavulanic Acid, Ampicillin
13.92 (22)	0	Pansusceptible*
5.70 (9)	9	Tetracycline, Streptomycin, Sulfisoxazole, Cefoxitin, Chloramphenicol, Ceftriaxone, Amoxicillin/Clavulanic Acid, Ampicillin, Azithromycin
5.70 (9)	8	Tetracycline, Streptomycin, Sulfisoxazole, Cefoxitin, Chloramphenicol, Amoxicillin/Clavulanic Acid, Ampicillin, Azithromycin
3.80 (6)	5	Sulfisoxazole, Cefoxitin, Amoxicillin/Clavulanic Acid, Ampicillin, Azithromycin

*Pansusceptible=susceptible to all antimicrobial drugs tested

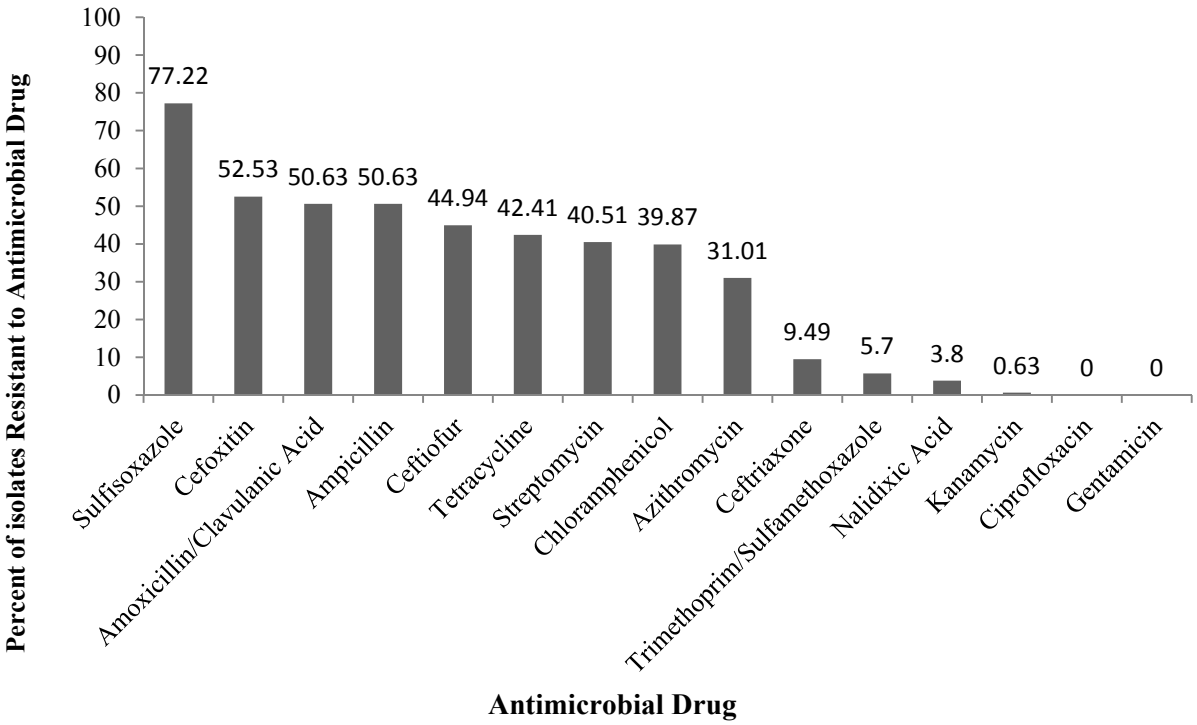


Figure 3. Percent of *Salmonella* spp. isolates sheep fecal and hide samples resistant to various antimicrobial drugs (n=158)

Table 7. Percentage of *Salmonella* spp. isolates (n=158) taken from sheep fecal and hide samples on the basis of minimum inhibitory concentration (MIC) ratio

	MIC ratio										
Antimicrobial	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Lowest concentration tested (µg/ml)**
Ampicillin	40.51	1.90	1.90	2.53	2.53	2.53	48.10	-	-	-	1.0
Amoxicillin/Clavulanic Acid	41.77	2.53	1.90	1.90	1.27	41.77	8.86	-	-	-	1/0.5
Azithromycin	0.63	0.00	0.00	0.63	14.56	53.16	13.92	5.70	11.39	-	0.12
Ceftriaxone	48.73	1.90	0.63	2.53	0.63	1.90	6.96	27.22	8.86	0.63	0.25
Chloramphenicol	1.27	27.85	27.22	3.80	0.63	39.24	-	-	-	-	2.0
Ciprofloxacin	57.59	19.62	8.86	10.76	0.63	0.00	1.90	0.63	0.00	-	0.015
Trimethoprim/Sulfamethoxazole	77.22	8.86	2.53	2.53	3.16	3.16	2.53	-	-	-	0.12/2.38
Cefoxitin	0.63	0.00	12.03	27.22	6.33	1.27	3.80	48.73	-	-	0.5
Gentamicin	3.80	58.86	31.01	4.43	1.90	0.00	0.00	-	-	-	0.25
Kanamycin	92.41	3.16	3.80	0.00	0.63	-	-	-	-	-	8.0
Nalidixic Acid	0.00	21.52	59.49	13.92	0.00	1.27	1.90	1.90	-	-	0.5
Sulfisoxazole	0.63	0.63	4.43	6.33	10.76	77.22	-	-	-	-	16.0
Streptomycin	59.49	1.90	38.61	-	-	-	-	-	-	-	32.0
Tetracycline	56.96	0.63	0.63	5.70	36.08	-	-	-	-	-	4.0
Ceftiofur	0.00	0.00	8.23	38.61	6.96	1.27	0.00	44.94	-	-	0.12

[1] Ratios were obtained by dividing each MIC by the lowest concentration on the specific antimicrobial tested. The ratio was then turned into a log base 2 for comparison.

- = Value greater than the highest number tested on the panel.

*Shaded values represent isolates considered resistant for individual drugs based on breakpoints established by the National Antimicrobial Resistance Monitoring System (NARMS)

Table 8. Minimum Inhibitory Concentrations for antimicrobial drugs used based on trial

	Trial		
Antimicrobial Drug	**Trial 1**	**Trial 2**	***P*-value**
Ampicillin	1.45[1]	4.81[2]	< .0001
Amoxicillin/Clavulanic Acid	1.18[1]	4.02[2]	< .0001
Azithromycin	2.06	2.36	0.40
Ceftriaxone	-1.03[1]	2.55[2]	< .0001
Chloramphenicol	2.75[1]	4.85[2]	< .0001
Ciprofloxacin	-5.42	-5.27	0.70
Trimethoprim/Sulfamethoxazole	-2.71	-2.67	0.93
Cefoxitin	2.35[1]	5.02[2]	< .0001
Gentamicin	-0.47	-0.68	0.34
Kanamycin	3.15	3.19	0.79
Nalidixic Acid	1.03	1.16	0.70
Sulfisoxazole[*]	8.61	8.24	
Streptomycin	5.13[1]	6.40[2]	< .0001
Tetracycline	2.50[1]	4.88[2]	< .0001
Ceftiofur	0.43[1]	2.59[2]	< .0001

[1,2]Values within antimicrobial with different superscript differ ($P \leq 0.05$)
[*]MIC values of antimicrobial Sulfisoxazole were dependent on a trial x sample type interaction ($P=0.24$)

DISCUSSION

Very little research has been conducted on the prevalence and antimicrobial susceptibility of *Salmonella* spp. from feedlot hair sheep in the United States. Studies on different species such as cattle and swine, as well as studies focusing on different gram-negative bacteria have been conducted and can be a useful tool in comparing related research experiments. The prevalence of *Salmonella* spp. was found to be lower in sheep fecal samples than those of cattle and even previous sheep studies (Corrier et al., 1990; Elder et al., 2000; Beach et al., 2002; Edrington et al., 2006; Edrington et al., 2009). All positive fecal samples were found in the first trial which indicates either contamination only at the hide level, or other *Salmonella* spp. isolates were below the detectable level of this projects analysis.

The prevalence of *Salmonella* on the hide at both the feedlot and the plant were much higher than fecal samples (P=0.05). Seventy percent of all positive isolates were found on the hide at the plant with 7.5% of the total positive isolates found on the hide at the feedlot (Table 2). Both trials had an increase in prevalence from feedlot to plant. This demonstrates the impact contamination during transportation can have on the spread of the bacteria Elder et al. (2000) found that hides were major contaminators of cattle carcasses for another similar gram-negative bacterium, *Escherichia coli* O157:H7 (*E. coli* O157:H7). Although carcass samples were not taken in this study, it is expected to play a similar role in sheep carcass contamination. A study by Edrington et al. in 2009 found only one of fifty-one carcasses to be positive for *E. coli* O157:H7 indicating effective intervention methods during slaughter.

Future research in this field is encouraged, in order to measure the potential human health risk caused by contamination of *Salmonella* spp. from hides onto carcasses.

Serogrouping the positive isolates presented interesting results within trials, as well as between sampling type (fecal, hide-feedlot, or hide-plant). Both trials exhibited a different predominant serogroup; Trial one being predominantly serogroup C (S. Newport and S. Heidelberg) (88%) and Trial two having predominantly serogroup B (*S. Typhimurium*) (76%) isolates (Table 3). This change in serogroup between the trials offers the idea that there was a change in *Salmonella* population make-up at the feedlot level. In 2006, a surveillance study conducted by Rodriguez et al. on the world-wide distribution of *Salmonella* during 2000-2002 found *S. Typhimurium* to be the most common isolate (29%) found in humans in North America. The study also found a large group of the isolates found in the United States belonging to *S. Newport* (15%) and *S. Heidelberg* (10%) as well as *S. Enteritidis* (21%) all of which were found in this study. There was also a change in population makeup between the sampling type during each trial suggesting that one or two shedders had a large impact on spreading contamination throughout the sample group (Table 4 and 5).

In regards to antimicrobial susceptibility, this study found differences in MIC levels dependent on trial for many of the antimicrobials tested. There were no isolates that were resistant to all of the drugs tested, but 1 out of the 158 isolates was resistant to 12 antimicrobial drugs used (Figure 2). This same isolate was found to be in Serogroup B+C. Around 13% of the isolates were susceptible to all of the drugs with all isolates being susceptible to Kanamycin, Ciprofloxacin, and Gentamicin (Figure 3). The most common resistance was to Sulfisoxazole with 77.2% of isolates exhibiting resistance (Figure 3).

Sulfisoxazole was interestingly the only antimicrobial that had an interaction between trial and sample type on the MICs, indicating a dependency on a combination of the two factors (Table 8). *S.* Typhimurium, has been found to be resistant to several antimicrobials including some used in this study: ampicillin, chloramphenicol, streptomycin, and tetracycline (Molla et al., 2006; Boyen et al., 2008; Perron et al., 2008). These findings are consistent with the results from the present study where a majority of Trial 2 was identified as serogroup B (*S.* Typhimurium) and had high levels of resistance patterns for those drugs listed above (Table 8).

This research indicates that feedlot hair sheep naturally harbor the foodborne pathogen *Salmonella* spp. in their feces and more importantly their hides which has the potential for cross contamination onto carcasses. It also suggests that cross contamination can come as a result from transporting livestock from feedlots to their final destination at the plant. Either *Salmonella* isolates fly under the radar below the level of detection or more likely the animals pick up new isolates during transport. It will be important as a next step in finding the prevalence of Salmonella isolates on sheep carcasses to guarantee pre- and post-harvest and transportation intervention methods when ensuring food safety to lamb consumers.

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