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Antimicrobial Drug Resistance and Molecular Characterization of *Salmonella* Isolated from Domestic Animals, Humans and Meat Products

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1. Introduction

Infections with nontyphoid *Salmonella enterica* serovars represent an important public health problem worldwide (Zhao et al. 2003) and an economic burden in many parts of the world today (Gomez et al 1997; Vugia et al, 2004). In the United States (US), *Salmonella* is the second most common identifiable cause of illness, and the leading cause of hospitalizations and deaths, due to food-borne bacterial infection (Mead et al, 1999). Each year, 31 major known pathogens acquired in the US caused an estimated 9.4 million episodes of foodborne illness (Scallan et al, 2011), and an estimated 38.4 million episodes of domestically acquired foodborne illness were caused by unspecified agents, resulting in 71,878 hospitalizations and 1,686 deaths (Scallan et al, 2011). The annual economic cost due to foodborne *Salmonella* infections in the US alone is estimated at $2.4 billion (http://www.ers.usda.gov) with an estimated 1.4 million cases of salmonellosis and over 500 deaths annually (Arshad et al. 2007). In 2004 for instance, among 3686 *Salmonella* isolates serotyped, 862 (23%) were serotype Typhimurium, 565 (15%) Enteritidis, 399 (11%) Newport and 248 (7%) Heidelberg (CDC, 2005). Similarly, the same *Salmonella enterica* serovars were reported as major causes of salmonellosis in humans in another study (Oloya et al. 2007). The predominance of *S. Typhimurium* and *S. Newport* in both domestic animals and human case reports further highlights their role in causing cross infections (Arshad et al. 2007; Bacon et al. 2002; Besser et al. 2000).

Although human salmonellosis has been associated with exposure to other vehicles of transmission (e.g. pets, reptiles, and contaminated water), about 95% of human infections have been found to be associated with ingestion of contaminated foods; namely animal products (Gaul et al. 2007; McLaughlin et al. 2006; Padungtod and Kaneene 2006), poultry products (Plym and Wierup 2006; Mead et al. 1999), sea foods (Duran and Marshall 2005; Ozogul et al. 2007; Shabarinath et al. 2007) and fresh produce (Johnston et al. 2006; Puohiniemi et al. 1997). Direct contact with companion and food animals has also been documented as another important route of *Salmonella* transmission to humans (Coburn et al. 2006; Doyle and Erickson 2006; Gorman and Adley 2004; Mead et al. 1999; Padungtod and Kaneene 2006). Consumption of raw or undercooked ground beef and lack of safe food handling practices to prevent cross contamination are considered critical in infections at household levels (Ling et al. 2001). These reports highlight the possibility of increased

transmission of these organisms to humans through the food chain (Zhao et al. 2003). Understanding the association between human salmonellosis cases, animal sources and the environment is an important epidemiological factor needed to successfully control the spread of the infection within communities (Ling et al. 2001).

Recently, emergence of resistant and multi-resistant bacteria has become an important worldwide sanitary problem, impacting both veterinary medicine and public health through the potential for therapeutic failures (Lathers, 2001). Antimicrobial resistance among bacterial isolates from animals is also of concern because of the potential for these organisms to be food-borne or zoonotic pathogens or to be donors of resistance genes to human pathogens (Lathers, 2001). For instance, multidrug-resistant Salmonella enterica serovar Typhimurium phage type DT104, resistant to ampicillin, chloramphenicol/florfenicol, streptomycin, sulfonamides, and tetracycline, has disseminated worldwide (Mulvey et al, 2006). The resistance genes reside on the 43-kb Salmonella genomic island 1 (SGI1), which is transferable. Drug-resistant variants of SGI1 have been identified in numerous serotypes. Strains harboring SGI1 may be more virulent and have a tendency to rapidly disseminate (Mulvey et al, 2006).

International agencies, such as the World Health Organization (WHO) have recommended improving resistance surveillance studies in not only human but also animal origin strains (WHO, 2005). Because of its ubiquitous characteristics and zoonotic nature, *Salmonella spp*. can be used as a good indicator microorganism for resistance surveillance studies (Usera, et al, 2002). Yet there is little information available on *Salmonella* isolates from healthy animals on farms across a wide geographic area that uses various production practices (Dargatz, et al, 2002). This chapter will examine the genotypic relatedness of *Salmonella* serovars commonly isolated from domestic animals raised under different production systems, meat products and humans in order to quantify their role in causing human infection. Antimicrobial drug resistance (AMR) and genetic profiles of *Salmonella* will be used to assess their role in transferring drug resistance to humans.

Reliable and powerful typing methods are necessary in order to gain insight into the infection routes of pathogenic microorganisms. Traditionally, *Salmonella* serotyping combined with various molecular techniques such as phage typing, plasmid profiles, pulsed field gel electrophoresis (PFGE) (Gaul et al. 2007; Guerra et al. 2000; Pickard et al. 2008; Rabsch 2007; Trung et al. 2007) have been used to establish this association. The PFGE method particularly has been found to be very discriminatory and reproducible (Guerra et al. 2000; Tsen et al. 2002) and useful in epidemiological analysis of *Salmonella* infections (Refsum et al. 2002) to determine the relatedness of individual cases (Kim et al. 2007), detect and establish outbreaks (Puohiniemi et al. 1997; Xercavins et al. 1997) and determine linkage between human salmonellosis and consumption of foods of animal origin (McLaughlin et al. 2006). PFGE is increasingly being used as well to identify multidrug resistant strains (Bacon et al. 2002; Besser et al. 2000; McLaughlin et al. 2006; Santos et al. 2007). In fact, the method allows for the detection of DNA polymorphisms that were previously undetected by other techniques (Santos et al. 2007). Also, PFGE has been widely used to investigate the ecology of foodborne pathogens at various points along the food chain (Avery et al., 2002; Vali et al., 2005). This technique has also been used to evaluate the genetic diversity in *Salmonella* isolates from humans, animals, and the environment (Refsum et al., 2002; Gaul et al,2007), and from oysters (Brands et al., 2005). PFGE using XbaI restriction was used by Gaul et al (2007) for screening and identifying swine Salmonella serotypes. Additionally, in the US, molecular subtyping network for foodborne bacterial diseases

including non-typhoidal *Salmonella* serotypes has been using standardized PFGE technique (Swaminathan, et al., 2001).

Most people who suffer from *Salmonella* infections usually present with temporary gastroenteritis that usually does not require treatment. However, when infection becomes invasive, antimicrobial treatment is mandatory (Winokur et al, 2000). As a result, traditionally ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole were used to treat such severe cases. However, the increasing number of antimicrobial-resistant *Salmonella* strains has led to a decrease in the efficacy of these treatments (Angulo et al, 2000). Additionally, the frequency of isolation of *Salmonella* strains resistant to one or more antimicrobial agents has risen in the US (Fey et al, 2000), and elsewhere in the world (Al-Tawfiq, 2007). Fluoroquinolones and broad-spectrum cephalosporins have been employed most recently, as the preferred drugs for treatment of adults and children, respectively, due to the low number of *Salmonella* isolates showing resistance to these drugs (Angulo et al, 2000; Chiappini et al, 2002*)*. However, the viability of these drugs may be diminishing as *Salmonella* strains producing β-lactamases conferring resistance to broad-spectrum cephalosporins have been isolated from clinical patients (Dunne et al, 2000; Winokur et al, 2000), some of which have been acquired from cattle (Fey et al, 2000). The situation is reported to be more complex and difficult in developing countries where there is a widespread misuse of antimicrobials both in human and veterinary medicine practices (Okeke et al, 2005). Furthermore, resistance to combinations of several classes of antimicrobials has led to the emergence of multidrug-resistant (MDR) strains that may pass from food animals to humans (Fey et al, 2000).

The spread of antibiotic resistance among bacteria have been associated with mobile genetic elements such as plasmids, transposons (Zhang et al, 2004) and integrons (Miko et al, 2005). Notably, MDR has been frequently linked with microbial genomic elements known as integrons, which have the ability to distribute genes encoding resistance to a number of antimicrobial drugs (Miko et al, 2005). Integrons do have specific structures and can capture genes notably those encoding antimicrobial resistance by a site-specific recombination system and have been located in both chromosomal and extra chromosomal DNA (Bennet, 1999; Hall and Collis 1995). The main classes of integrons are found in the family *Enterobacteriaceae* with class 1 integrons being the most extensively studied. Class 1 integrons are characterized by presence of two conserved segments, the 5′ -conserved segment (5′ -CS) and 3'-conserved segment (3'-CS) (Bennet, 1999), and are defined by an *intI* gene encoding integrase, a recombinant site *attI,* and a strong promoter. Previous studies (Zhang et al, 2004; Zhao et al, 2005) on integrons and associated antimicrobial resistance genes in *Salmonella* revealed a predominance of gene cassettes that confer resistance to aminoglycosides and trimethoprim, with *aadA* genes carried by all the integrons-containing *Salmonella* serovars. The investigation of multi-drug-resistance in foodborne pathogens in general and *Salmonella* in particular is essential for the proper understanding of the epidemiology of emerging multidrug resistance in *Salmonella* serovars (Winokur et al, 2000). The implications of therapeutic failure in public health due to multidrug resistance is particularly important given that *Salmonella* is the leading cause of hospitalizations and deaths, due to food-borne bacterial infection in the US (Mead et al, 1999).

1.1 Aim of chapter

This chapter will 1) describe prevalence, antimicrobial drug resistance (AMR) and molecular characterization of *Salmonella* commonly isolated from domestic animals, humans and meat

products and 2) assess the relatedness of AMR and genetic profiles of *Salmonella* from various sources and their role in transferring antimicrobial resistance to humans.

2. Research methods

2.1 *Salmonella* from domestic animals sources

2.1.1 *Salmonella* from feedlot cattle

One hundred and thirty eight (138) 1-year-old steers distributed in 24 pens (6 steers/pen) were used in this study (Tabe et al (2010a, 2010b). Cattle from various private farms were housed at the North Dakota State University feedlot facility in October 2006. From October 2006 to March 26, 2007 cattle were placed on growers diet and then on finishing diet from March 27 to June 2007. Cattle in different pens could not directly contact each other, and there was no sharing of feed or water sources between pens. Fecal samples were collected from cattle every three weeks from March 2007 to June 2007. During the first and second sampling periods (March and April 2007 respectively), one-hundred-thirty eight cattle were available for the study. At the third sampling period (May 2007), two unhealthy cattle were withdrawn from the study while at the last sampling period (June 2007), forty six cattle were available as the rest had been taken for slaughter.

Samples were collected in accordance with the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) following a previously described protocol (Khaitsa et al., 2007a). The feces were put into sterile plastic cups and placed in iced-pack coolers before transport to the laboratory for processing. The sampling procedure was repeated every three weeks for the entire finishing period. For the isolation of *Salmonella*, fecal samples were cultured using conventional culture methods optimized for the detection of *Salmonella* (Khaitsa et al., 2007). Briefly, a sterile swab was loaded with fecal sample and pre-enriched in buffered peptone water (Difco, Becton Dickinson) at 37°C overnight followed by immunomagnetic beads separation specific for *Salmonella* species (Dynabeads anti-*Salmonella,* Dynal Biotech, Inc., Lake Success, N.Y.) according to the **manufacturer's** instructions. After the final wash, the beads were transferred to 10 ml of Rappaport Vassiliadis R10 (RV) broth (Becton Dickinson) and incubated (with constant gentle shaking) at 42^0C for 24 h. Following incubation, the RV cultures were streaked onto modified brilliant green agar (Becton Dickinson) and mannitol lysine crystal violet brilliant green agar (Oxoid, Basingstoke, UK). Colonies with typical *Salmonella* characteristics (Fratamico et al., 2000) were stabbed in 10-ml triple sugar iron agar slants (Becton Dickinson), and the biochemical results read after 24-h incubation as described. Presumptive *Salmonella* isolates were stabbed into 2 ml tryptic soy agar (Difco, Becton Dickinson) slants and shipped to the National Veterinary Service Laboratories, Animal and Plant Health Inspection Services, US Department of Agriculture, Ames, Iowa, for serotyping. The detection sensitivity culture post immunomagnetic separation and enrichment using culture media for *Salmonella* was based on growth of bacteria of interest on the culture plates. Fifty eight (58) isolates of *Salmonella* were shipped to the *E. coli* reference center (University Park, PA) for PFGE.

2.1.2 *Salmonella* from ranch cattle

The objective of this study (Theis et al 2005, 2006, 2007) was to determine the prevalence, serotypes, and antimicrobial resistance patterns of *Salmonella* isolates recovered from grass

fed cattle in North Dakota. A total of 212 cattle (97 calves and 115 cows) originating from 7 cow-calf farms in the ND counties of Billings, Dunn, Mercer and Stark participated in the study. A random sample of at least 30 cattle (15 calves and 15 adult cows) were selected from each of the 7 herds that participated in the study except where less than 30 animals in each category were available; in that case all of them were sampled. One herd had only calves and 2 herds had only adult cows and so 30 animals of one category were sampled from each of these herds. Approximately 20 grams of feces were obtained from the rectum of individual cattle and shipped by Fedex overnight to the department of Veterinary and Microbiological Sciences, at North Dakota State University. The fecal samples were processed within twenty-four hours of their arrival to the laboratory. The fecal samples were cultured in the laboratory using culture methods optimized for the detection of *Salmonella* (Khaitsa et al., 2007a) in fecal specimens. Presumptive *Salmonella* isolates were sent to NVSL in Ames, IA for serotyping. Antimicrobial susceptibility of *Salmonella* isolates was determined using a custom designed panel according to the manufacturer's instructions (Sensititre, Trek Diagnostics, Westlake, Ohio).

2.1.3 *Salmonella* from dairy cattle

A study (Khaitsa et al, 2004) investigated the prevalence of cattle shedding *Salmonella* in their feces at the NDSU dairy and to test antimicrobial susceptibility of *Salmonella* isolates. In June, 2004, fecal samples from a random sample of thirty cows out of 60 at the NDSU dairy were collected and cultured for *Salmonella* at the Department of Veterinary and Microbiological Sciences. Approximately 20g of fecal matter was obtained from the rectum of each cow and transported on ice to the Department of Veterinary and Microbiology Sciences at NDSU for microbiologic culture. The fecal samples were cultured in the laboratory using culture methods optimized for the detection of *Salmonella* (Khaitsa et al 2007a) in fecal specimens.

2.1.4 *Salmonella* from bison

Twenty bison from one herd in North Dakota, US were run through a chute and approximately 20 grams of feces obtained from the rectum of each animal. Fecal samples were transferred into sterile plastic cups, placed on ice and transported to the laboratory for culturing. *Salmonella* spp were cultured using the procedure described by Khaitsa et al (2007a). All suspect colonies were sent to National Veterinary Services Lab, Ames, IA for serotyping. Antimicrobial susceptibility testing was carried out using Sensititre Trek Diagnostic Systems, Westlake, OH.

2.2 *Salmonella* from meats

A study (Khaitsa et al 2007b) investigated the occurrence of *Salmonella* in raw and ready to eat turkey meat products, and factors associated with its occurrence in 959 turkey meat products (raw, n =614; and ready to eat (RTE), n = 345) purchased from four retail outlets in one city in the Midwestern United States. Another study (Kegode et al, 2008) investigated occurrence of *Salmonella* species, in 456 fresh raw meat products (turkey (n=87, 19.1%) chicken (n=123, 27.0%) chicken, pork (n=113, 24.8%) and beef (n=133, 29.2%)) purchased from five retail outlets in the Midwestern United States during a 12-week period (July 11, 2005 to October 3, 2005). Three stores were visited each week until all the stores had been visited a total of five times. The stores were sampled on different

days of the week during subsequent sampling times in order to minimize systematic bias associated with a particular day of the week. On each visit to a store, an average of 18 (range 11 to 23) fresh raw samples of all meat types (turkey, chicken, pork, and beef) and different meat products were obtained. Turkey products sampled included: ground breast, breast, breast cutlets, breast tenderloin, drumstick, and thigh. Chicken products comprised whole, quarter, breast, drumstick, thigh, wing, and kebab; pork products included ground, chops, steak, ribs, neck bones, roast, and stew; beef products consisted of ground beef-store brand, steak, stew, chuck, roast, ribs, round, loin, and kebab. Where available, different brands were selected including in-store packaged products. All products were raw and unfrozen. Samples were immediately transported to the laboratory on ice and processed within one hour of purchase.

For *Salmonella* isolation, meat samples were aseptically placed in a plastic WhirlPak bag (Nasco, Fort Atkinson, WI) with 200-400ml buffered peptone water, depending on the size of the meat sample. Approximately 200 ml and 400 ml of buffered peptone water added to any meat sample that was ≤ 1 Ib and > 1Ib, respectively. The bags were shaken manually for 3 minutes and left on ice for 20 minutes. All samples were subjected to an enrichment procedure. The buffered peptone water (BPW) rinse solution (20ml) was mixed with the same volume of double-concentrated lactose broth and enriched overnight at 35^{o}C. To culture *Salmonella,* 1.0 ml of the lactose enrichment broth was transferred into 9.0 ml of tetrathionate broth and incubated (42^{o}C for 24 hr.) The broth culture was then streaked onto XLT4 agar plates and incubated (24h at 37^{o}C). Suspect colonies (yellow with black centers) were stabbed in Triple Sugar Iron (TSI) agar slants and incubated (37^{o}C for 24 hr.) Presumptive *Salmonella* isolates, which formed red slants with black butts, were sent for serotyping to the US National Veterinary Services Laboratories (NVSL, Ames, IA).

Additionally, Tumuhairwe et al (2007) reviewed the temporal and spatial distribution of 1465 human salmonellosis cases associated with consumption of turkey meat in the US during the period 1990 to 2003 involving 49/50 states. Tumuhairwe et al (2007) also described the distribution of salmonellosis cases by vehicle and serotype. Trends in the outbreak numbers over time, and major serotypes across vehicles were tested by Cox-Stuart and chi-square test, respectively. Also, a study (Tumuhairwe et al, 2008) characterized 386 non-typhoidal salmonellosis cases in North Dakota from 2000 to 2005. Salmonellosis cases were extracted from the enteric disease investigation database of the North Dakota Department of Health (NDDoH) for the period 2000 to 2005.

2.3 *Salmonella* from clinical cases of humans and animals (cattle, chicken, ducks, swine, turkeys, elk and bison)

A total of 434 frozen presumptive *Salmonella* isolates were included in the study. The isolates were previously obtained from 4 different sources comprising; 1) feces from apparently healthy feedlot, range and dairy cattle in an ongoing surveillance program in ND; 2) Clinical isolates from sick or dead cattle, chicken, ducks, swine, turkeys, elk and bison submitted to North Dakota State University-Veterinary Diagnostic Laboratory (NDSU-VDL) (2000-2005); 3) Frozen isolates from S*almonella* data bank in the NDSU-Veterinary and Microbiological Services (VMS) Department from previous food surveillance studies involving turkey, chicken and bison meat sold at the grocery stores at ND; and 4) 183 S*almonella* isolated from stools of human patients in ND (2000-2005) and stored at North Dakota Department of Health (NDDoH) (Table 1).

Source	Nature/state of the sample	Number	Percent
Humans	sick	179	41.2
Cattle	feedlot (feces)	112	25.8
	dairy (feces)	5	1.2
	range(feces)	17	3.9
	sick or dead cattle	59	13.6
Chicken	retail chicken	4	0.9
Ducks	ill/dead	1	0.2
Swine	ill/dead	5	1.2
Turkeys	ill/dead	3	0.7
	meat	32	7.4
Elk	ill/dead	1	0.2
Bison	fecal samples	1	0.2
	meat	1	0.2
Humans	sick	179	41.2
Others	beddings, linx etc	14	3.2
Total		434	100

Table 1. Sources of *Salmonella* isolates from clinical cases of humans and animals

2.4 Antimicrobial resistance (AMR) testing

Antimicrobial susceptibility of *Salmonella* isolates from the various sources was determined using the National Antimicrobial Resistance Monitoring System (NARMS) panel according to Food and Drug Administration and National Committee for Clinical Laboratory Standards (NCCLS) recommendation (Sensititre®, Trek Diagnostics System, Inc, Westlake, Ohio). Each isolate was screened for resistance using full-range minimum inhibitory concentration. The US National Antimicrobial Resistance Monitoring System (NARMS) panels were used to compare AMR levels between domestic animal and human isolates of the same genotype in order to assess a possible role of domestic animals in transfer of AMR of *Salmonella* isolated from human cases. The antimicrobials tested included ampicillin, apramycin, ceftiofur, chlortetracycline, clindamycin, enrofloxacin, erythromycin, florfenicol , gentamicin, neomycin, oxytetracycline, penicillin, spectinomycin, sulphachloropyridazine, sulphadimethoxime, sulphathiazole, tiamulin, tilmicosin, trimethoprim/ sulphamethoxazole and tylosin. Isolates were defined as resistant according to FDA recommended breakpoints. Breakpoints were defined as minimum drug concentration above which growth of the test isolate should not occur (Logue et al. 2003).

2.5 *Salmonella* serotyping and genotyping

Frozen (-70°C) presumptive *Salmonella* cultures from the above sources were thawed and stabbed into 2ml tryptic soy agar (Difco, Becton Dickinson) deeps and shipped to the

National Veterinary Service Laboratories, Animal and Plant Health Inspection Services, U.S. Department of Agriculture, Ames, Iowa, for serotyping. PFGE assays on *Salmonella* cultures to investigate their genotypic relatedness were performed at the *E. coli* Reference Centre, Pennsylvania State University, University Park. The sample preparation, restriction digestion, electrophoresis, and gel staining for PFGE were accomplished following the CDC-standardized procedure as described (CDC, 2004) (http://www.cdc.gov/pulsenet/protocols.htm). Restriction endonuclease *XbaI* (Roche Diagnostics Corporation, Indianapolis, IN) was used for restriction digestion of genomic DNA. The size standard used for all gels was *XbaI*-digested DNA from *Salmonella* Braenderup strain H9812 (American Type Culture Collection catalogue no. BAA-664), i.e. the universal size standard used by all PulseNet laboratories. Fingerprints were analyzed using BioNumerics software version 3.5 (Applied Maths, Austin, Texas). Strain relatedness was done based on previously recommended criteria (Gebreyes et al. 2006) using 'different bands' algorithm for clustering and the unweighted pair group for arithmetic means (UPGMA) tree-building approach with optimization of 1 and 0.5% position tolerance. Visual inspection of the patterns was performed as a final step for analysis.

2.6 PCR amplification of class 1 and 2 integrons

The bacterial DNA template preparation and the PCR conditions for the detection of class 1 and class 2 integrons were undertaken as previously described (Miko et al, 2005). The screening for the presence of class 1 and class 2 integrons was carried out using PCR with primers specific for the *intI1* (and *intI2* (Goldstein et al, 2001)). The primer sequences used are shown in Table 2. Amplifications were performed in 10 µL of 5x Taq PCR Master Mix (Qiagen, Valencia, CA, USA), 2 pmol/L each primer, and 2 µg template DNA. Amplification specifications were as follows: 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 30 s at 72°C. PCR products were analyzed by gel electrophoresis with 2 % agarose gels. All PCRs included DNA ladder, positive and negative controls.

Primers	Sequence [a`]	Size (bp)	PCR Annealing Temp (^{0}C)	References
intI1	F: CCTCCCGCACGATGATC	280	55	*Kraft et al., 1986*
	R: TCCACGCATCGTCAGGC			
intI2	F: TATTGCTGGGATTAGGC	233	50	*Goldstein et al., 2001*
	R: ACGGCTACCCTCTGTTATC			

Table 2. PCR primers and conditions used in Screening *Salmonella* isolates for presence of class 1 and class 2 integrons; [a] F, Forward; R, Reverse.

3. Key results

3.1 *Salmonella* from feedlot cattle

Salmonella was isolated from 58 out of 458 (12.7%) fecal samples tested (Tabe et al, 2010a, 2010b). All *Salmonella* belonged to the Typhimurium serotype and the majority 53/58 (91.4%) were Typhimurium *vars* Copenhagen. The rest (3/58, 5%) were reported as

Salmonella Typhimurium. AMR testing showed that all isolates were resistant to more than one of the antibiotics (Table 3). All but two of the isolates were resistant to more than two of the antibiotics tested with 96.6% (56 of 58) of the isolates showing MDR antibiogram. All isolates tested were susceptible to amikacin, cefoxitin, cetriaxone, ciprofloxacin, gentamycin, gentamycin, nalidixic acid, and trimethoprim-sulfamethoxazole (Table 3). Almost all the isolates recovered from this study had a similar antimicrobial pattern. Regardless of sampling period (1, 2, or 3), 29 (3 Salmonella serovars Typhimurium and 26 Salmonella serovars Typhimurium var Copenhagen) were positive for class I integron (280 bp product) while only two of the isolates showed a 233-bp PCR product using primers *intI2* thus suggesting the presence of integron 2. These two isolates also had integron 1. Upon PFGE analysis, 9 distinguishable *Salmonella* genotypes were identified. For clarity, the genotypes were numbered I to IX with genotype V (28 of 58; 46.6%) being the most prevalent followed by type VII (15 of 58; 25.9 %) (Figure 1). Genotypes I, II, and III had the least prevalence (1 of 58; 1.7 % each). From the 58 isolates, types IV, V, VII, VIII, and IX (38 of 58; 65.5 %) isolated from the cattle at two sampling periods were observed at a similarity level of 100 %. Type V (28 of 58 isolates; 48.2 %) genotypes comprised of the most common isolates; of the 28 isolates from type V, 8 of 28 (28.6%), 18 of 28 (64.3%), and 2 of 28 (7.1%) were derived from sampling 1, 2 and 3 respectively. (Figure1). The 2 isolates which were positive for both *Int* 1 and 2 belonged to genotypes I and IV, respectively. Sampling time had a significant effect on the recovery of *Salmonella* (P = 0.004) while pen (P = 0.79) did not. All 58 *Salmonella* isolates which were grouped into two clusters (d and e) and five single isolates (a, b, c, f, and g) were observed at a similarity level of 80% (Figure 1).

Antibiotics	Susceptible Isolates (%)	Intermediate Isolates (%)	Resistant Isolates (%)
Amikacin (0.5–64),	58(100.0)	-	-
Amoxicillin/clavulanic acid (1/0.5–32/16)	2(3.5)	1(1.7)	55(94.8)
Ampicillin (2–32)	2(5.3)	-	56(94.7)
Cefoxitin (0.5–32)	58(100.0)	-	-
Cetriaxone (0.25–64)	58(100.0)	-	-
Chloramphenicol (2–32)	-	2(5.3)	56(94.7)
Ciprofloxacin (0.015–4)	58(100.0)	-	-
Gentamycin (0.25–16)	58(100.0)	-	-
Kanamycin (6–64)	58(100.0)	-	-
Nalidixic acid (0.5–32)	58(100.0)	-	-
Streptomycin (32–64)	NI	NI	56(94.7)
Sulfizoxazole (16–512)	2(5.3)	-	56(94.7)
Tetracycline(4–32),	2(5.3)	-	56(94.7)
Trimethoprim-sulfamethoxazole (4-76)	58(100.0)	-	-

Table 3. Number (%) of *Salmonella* isolates resistant/susceptible to various antimicrobials (N = 15)

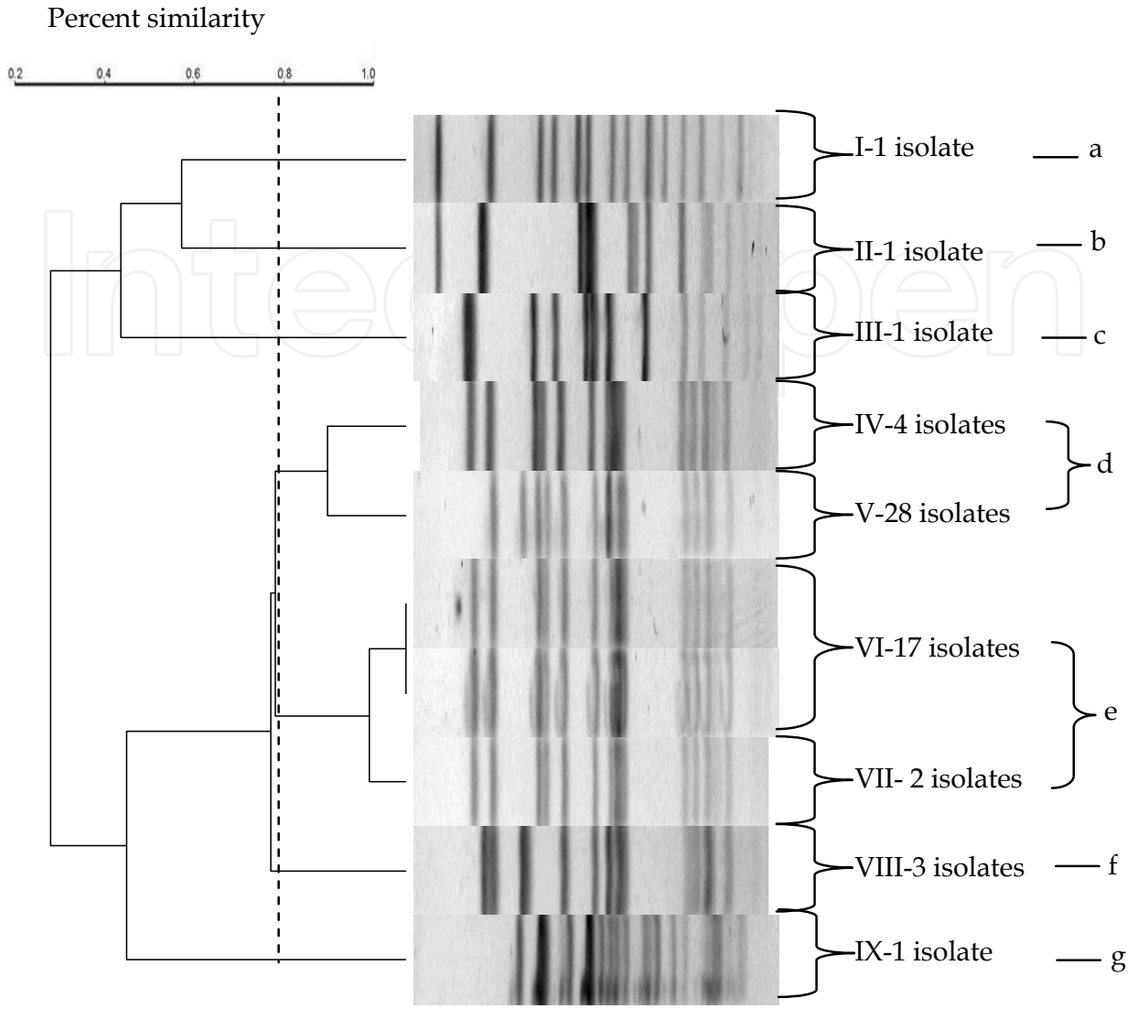

Fig. 1. Dendogram generated from the *Xbal* patterns of the 58 *Salmonella* isolates using UPGMA clustering analysis with the BioNumerics software. A positive tolerance of 1.5 % was chosen.

3.1.1 *Salmonella* from ranch cattle

Of the 212 cattle (115 adult cattle, 97 calves) investigated by Theis et al (2007), 15 (7%) tested positive for *Salmonella*. The prevalence of *Salmonella* among adult cattle and calves was 9/115 (7.8%) and 6/97 (6.2%), respectively. The 15 cattle that tested positive to *Salmonella* were distributed in three of the four counties with the majority originating from Billings county and no animal from Mercer county as follows: Stark (7/92, 7.6%), Billings (5/30, 6.6%), Dunn (3/60,5.0%), and Mercer (0/30, 0.0%). Thirteen (87%) of the 15 *Salmonella* isolates recovered were *Salmonella* Typhimurium (Copenhagen) and the rest (2/15, 13%) were *Salmonella* Worthington. All 15 *Salmonella* isolates from healthy cattle were susceptible to Apramycin, Ceftiofur, Entrofloxacin, Gentamicin, and Neomycin. All samples were resistant to Chlortetracycline, Clindamycin, Erythromycin, Florfenicol, Oxytetracycline,

Penicillin, Sulphachlorophridazine, Sulphadimethoxime, Sulphathiazole, Tiamulin, Tilmicosin. Two isolates (both *Salmonella* Worthington), were susceptible to Ampicillin, whereas the other thirteen samples (all *Salmonella* Typhimurium (Copenhagen), were resistant to Ampicillin and to Spectinomycin.

3.1.2 *Salmonella* from dairy cattle

In a study of Salmonella occurrence in dairy cattle (Khaitsa et al, 2004), 5 out of 30 cows (17%) tested positive for *Salmonella.* A sensitivity test to 20 antibiotics was performed on the 5 *Salmonella* isolates and the results were similar for all the 5 isolates except for only one isolate that was sensitive to Sulphachloropyridazine and Sulphadimethoxime and gave an intermediate result to Sulphathiazole (Table 4).

Antimicrobial	All 5 Salmonella Isolates
Ampicillin	R
Apramycin	S
Ceftiofur	S
Chlortetracycline	R
Clindamycin	R
Enrofloxacin	S
Erythromycin	R
Florfenicol	R
Gentamicin	S
Neomycin	S
Oxytetracycline	R
Penicillin	R
Spectinomycin	R
Sulphachloropyridazine	R (S)*
Sulphadimethoxime	R (S)*
Sulphathiazole	R *(I)**
Tiamulin	R
Tilmicosin	R
Trimethoprim/Sulphamethoxazole	S
Tylosin (Tartrate/Base)	R

S = Sensitive, I = Intermediate. *These 3 antimicrobials are the only ones that gave a different result (sensitive or intermediate) to 1 of the 5 isolates; the other 4 isolates were all resistant to them).
For all other antimicrobials the results were the same for all 5 Salmonella isolates.

Table 4. Antimicrobial Sensitivity Results to 5 *Salmonella* Isolates from dairy cattle. R = Resistant,

3.1.3 *Salmonella* from bison

The prevalence of *Salmonella* in the bison feces was 15% (3/20). The *Salmonella* isolates belonged to the serotypes *Salmonella* Typhimurium (Copenhagen) and *Salmonella* Worthington. In a panel of 20 antimicrobials, *Salmonella* Typhimurium (Copenhagen) was resistant to 13 of 20 antimicrobials (65% resistance), including macrolides (erythromycin, tilmicosin, tylosin), tetracyclines (chlortetracycline, oxytetracycline), chloramphenicol

analog – florfenicol, most sulphonamides, and penicillin, and susceptible to 7 antimicrobials including the cephalosporin – ceftiofur, the quinolone – enrofloxacin some aminoglycosides, and ampicillin (Table 5). *Salmonella* Worthington was resistant to 14 of 20 antimicrobials (70% resistance), including macrolides (erythromycin, tilmicosin, tylosin), tetracyclines (chlortetracycline, oxytetracycline), chloramphenicol analog – florfenicol, some sulphonamides, and penicillins (penicillin and ampicillin), and susceptible to 6 antimicrobials including the cephalosporin – ceftiofur, the quinolone - enrofloxacin and some aminoglycosides (Table 5). Except for ampicillin, both *Salmonella* isolates were resistant to similar antimicrobials (Table 5). None of the *Salmonella* isolates were resistant to clinically important antimicrobials.

	***Salmonella* Isolates**		
Antibiotics	**18S**	**24S**	**53S**
Aminoglycosides			
Apramycin	S	S	S
Gentamycin	S	S	S
Neomycin	S	S	S
Spectinomycin	R	R	S
Sulphanamides/Potentiated Sulphonamides			
Trimethoprim/Sulphamethoxazole			
Sulphadimethoxime	S	S	S
Sulphachloropyridazine	R	R	S
Sulphathiazole	R	R	S
Cephalosporins	R	R	I
Ceftiofur			
Quinolones/Fluoroquinolones	S	S	S
Enrofloxacin			
Pleuromutilins	S	S	S
Tiamulin			
Chloramphenicol Analog	R	R	R
Florfenicol			
Penicillins	R	R	R
Ampicillin			
Penicillin	S	R	R
Tetracyclines	R	R	R
Chlortetracycline			
Oxytetracycline	R	R	S
Macrolides	R	R	S
Erythromycin			
Tilmicosin	R	R	R
Tylosin (Tartrate/Base)	R	R	R
Misc.	R	R	R
Clindamycin			
	R	R	R

R = Resistant S = Susceptible I-Intermediate

Table 5. Antibiotic sensitivity and resistance of *Salmonella* isolates from a bison herd

3.2 *Salmonella* from meats

In the Khaitsa et al (2007b) study, 2.4% (23/959) of the samples were contaminated with *Salmonella*; with 5% (16/329), and 1% (7/607) of the raw and ready to eat meat samples testing positive for *Salmonella*, respectively. There was a significant difference in recovery of *Salmonella* ($P < 0.05$), between meat type (raw vs RTE; OR =4.2, 95% CI = 1.6, 10.8); and sampling time (OR = 0.4, 95% CI = 0.2, 0.7). Retail store and product brand did not affect *Salmonella* recovery. The twenty three *Salmonella* isolates recovered from meat products were confirmed to belong to 6 different serotypes; the predominant one being *S. hadar* followed by *S. Heidelberg, S.* typhimurium var Copenhagen, *S. newport*, *S. saintpaul* and *S. agona.* Overall, *Salmonella* isolates from raw turkey products exhibited a higher antimicrobial resistance rate (53%) compared to those from RTE products (33%). Multidrug resistance was exhibited by 54% of the *Salmonella* isolates with the majority (62%) originating from RTE meats compared to 45% from raw ones.

In the Kegode et al (2008) study the distribution of samples that tested positive for *Salmonella* by meat type and meat part is summarized in Table 6. *Salmonella* was recovered from turkey breast (1/8, 13%), ground turkey breast (1/15, 7%), and turkey drumsticks (1/20, 5%) (Table 6). For chicken products *Salmonella* (2/5, 40%) were recovered from whole chicken. Thirteen *Salmonella* isolates recovered from the meat samples were confirmed by NVSL to belong to eight different *Salmonella enterica* serotypes (Table 7). The predominant serotype was *S. enterica s*erotype Heidelberg recovered from turkey from which *S.* Typhimurium, *S.* Newport, *S.* Saintpaul and *S.* Senftenberg were also recovered. *S.* Kentucky, *S.* Typhimurium var Copenhagen, *S.* Blockley, and one undetermined serotype were recovered from chicken.

In the study by Tumuhairwe et al, 2007) that investigated the temporal and spatial distribution of 1465 salmonellosis outbreaks involving 49/50 states in the US, turkey meat associated outbreaks (TMAOs) were reported by 24 states, mostly from California and New York. Additionally, turkey meat was implicated in 4.2% of outbreaks, sea-foods (5.8%), pasta (8.3%), milk-products (8.6%), chicken (13.4%), red-meats (15.4%), eggs (21.3%), and fresh-produces (22.9%). Most outbreaks were at restaurants and private-homes for TMAOs (23.2% and 21%). The major serotypes were: S. Enteritidis, S. Heidelberg, S. Reading and S. Newport for TMAOs,

In the study by Tumuhairwe et al (2008), there were 45 different serotypes that were recovered from 71.8% (277/386) of the salmonellosis cases in North Dakota (2000 to 2005). The four major ones contributing over 70% of the cases were: *S.* Typhimurium (93, 33.1%), *S.* Enteritidis (40, 14.2%), *S.* Heidelberg (33, 11.7%) and *S.* Newport (32, 11.4%). The rest of the serotypes were: *S.* Saintpaul and *S.* Montevideo from eight cases each, *S.* Thompson was recovered from five cases, *S.* Hadar from four cases, *S.* Stanley, *S.* Poona, *S.* Mbandaka, *S.* Javiana, *S.* Braenderup, and *S.* Bredeney from three patients each. *S.* Reading, *S.* Oranienburg, *S.* Hillington, *S.* Derby, *S.* Urbana, and *S.* Albany were each recovered from 2 cases. One case each was diagnosed with *S.* Agona, *S.* Berta, *S.* Bleadon, *S.* Blockley, *S.* Chameleon, *S.* Ealing, *S.* Edinburgh, *S.* Havana, *S.* Ibadan, *S.* Indiana, *S.* Infantis, *S.* Istanbul, *S.* Lexington, *S.* Litchfield, *S.* Manhattan, *S.* Marina, *S.* Miami, *S.* Mississippi, *S.* Muenchen, *S.* Newport, *S.* Othmarschen, *S.* Sandiego, *S.* Schwarzengrund, *S.* Senftenberg, *S.* Sepsis, *S.* Syrsis, *S.* Tripoli, *S.* Uppsala, and *S.* Weltevereden.

Store/Meat Type	*Salmonella*
Store A (n=97)	
whole chicken	0
ground turkey	1
turkey breast	1
Total	2
	2/97 (2%)
Store B (n=108)	
turkey drumstick	1
chicken drumstick	1
chicken thigh	0
whole chicken	2
Total	4
	4/108 (4%)
Store C (n=95)	
chicken breast	0
chicken thigh	0
chicken wings	0
whole chicken	0
ground turkey breast	1
Total	1
	1/95 (1.1%)
Store D (n = 93)	
ground turkey	4
chicken thigh	1
whole chicken	0
chicken wings	0
turkey thigh	0
Total	5
	5/93 (5.4%)
Store E (n = 63)	
chicken breast	1
Total	1
	1/63 (1.6%)
Grand Total (n =456)	13
	13/456 (2.9%)

Table 6. Number and percentage of retail meat samples that tested positive for *salmonella* by store and meat type, 2005 (n = 456).

***Salmonella* serotypes**	n (%)	Chicken	Turkey
S. Heidelberg	4 (30.8)	0	4
S. Kentucky	2 (15.4)	2	0
S. Typhimurium (Copenhagen)	1 (7.7)	1	0
S. Typhimurium	1 (7.7)	0	1
S. Blockley	1 (7.7)	1	0
S. Newport	1 (7.7)	0	1
S. Saintpaul	1 (7.7)	0	1
S. Senftenberg	1 (7.7)	0	1
Unknown	1 (7.7)	1	0
Total	13 (100)	5	8

United States metropolitan area, 2005.

Table 7. *Salmonella enterica* serotypes recovered from retail meats in the Midwestern

3.3 *Salmonella* from clinical cases of humans and animals (cattle, chicken, ducks, swine, turkeys, elk and bison)

3.3.1 *Salmonella* serotypes

A total of 434 isolates were serotyped, including the 255 (58.8%) isolates from NDSU (from apparently healthy cattle, sick or dead animals and meat products) and 179 (41.2%) isolates from NDDoH (Table 8).

Serotypes	Cattle	Human	Chicken	Ducks	Swine	Turkey	Bison	Elk	Others	Total
Agona	-	3(0.7)	-	-	-	1(0.2)	-	-	-	4(0.9)
Anatum	-	1(0.2)	-	-	-	2(0.5)	-	-	-	3(0.7)
Arizona	3 (0.7)	-	-	-	-	-	-	-	-	3(0.7)
Blockley	-	-	2(0.5)	-	-	-	-	-	-	2(0.5)
Braenderup	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Brandeberg	-	2(0.5)	-	-	-	-	-	-	-	2(0.5)
Bredeney	-	2(0.5)	-	-	-	-	-	-	-	2(0.5)
Derby	-	-	-	-	2(0.5)	-	-	-	-	2(0.5)
Dublin	2 (0.5)	-	-	-	-	-	-	-	-	2(0.5)
Ealing	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Enteritidis	-	-	-	1(0.2)	-	-	-	-	-	1(0.2)
Give	4 (0.9)	-	-	-	-	-	-	-	-	4(0.9)
Hadar	-	-	-	-	-	10(2.3)	-	-	-	10(2.3)
Havana	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Heidelberg	-	5(1.2)	-	-	-	9(2.1)	-	-	-	14 (3.2)
Indiana	-	2(0.5)		-	-	-	-	-	-	2(0.5)

Serotypes	Cattle	Human	Chicken	Ducks	Swine	Turkey	Bison	Elk	Others	Total
Infantis	2(0.5)	2(0.5)		-	-	-	-	-	-	4(0.9)
Java	-	1(0.2)		-	-	-	-	-	-	1(0.2)
Kentucky	-	1(0.2)	2(0.5)	-	-	-	-	-	-	3(0.7)
Litchfield	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Mbandaka	2(0.5)	2(0.5)	-	-	-	-	-	-	-	4(0.9)
Mississipi	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Montevideo	-	3(0.7)	-	-	-	-	-	-	-	3(0.7)
Muenchen	-	3(0.7)	-	-	-	-	-	1 (0.2)	-	4(0.9)
Muenster	15 (3.5)	-	-	-	-	-	-	-	-	15(3.5)
Newport	9(2.1)	17(3.9)	-	-	-	2(0.5)	-	-	-	28(6.5)
Oranienburg	-	2(0.5)	-	-	-	-	-	-	-	2(0.5)
Paratyphi	-	2(0.5)	-	-	-	-	-	-	-	2(0.5)
Reading	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Reno	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Sandiego	-	2(0.5)	-	-	-	-	-	-	-	2(0.5)
Senftenberg	-	-	-	-	-	1(0.2)	-	-	-	1(0.2)
Soesterberg	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Sonnei	-	3(0.7)	-	-	-	-	-	-	-	3(0.7)
Sovenga	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
St paul	-	7(1.6)	-	-	-	3(0.7)	-	-	-	10 (2.3)
Stanley	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Thompson	-	1(0.2)	-	-	-	-	-	-	11(0.2) (bedding)	1(0.2)
Tripoli	-	2(0.5)	-	-	-	-	-	-	-	2(0.5)
Typhi	-	1(0.2)	-	-	-	-	-	-	-	1(0.2)
Typhimurium	140 (32.3)	58 (13.4)	-	-	3(0.7)	4(0.9)	1(0.2)	-	11(0.2) (lynx)	207 (47.7)
Worthingtom	2 (0.5)	-	-	-	-	-	1(0.2)	-	-	3(0.7)
unidentified	14 (3.2)	47 (10.8)	-	-	-	3(0.7)	-	-	12(2.8)	76 (17.5)
Total	193 (44.5)	179 (41.2)	4(0.9)	1(0.2)	5(1.2)	35(8.1)	2(0.5)	1 (0.2)	14(3.2)	434(100)

Table 8. *Salmonella* serotypes isolated from different animal species and human cases in North Dakota.

The total number of isolates that were common between domestic animals and humans were 183 (42.2%) and 90 (20.7%) respectively (Table 8). *S. Typhimurium* was the predominant serotype in both humans (13.4%, n=58) and domestic animals (34.3%, n= 159), followed by Newport with 11 (2.6%) and 17(3.9%) isolated in animals and human, respectively. *S. Arizona* (n=3, 0.7%), *S. Give* (n=4, 0.9%) *and S. Muenster* (n=15, 3.5%) were isolated mostly

from sick or dead animals submitted to the NDSU-VDL. Of the 42 serotypes involved in animal and human infection, human isolates were highly diverse with 32 serotypes involved compared to cattle (9), turkeys (8), chickens (2), bison (2), swine (2), ducks (1) and elk (1). The detailed distribution of the different serotypes between different host species is provided in Table 8.

3.3.2 PFGE Results

The initial 434 *Salmonella* isolates were grouped into 113 distinct PFGE profiles at 85% similarity (Tables 9, 10, 11; Figure 2). The 179 human isolates were distributed within the 98 of the 113 PFGE fingerprint patterns or profiles at the same level of similarity. A detailed examination of the 273 isolates from serotypes commonly isolated from man (n=90) and domestic animals (n=183), revealed that 40 of the human and 55 animal isolates were distributed amongst 8 distinct (i.e. with 100% similarity) PFGE fingerprint profiles. The 40 isolates from the human cases were linked to 2 serotypes – *S. Typhimurium Copenhagen* and *S. Heidelberg* that shared indistinguishable genetic fingerprint patterns (100% homology) with some animal isolates. The biggest clonal group involving *S. Typhimurium Copenhagen* with 100 % similarity in the PFGE fingerprint patterns involved 22 isolates from cattle, 17 Humans and 1 from a sick swine (Figure 2). The second PFGE profile involved 19 isolates of *S. Typhimurium Copenhagen* with indistinguishable fingerprints, isolated from 7 feedlot cattle, 2 range cattle and 10 human cases (Figure 2). The third profile had 10 cattle and 4 humans, fifth profile had 1 human, 1 swine and 1 turkey, sixth profile was identified as *S. Heidelberg* form human (1) and turkey meat (1), seventh profile had *S. Typhimurium Copenhagen* from cattle (4), human (5) and chicken (1) and the eighth profile had S. *Heidelberg,* isolated from human (1) and turkey meat (1). Figure 2 shows details of human and domestic animal serotypes in the eight distinct profiles each with indistinguishable PFGE fingerprint patterns. The isolation of serovars with similar PFGE patterns in cattle preceded those in humans. Most outbreaks were recorded in 2004 (58%), while a few turkey isolates with similar PFGE profile were recorded after (Table 9).

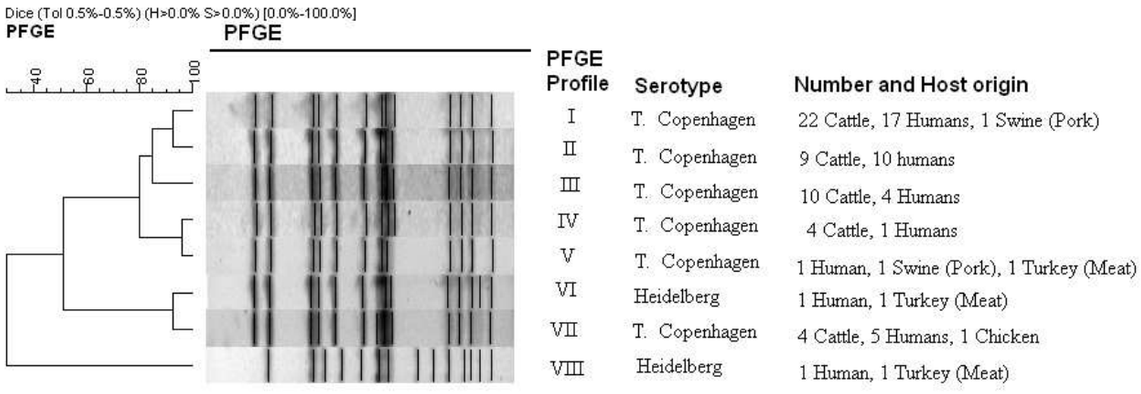

Fig. 2. PGFE profile of the commonly serotypes isolated from domestic animals or their products and humans.

Xbal profiles with indistinguishable fingerprint	Host	Year of isolation and number isolated (%)				
		2003	2004	2005	2006	Total
I	Cattle	-	19 (19.8)	2 (2.1)	1 (1.0)	22 (22.9)
	Human	-	4 (4.2)	12 (12.5)	1 (1.0)	17(17.7)
	Swine	-	-	-	1(1.0)	(1.0)
II	Cattle	2 (2.1)	7(7.3)	-	-	9(9.4)
	Human	-	2 (2.1)	8(8.3)	-	10(10.4)
III	Cattle	-	10(10.4)	-	-	10(10.4)
	Human	-	-	4(4.2)	-	4(4.2)
IV	Cattle	-	4(4.2)	-	-	4(4.2)
	Human	-	-	1(1.0)	-	1(1.0)
V	Turkey meat	-	-	1(1.0)	-	1(1.0)
	Human	-	-	-	1(1.0)	1(1.0)
	swine	-	-	-	1(1.0)	1(1.0)
VI	Turkey meat	-	-	-	1(1.0)	1(1.0)
	Human	-	-	1(1.0)	-	1(1.0)
VII	Cattle	-	4(4.2)	-	-	4(4.2)
	Human	-	-	6(15)	-	6(12.5)
	Chicken	-	-	1(1.0)	-	1(1.0)
VIII	Turkey meat	-	-	-	1(1.0)	1(1.0)
	Human	-	1(1.0)	-	-	1(1.0)
	Total	2(2.1)	51(53.1)	36(37.5)	7(7.3)	94(100)

Table 9. PFGE profiles, host species and year of isolation of the *Salmonella* serotypes.

3.3.3 Antimicrobial resistance (AMR) patterns

A comparison of the AMR patterns of isolates with indistinguishable PFGE profiles revealed variations within the groups (Table 11). In profile 1, 2 bovine and 1 human isolates shared similar AMR and PFGE profiles. Second observation was recorded for 1 swine and 20 cattle.

Xbal patterns with indistinguishable fingerprint	*Salmonella* Serotype	Origin	Antimicrobial resistance profile	Number of matching isolates
I	Typhimurium Copenhagen	Cattle	AM,AMP,CL,STR,SU,TET	20
		Cattle	AM,AMP,KAN,STR,SU,TET	2
		Swine	AM,AMP,CL,STR,SU,TET	1
		Human	AM,AMP,KAN,STR,SU,TET	1
		Human	CL	4
		Human	-	5
		Human	AM,AMP	3
		Human	GE,STR,SU	1
		Human	CL,KAN,TET	3
II	Typhimurium Copenhagen	Cattle	AM,AMP,CL,STR,SU,TET	8
		Cattle	AM,AMP,KAN,STR,SU,TET	1
		Human	-	5
		Human	CL	4
		Human	CL,KAN,TET	1
III	Typhimurium Copenhagen	Cattle	AM,AMP,CL,STR,SU,TET	10
		Human	CL	2
		Human	-	2
IV	Typhimurium Copenhagen	Cattle	AM,AMP,CL,STR,SU,TET	4
		Human	-	1
V	Typhimurium Copenhagen	Human	CL	1
		Swine	AM,AMP,CL,STR,SU,TET	1
		Turkey meat	AM,AMP,CL,STR,SU,TET	1
VI	Heidelberg	Human	-	1
		Turkey meat	AMP,CL,SU,TET	1
VII	Typhimurium Copenhagen	Cattle	AM,AMP,CL,STR,SU,TET	4
		Chicken	AM,AMP,CL,STR	1
		Human	CX	1
		Human	CL	1
		Human	-	2
		Human	AM,AMP	1
VIII	Heidelberg	Human	CL	1
		Turkey meat	SU	1
			Total	95

AM-Amoxacillin/Clavulonic acid, AMP-Ampicillin,CX-Ceftixiaxone, CL-Chloramphenicol, GEN-Gentamicin, KAN-Kanamycin, STR-Streptomycin, SU-Sulfizoxazole,TET-Tetacycline

Table 10. Relationship of molecular types and antibiotic resistance patterns of *Salmonella enterica* serotype isolated from man, domestic animals and animal products.

The rest shared the PFGE but not the AMR profiles. The AMR profile AM,AMP,CL, STR,SU,TET appeared the most common across many PFGE profiles, recorded in 20 bovines and 1 human (profile 1), 8 bovines (profile II), 10 bovines (profile III), 4 bovine (profile IV), 1 swine and 1 turkey (profile V) and 4 bovines (profile VII). Details of AMR profiles of other PFGE profiles will be provided. For the antibiotic susceptibility tests, a total of 9 antibiotic resistant patterns were found for the 55 animal isolates and 40 human isolates with identical PFGE profiles. Of these, cattle isolates accounted for 7, human 19, swine 2, turkey meat 3 and chicken 1 AMR patterns. A review of susceptibility levels of different isolates was summarized. All human (62), swine (2) and turkey (3) and 97 out of 98 cattle isolates were susceptible to amikacin. Resistance to amoxicillin/clavulanic acid was observed in swine (all 2 isolates) and 75 cattle isolates (76.5%) while turkey (n=2, 100%) and human (n=59, 95.2%) were mostly susceptible. All the 2 swine isolates were susceptible to cefoxitin, ceftriaxone, ciprofloxacin, gentamycin, nalidixic acid and trimethoprim/sulfamethoxazole, while resistant to amoxicillin/clavulanic acid, ampicillin, streptomycin, sulfizoxazole and tetracycline. Detailed antimicrobial susceptibility profiles of the different isolates by origin are shown in Tables 10 and 11.

	No of susceptibility isolates (%)				No. of intermediate isolates (%)			No. of resistant isolates (%)			
	Cattle	Human	Swine	Turkey	Cattle	Human	Turkey	Cattle	Human	Swine	Turkey
Amikacin (0.5–64),	97 (99.0)	62 (100.0)	2 (100.0)	3 (100.0)	-	-	-	1(1.0)	-	-	-
Amoxicillin /clavulanic acid (1/0.5–32/16)	2 (2.0)	59 (95.2)	-	2 (66.7)	21 (21.4)	2(3.2)	1 (33.3)	75 (76.5)	1(1.6)	2 (100.0)	-
Ampicillin (2–32)	-	59 (95.2)	-	1 (33.3)	-	-	-	98 (100.0)	3(4.8)	2 (100.0)	2 (66.7)
Cefoxitin (0.5–32)	97 (99.0)	60 (96.8)	2 (100.0)	3 (100.0)	-	1(1.6)	-	1(1.0)	1(1.6)	-	-
Ceftriaxone (0.25–64)	97 (99.0)	60 (96.8)	2 (100.0)	3 (100.0)	-	1(1.6)	-	1(1.0)	1(1.6)	-	-
Chlora-mphenicol (2–32)	1(1.0)	35 (56.5)	1 (50.0)	1 (33.3)	4(4.1)	26(41.9)	-	93 (94.9)	1(1.6)	1 (50.0)	2 (66.7)
Ciproflo-xacin (0.015–4)	97 (99.0)	62 (100.0)	2 (100.0)	3 (100.0)	-	-	-	1(1.0)	-	-	-
Gentamicin (0.25–16)	97 (99.0)	60 (96.8)	2 (100.0)	3 (100.0)	-	-	-	1(1.0)	2(3.2)	-	-
Kanamycin (6–64)	88 (89.8)	58 (93.5)	1 (50.0)	3 (100.0)	-	-	-	10 (10.2)	4(6.5)	1 (50.0)	0.0

	No of susceptibility isolates (%)				No. of intermediate isolates (%)			No. of resistant isolates (%)			
	Cattle	Human	Swine	Turkey	Cattle	Human	Turkey	Cattle	Human	Swine	Turkey
Nalidixic acid (0.5–32)	97 (99.0)	62 (100.0)	2 (100.0)	3 (100.0)	-	-	-	1(1.0)	-	-	-
Streptomycin (32–64)	2 (2.0)	58 (93.5)	-	2 (66.7)	-	-	-	96 (98.0)	4(6.5)	2 (100.0)	1 (33.3)
Sulfizoxazole (16–512)	-	58 (93.5)	-	-	-	-	-	98 (100.0)	4(6.5)	2 (100.0)	3 (100.0)
Tetracycline (4–32),	-	57 (91.9)	-	1 (33.3)	-	-	-	98 (100)	5(8.1)	2 (100)	2 (66.7)
Trimethoprimsulfamethoxazole (4-76)	97 (99.0)	62 (100)	2(100)	3(100)	-	-	-	1(1.0)	-	-	-

Table 11. Drug susceptibility patterns of the common *salmonella* serotypes isolated from domestic animals and human.

4. Discussion

4.1 *Salmonella* from animals

4.1.1 *Salmonella* in feedlot cattle

The study by Tabe et al (2010a, 2010b) reported *Salmonella* prevalence of 12.7% in fecal samples tested. A larger study (Dargatz et al 2003) that evaluated presence of *Salmonella* in fecal samples from cattle in US feedlots (73 feedlots in 12 states during the period from October 1999 to September 2000) had earlier reported a lower overall *Salmonella* prevalence of 6.3%. However, *Salmonella* prevalence at pen and feedlot level was higher. In that study (Dargatz et al 2003) although overall individual animal prevalence was 6.3% (654/10,417), 22.2% (94/422) of pens and 50.7% (37/73) of feedlots had one or more positive samples. Samples collected during the period of April to June (6.8%, 209/3054) and July to September (11.4%, 286/2500) were more likely to be positive than those collected during October to December (4.0%, 73/1838) and January to March (2.8%, 86/3025). The study by Tabe et al (2010a, 2010b) was conducted from October 2006 to March 26, 2007.

An understanding of the genetic diversity of *Salmonella* isolated from cattle could help determine if contamination at a feedlot is due to bacteria that are transient or resident (Galland et al., 2001) in their gut. Transient bacteria can be introduced into the feedlot by arriving cattle, in ingredients for cattle rations such as legume hay, from contaminated water sources, or by other animals (wild or domestic), motor vehicles, and employees (Galland et al., 2001). In the study by Tabe et al (2010a, 2010b), the isolation of *S.* Typhimurium vars Copenhagen as the major *Salmonella* serovar 95% of the time supported previous reports (Hegde et al., 2005; Khaitsa et al., 2007a) of the existence of common genotypes circulating among the steers. *Salmonella* Typhimurium vars Copenhagen which was primarily reported to be found in pigeons is now frequently isolated from cattle, swine,

and other animals (Frech et al., 2003). Another study (NARMS-EB, 2003) reported Typhimurium variant Copenhagen as the most predominant serotype accounting for 16.9% of the total number of isolates examined by U.S. Department of Agriculture's National Animal Health Monitoring System for Enteric Bacteria and reported over a 7-year period (1997 to 2003).

The study by Tabe et al (2010a), reported widespread AMR among the *Salmonella* isolated; all but two of the *Salmonella* isolates were resistant to more than two of the antimicrobials tested with 96.6% of the isolates showing multidrug resistant antibiograms. The widespread AMR of *Salmonella* isolated from cattle in North Dakota had been reported before (Oloya, et al, 2009*)* with most animal strains showing more multidrug resistance compared to human *Salmonella* isolates possibly due to a difference in antimicrobial selection pressure exerted to the microorganisms in the two populations. Isolation of *S*. Typhimurium vars Copenhagen as the major *Salmonella* serovar 95% of the time supports previous reports of the existence of common genotypes circulating among the steers. This similarity in clonal relationship and antimicrobial resistance of *S*. Typhimurium vars Copenhagen was reported in a study that characterized *Salmonella* isolates from feedlot cattle (Khaitsa et al, 2007a), humans, and ready to eat turkey produce (Oloya et al, 2007, 2009). This could possibly be responsible for the spread of such resistant genes among bacteria, a characteristic typical of gram negative bacteria. Surveillance of antibiotic resistance, especially of integrons distribution among bacteria is therefore critical. The genotypic variation in *Salmonella* isolated in healthy feedlot steers reported in this study plus variation in MDR antibiogram supports previous reports that not all MDR *salmonella* Typhimurium do carry a wide variety of resistance genes (Khaitsa et al, 2007a*;* White, 2005). Additionally, isolates with the same resistance phenotypes often had different resistance genotypes, a phenomenon that had been observed before by other studies (Frye and Fedorka-Cray, 2007).

In the study by Tabe et al (2010a), although the prevalence of class 1 and 2 integrons were 50% (29/58) and 35% (2/58), respectively, more than 90% of the isolates were multidrug resistant to Amoxicillin/clavulanic acid, Ampicillin, Chloramphenicol, Streptomycin, Sulfizoxazole, and Tetracycline. The lower frequency of class 2 integron relative to class 1 as seen in this study could probably result from lower exposure to selective pressure of antibiotics among the isolates (Zhao et al, 2005). Additionally, two isolates positive for integron 1 had integron 2. These isolates belonged to genotypes I and IV and showed only about 67% genomic similarity (Figure 1). Additionally, these isolates were recovered from different sampling periods (sampling time one and two respectively). It is important to note that, all 29 isolates with integron 1, were susceptible to Amikacin, Cefoxitin, Cetriaxone, Ciprofloxacin, Gentamycin, Kanamycin, Nalidixic acid, and Trimethoprim-sulfamethoxazole possibly due to the presence of defective resistant genes or the presence of quiescent integrons as reported in a previous study (Khaitsa et al, 2008). The fact that integrons 1 and 2 were not detected in some of the isolates (n=29), 93% (27/29) which were resistant to two or more of the antibiotics, with patterns similar to the positive integron isolates, may be an indication that integrons may play a sufficient but not a necessary role in antibiotic resistance in bacteria. This observation is similar to what has been reported in a previous study where class 1 integron was not always involved in the resistance of *E. coli* isolates to antimicrobial agents (Khaitsa et al, 2008). However integrons have been often associated with broad antibiotic resistance, even if they do not encode multiple drug resistant determinants (Zhang et al, 2004). This was also evident in our study as not all integron bearing strains expressed resistance to antibiotics. Additionally, it is possible that our PCR analysis as designed in this

study missed some large amplicons and most especially integron 2, which contains some gene cassettes encoding antibiotic resistance (Zhang et al, 2004).

The emergence and dissemination of MDR among *Salmonella* isolates from health cattle may have potential adverse implication in public health. Since the first description of class 1 integron by Stokes and Hall (Stokes, H.W., and R.M. Hall. 1989), integron-mediated resistance has been reported in clinical isolates of various organisms including *K. pneumoniae, K. oxytoka, Pseudomonas aeroginosa, E. coli, C. fruedii* and *V. cholerae* (Orman,et al 2002; Sallen *et al, 1995*). It has been reported (Collis, et al, 2002) that classes 1 and 2 are most common in resistant bacteria, and the mobility of these integrons was undoubtedly important in facilitating their spread into many different bacterial species. A study (Krauland et al, 2009) reported that Salmonella enterica bacteria have become increasingly resistant to antimicrobial agents, partly as a result of genes carried on integrons, and that clonal expansion and horizontal gene transfer may contribute to the spread of antimicrobial drug-resistance integrons in these organisms. Krauland et al (2009) investigated this resistance and integron carriage among 90 isolates with the ACSSuT phenotype (resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline) in a global collection of S. enterica isolates. Four integrons, dfrA12/orfF/aadA2, dfrA1/aadA1, dfrA7, and arr2/blaOXA30/cmlA5/aadA2, were found in genetically unrelated isolates from 8 countries on 4 continents, which supports a role for horizontal gene transfer in the global dissemination of S. enterica multidrug resistance. Serovar Typhimurium isolates containing identical integrons with the gene cassettes blaPSE1 and aadA2 were found in 4 countries on 3 continents, which supports the role of clonal expansion. The study by Krauland et al (2009) demonstrated that clonal expansion and horizontal gene transfer contribute to the global dissemination of antimicrobial drug resistance in S. enterica.

The 58 isolates of *Salmonella* Typhimurium var. Copenhagen reported by Tabe et al (2010a) belonged to nine PFGE profiles. Multiple genotypes were frequently observed among *Salmonella* isolated within and between pens sampled in one feedlot in this study (Tabe et al, 2010a). A similar result was reported by a previous study (Edrington et al., 2004) which highlighted the genotypic variation in *Salmonella* isolated from cattle within a farm and among four farms. Another study (Alam et al 2009) that investigated antimicrobial susceptibility profiles of 530 Salmonella enterica serotypes recovered from pens of commercial feedlot cattle reported tremendous strain diversity and multidrug resistance (MDR) among *Salmonella* recovered. This study determined antimicrobial susceptibility profiles, serotype, and presence or absence of the integron-encoded intI1 gene for 530 Salmonella isolates recovered using composite rope (n = 335), feces (n = 59), and water (n = 136) samples from 21 pens in 3 feedlots. Most isolates (83.0%) of the 19 Salmonella serotypes identified were susceptible or intermediately susceptible to all the antimicrobials evaluated. Resistance to sulfisoxazole (14.9%), streptomycin (3.8%), and tetracycline (3.6%) were the most common. None of the isolates tested positive for a class 1 integron, and only 2.5% were resistant to multiple antimicrobials. All the MDR isolates, namely, serotypes Uganda (n = 9), Typhimurium (n = 2), and Give (n = 2), were resistant to at least five antimicrobials. Most MDR isolates (n = 11) were from two pens during 1 week within one feedlot. Overall, many Salmonella isolates collected within a pen were similar in terms of serotype and antimicrobial susceptibility regardless of sample type. However, MDR Salmonella and rare serotypes were not recovered frequently enough to suggest a general strategy for appropriate composite sampling of feedlot cattle populations for Salmonella detection and monitoring. This observation offers an insight into the complexity of the population

dynamics of foodborne pathogens in food animals preharvest and demonstrates their variability in terms of shedding and environmental contamination (Edrington et al., 2004). In order to reduce the prevalence of foodborne pathogens in food animals at slaughter (which could produce significant reductions in the food supply; Hynes et al., 2000), a thorough understanding of the population dynamics of *Salmonella* at the farm level is crucial before implementation of pathogen reduction strategies can be expected to be successful (Edrington et al., 2004).

4.1.2 *Salmonella* from ranch cattle

The study by Theis et al (2005, 2007) reported a prevalence of *Salmonella* in ranch cattle of 7.1%. Other researchers (Dargatz et al., 2000) have reported a lower prevalence (1.4 to 4.5%) than that observed by Theis et al (2005, 2007) while others (Fegan et al, 2004) have reported *Salmonella* prevalence as high as 16%. It is possible that the lower prevalence reported by Theis et al (2005, 2007) could have been attributed to the smaller sample (N =212) of cattle compared to that of other researchers. It is also possible that the time of sampling may have influenced the prevalence of *Salmonella* reported. Seasonal changes have been reported to affect *Salmonella* prevalence. Samples collected during the period of April to June and July to September were more likely to be positive than those collected during October to December and January to March (Fegan et al, 2004). The study by Theis et al (2005, 2007) was conducted from September to November, 2004.

The *Salmonella* serotypes identified in beef cattle (Theis et al, 2005, 2007) were *Salmonella* Typhimurium (Copenhagen) (87%) and *Salmonella* Worthington (13%). The presence of *S.* Typhimurium in cattle and the consequent cross contamination of beef carcass tissue are of particular concern as this serotype is one of the most common causes of *Salmonella* infection in developed countries (Gomez et al, 1997). Of the twenty most common *Salmonella* serotypes identified by the Centers for Disease Control and Prevention (CDC) eight (*Salmonella* Typhimurium, Heidelberg, Agona, Montevideo, Braenderup, Enteritidis, Saint Paul, and Thompson) are found in both human and non-clinical nonhuman isolates (Chen et al, 2004). All 15 *Salmonella* isolates recovered by Theis et al (2005, 2007) were resistant to more than 10 antimicrobials which is an indication that multiple antimicrobial resistance was widespread. This should be of concern because of the potential for therapeutic failures. Other studies have found various levels of antimicrobial resistance. For example one study of *Salmonella* isolates in food animals found that of the 209 *Salmonella* isolates tested 112 (53.6%) were resistant to more than one antimicrobial (Johnson et al., 2005). AMR has been a topic of interest in many studies and the results of those studies vary widely. For instance one study of AMR patterns of *Salmonella* isolated from beef cattle (Dargatz et al., 2000) showed that all of the 1314 *Salmonella* isolates tested were susceptible to amikacin, cefotaxime, and ciprofloxacin with only 14% susceptible to all antimicrobials tested. The remaining 86% showed resistance to at least one antimicrobial agent. The most common resistance observed was to tetracycline with ampicillin, and co-amoxiclav was the second most common class that the *Salmonella* serotypes were resistant.

4.1.3 *Salmonella* from dairy cattle

In the study by Khaitsa et al (2004) five out of 30 (17%) of the cattle sampled tested positive for *Salmonella*. This result was similar to what had been reported in other dairies (NAHMS, 1996; USDA, 2001) with prevalence values ranging from 5.4% to 75%. This result demonstrated that dairies are a potential source of *Salmonella* for susceptible animals/humans.

The United States National Animal Health Monitoring System's Dairy '96 study reported 5·4% of milk cows shed *Salmonella* and 27·5% of dairy operations had at least one cow shedding *Salmonella* [Wells et al, 1998; NAHMS, 1996]. *Salmonella* has been isolated from all ages of dairy cattle and throughout the production process. Mature dairy cattle typically appear asymptomatic while shedding this pathogen in their faeces (Richardson, 1975; McDonough, 1986; Edrington, 2004; Edrington et al, 2004) and while young calves are more susceptible to salmonellosis, cases in adult cattle have been reported (Gay and Hunsaker, 1993; Anderson, 1997; Sato, 2001). Previous research demonstrated significant variation in the prevalence of faecal *Salmonella* in healthy, lactating dairy cattle, not only among farms across the United States (Edrington et al, 2008) but also in farms within a small geographic area and in individual farms from season to season (Edrington et al, 2004) . Additional research examined production parameters (heifers *vs*. mature cows, lactation status, stage of lactation and heat stress) on *Salmonella* prevalence (Edrington, 2004; Fitzgerald et al, 2003). While minor differences were noted in *Salmonella* shedding, results were generally inconsistent with no significant trends noted.

As part of a national study of US dairy operations, another study (Blau et al 2005) conducted between March and September 2002, in 97 dairy herds in 21 states reported an overall prevalence of 7.3% of fecal samples that were culture positive for *Salmonella.* In another study of dairy cattle (Warnick et al. 2003) , *Salmonella* was isolated from 9.3% of 4049 fecal samples collected from a 2 months study of 12 dairy herds originating from Michigan, Minnesota, New York and Wisconsin(Warnick et al, 2003). Also, Fossler et al (2004) sampled dairy cattle to describe the occurrence of fecal shedding, persistence of shedding over time, and serogroup classification of *Salmonella* spp on a large number of dairy farms of various sizes. The design was that of a longitudinal study and the sample population comprised 22,417 fecal samples from cattle and 4,570 samples from the farm environment on 110 organic and conventional dairy farms in Minnesota, Wisconsin, Michigan, and NewYork. Five visits were made to each farm at 2-month intervals from August 2000 to October 2001. Fecal samples from healthy cows, calves, and other targeted cattle groups and samples from bulk tank milk, milk line filters, water, feed sources, and pen floors were collected at each visit. *Salmonella* spp were isolated from 4.8% of fecal samples and 5.9% of environmental samples; 92.7% of farms had at least 1 *Salmonella*-positive sample.

Results from the various studies conducted indicated some variability in the prevalence of fecal shedding of *Salmonella* among the different cattle and production systems sampled possibly due to several factors such as state of origin, treatment with antimicrobials, herd size and season that have previously been reported (Fossler et al, 2005). The study by Fossler et al (2005) that investigated environmental sample-level factors associated with the presence of *Salmonella* in a multi-state study of conventional and organic dairy farms reported that State of origin was associated with the presence of *Salmonella* in samples from cattle and the farm environment; Midwestern states were more likely to have *Salmonella*-positive samples compared to New York. Cattle treated with antimicrobials within 14 days of sampling were more likely to be *Salmonella*-negative compared with nontreated cattle (OR=2.0, 95% CI: 1.1, 3.4). Farms with at least 100 cows were more likely to have *Salmonella*-positive cattle compared with smaller farms (OR=2.6, 95% CI: 1.4, 4.6). Season was associated with *Salmonella* shedding in cattle, and compared to the winter period, summer had the highest odds for shedding (OR=2.4, 95% CI: 1.5, 3.7), followed by fall (OR=1.9, 95% CI: 1.2, 3.1) and spring (OR=1.8, 95% CI: 1.2, 2.6). Environmental samples significantly more likely to be *Salmonella*-positive (compared to bulk tank milk) included, in descending order,

were; samples from sick pens (OR=7.4, 95% CI: 3.4, 15.8), manure storage areas (OR=6.4, 95% CI: 3.5, 11.7), maternity pens (OR=4.2, 95% CI: 2.2, 8.1), hair coats of cows due to be culled (OR=3.9, 95% CI: 2.2, 7.7), milk filters (OR=3.3, 95% CI: 1.8, 6.0), cow waterers (OR=2.8, 95% CI: 1.4, 5.7), calf pens (OR=2.7, 95% CI: 1.3, 5.3), and bird droppings from cow housing (OR=2.4, 95% CI: 1.3, 4.4). Parity, stage of lactation, and calf age were not associated with *Salmonella* shedding. Another study (Fitzgerald et al, 2003)
that examined factors affecting fecal shedding of *Salmonella* in dairy cattle reported that multiparous lactating cows tended to shed more ($P = 0.06$) *Salmonella* than primiparous lactating cows (39% vs 27%, respectively), and that parity did not influence ($P > 0.10$) *Salmonella* shedding in non lactating cows. Unfortunately, information on parity of the cows in Khaitsa et al (2004) was not obtained so comparisons of *Salmonella* prevalence by parity could not be made.
The fact that *Salmonella* isolates recovered by Khaitsa et al (2004) were resistant to more than 10 out of the 20 antimicrobials tested was a concern. Dairy cattle serve as an important reservoir for *Salmonella* and have been implicated in cases of human salmonellosis [CDC, 2003]. In the study by Edrington et al (2008), seven and nine different *Salmonella* serotypes were identified in the healthy and sick dairy cattle, respectively. The serotypes Senftenberg and Kentucky were not detected in any of the healthy cattle and accounted for 34% of the sick isolates. No differences in antimicrobial susceptibility patterns were observed in any the *Salmonella* isolates from sick and healthy cattle. Isolates were susceptible to all antimicrobials examined with the exception of spectinomycin, with three and five isolates resistant in the healthy and diarrhoeic groups, respectively. PFGE was used to compare the genetic relatedness of isolates cultured from the faecal samples of healthy and sick cattle. Seventeen serotypes representing 84 isolates were examined. No genotypic differences were noted when comparing sick *vs*. healthy isolates However, multiple genotypes within serotype were observed for a number of the isolates examined.

4.1.4 *Salmonella* from bison

Salmonella prevalence of 15% reported in the bison herd was comparable to that reported in cattle herds (Beach et al, 2002; Huston et al, 2002; Warnick et al, 2003) and other livestock (Branham et al, 2005) from the US. This is an indication that *Salmonella* prevalence in bison may be more widespread than is currently known. Unfortunately, not many studies of Salmonella occurrence in bison have been reported; it is possible, Khaitsa et al (2008) was the first of such studies reported. A cross-sectional study of 212 cattle from 7 cow-calf operations in North Dakota reported *Salmonella* spp. shedding point prevalence of 7% (15 of 212) of cattle sampled (Theis, 2006). This prevalence was similar to that reported for bison given the limitation of number of animals sampled in both studies. It is also possible that the time of sampling may have influenced the prevalence of *Salmonella* reported. Seasonal changes have been reported to affect prevalence of *Salmonella* fecal shedding in cattle (Dargatz et al, 2003). Samples collected during the period of April to June and July to September were more likely to be positive than those collected during October to December and January to March (Dargatz et al, 2003). In this study we sampled bison in June 2005 while Theis (2006) sampled cattle from September to November, 2004. Another longitudinal study (Branham et al, 2005) that assessed *Salmonella* spp. presence in white-tailed deer (*Odocoileus virginianus*) and livestock simultaneously grazing the same rangeland, reported *Salmonella* prevalence of 2/26 (7.69%) and 6/82 (7.32%) in deer and sheep, respectively, and

a lower prevalence of (3/81 (3.70%), and 1/80 (1.25%) in goats and cattle, respectively, all from samples taken in September.

The *Salmonella* isolated from bison feces (Khaitsa et al, 2008) belonged to the serotypes *Salmonella* Typhimurium (Copenhagen) and *Salmonella* Worthington. This was not a total surprise since bovine are a common source of *Salmonella* Typhimurium (Cray et al, 2006). It is interesting to note that the same serotypes, *Salmonella* Typhimurium (Copenhagen) and *Salmonella* Worthington, were recovered from cattle on cow-calf operations in North Dakota during the same year[35] (Theis, 2006). However, a larger study of beef cattle (Beach et al 2002), reported that the five serotypes most commonly associated with feedlot cattle and their environment were Salmonella Anatum (18.3% of the isolates), Salmonella Kentucky (17.5%), Salmonella Montevideo (9.2%), Salmonella Senftenberg (8.3%), and Salmonella Mbandaka (7.5%). The five serotypes most commonly associated with nonfeedlot cattle and their environment were Salmonella Kentucky (35.4%), Salmonella Montevideo (21.7%). Salmonella Cerro (7.5%), Salmonella Anatum (6.8%), and Salmonella Mbandaka (5.0%) (Beach et al 2002).

Other studies[9], (Edrington et al 2004) have reported different *Salmonella* serotypes recovered from cattle originating from other states, possibly due to regional differences. In one study (Edrington et al 2004)[9] mature dairy cattle were sampled over a 2-year period (2001-2002) on six farms in New Mexico and Texas. Fecal samples (n = 1560) were collected via rectal palpation and cultured for *Salmonella*, and one isolate from each positive sample was serotyped. Twenty-two different serotypes were identified from a total of 393 Salmonella isolates. Montevideo was the predominant serotype (27%) followed by Mbandaka (15%), Senftenberg (11.4%), Newport (6.4%), Anatum (4.8%), and Give (4.8%). Salmonella Typhimurium and Dublin, two frequently reported serotypes, accounted for only 1% of the observed serotypes in this study. A national *Salmonella* study of 97 dairy herds in 21 states in the US reported *Salmonella* Meleagridis (24.1%), Salmonella Montevideo (11.9%), and *Salmonella* Typhimurium (9.9%) as the three most frequently recovered serotypes (Blau et al 2005). It is noteworthy that *Salmonella enterica* serovar Hadar was the major *Salmonella* serotype isolated from processed bison carcasses originating in the same region as our sampled animals[25] (Li et al, 2006). In the absence of studies that correlate recovery of *Salmonella* from the same bison pre and post-harvest, it is difficult to ascertain the sources of contamination of bison carcasses post-harvest.

In the study Khaitsa et al (2008) both *Salmonella* isolates were susceptible to at least 6 antimicrobials on the panel including the cephalosporin - ceftiofur and the quinolone/fluoroquinolone - enrofloxacin that are clinically important. However, both isolates (100%) demonstrated widespread multi-drug resistance (resitance to ≥ 13 antimicrobials) in a panel of 20.antimicrobials with resistance most frequently to tetracycline, streptomycin, and/or ampicillin). In a larger study (Dargatz et al 2003) of 73 feedlots in 12 states the antimicrobial resistance patterns of *Salmonella* spp recovered were determined. The susceptibilities of all isolates were determined using a panel of 17 antimicrobials. The majority of isolates (62.8%, 441/702) were sensitive to all of the antimicrobials tested. Resistance was most frequently observed to tetracycline (35.9%, 252/702) followed by streptomycin (11.1%, 78/702), ampicillin (10.4%, 73/702) and chloramphenicol (10.4%, 73/702). Multiple resistance (resistance to > or =2 antimicrobials) was observed for 11.7% (82/702) of the isolates. However, overall, most of the *Salmonella* isolates were sensitive to all the antimicrobials tested. Interestingly, antimicrobial testing of *Salmonella enterica* serovar

Hadar recovered from bison carcasses originating from the same region as our sample bison also demonstrated resistance to tetracycline, gentamicin, sulfamethoxazole, and streptomycin[25], results that were quite similar to what we reported for isolates from apparently healthy bison. Additionally, both isolates recovered in our study were susceptible to apramycin. In comparison with human isolates, of the 2613 isolates tested in 1999-2000 at the 17 public health laboratories participating in NARMS, 26% (679) were resistant to >1 agent; 21% (546) were multidrug resistant (resistant to >2 agents)[1] (Angulo et al, 2001). Three multidrug resistant strains accounted for 10% (263/2613) of all Salmonella isolates, 38% (263/679) of the resistant isolates and 48% (263/546) of the multidrug resistant isolates. In particular, 30% (162/546) of multidrug resistant *Salmonella* were *S*. Typhimurium R-type ACSSuT, 12% (63/546) were S. Typhimurium R-type AKSSuT, and 7% (38/546) were S. Newport R-type ACSSuT; no other multidrug resistant patterns accounted for more than 5% of multidrug resistant Salmonellae.

It was interesting to note that in spite of the reports that antibiotics were not routinely used in the study herd, and that no other animals were raised on the farm together with the bison, antimicrobial resistance was detected in the *Salmonella* isolates recovered. It is possible that since the animals were not housed, and the pasture was not completely fenced, wild life, birds and other domestic livestock had access to the animals. It is possible therefore that even when antibiotics were not used in the bison, *Salmonella* isolated from the bison could have acquired resistance through horizontal transfer from other multidrug resistant organisms originating from wild life, birds or other domestic livestock that had access to the bison. Hoyle et al., 2005 discuss the problem of possible transfer of resistance, which may occur horizontally or vertically from enteric organisms such as *Salmonella* to other organisms. Many pathogenic and commensal organisms are multidrug resistant due to exposure to various antibiotics. Often, this antimicrobial resistance is encoded by integrons that occur on plasmids or that are integrated into the bacterial chromosome. Integrons are commonly associated with bacterial genera in the family *Enterobacteriaceae* (Goldstein et al 2001). Most of the resistance integrons found to date in clinical isolates of *Enterobacteriaceae* are class 1 integrons, which are highly associated with resistance to antimicrobial agents (Norrby 2005). Multi-drug resistant phenotypes have been associated with large, transferable plasmids such as integrons (Schoeder et al 2003). These plasmids are stable, transfer readily to other microorganisms in the same environment, and often contain cassettes encoding resistance to one or more classes of antimicrobials (Schoeder et al 2003) thus, resistance to an antimicrobial not routinely used in clinical medicine can mean resistance to one that is (Schoeder et al 2003). This finding has implications for animal and public health due to the potential for failure to treat some infections in animals and humans with the drugs that are currently on the market.

4.2 *Salmonella* from meats

In the study by Khaitsa et al (2007b) that investigated the occurrence of *Salmonella* in raw and ready to eat turkey meat products, in 959 turkey meat products (raw, n =614; and ready to eat (RTE), n = 345) purchased from four retail outlets in the Midwestern United States, overall, *Salmonella* was detected in 2.4% (23 of 959) of the retail meat samples with most 5% (16/329), recovered from raw meats and only 1% (7/607) from ready to eat meat samples. This finding was significant as it demonstrated that control strategies for this pathogen post-production are meeting with some success. However, recovery of *Salmonella* from the ready

to eat meat products was a concern as it indicated that control strategies for this pathogen post-processing in these ready to eat turkey products was not completely successful. This may be attributed to the way the meats are handled after processing (CDC, 1998).

Other researchers have reported similar low recovery of *Salmonella* in retail meats (Ono, 1999; , Mayrhofer et al, 2004, Whyte et al, 2004, Zhao et al, 2001*)*. It was also reported that among raw turkey meat products, ground turkey had higher *Salmonella* contamination rates than whole turkey or other turkey parts (drumsticks, thighs, breast, breast cutlets, wings, breakfast link, bratwurst, sausage and bacon). This was not a total surprise as ground turkey samples have traditionally had higher food borne pathogens compared to whole turkey or turkey parts *(*Cloak et al, 2001*)*. This is possibly due to the fact that ground turkey is an amalgamation of large numbers of meat parts from different sources that are eventually ground together. *Salmonella* contamination of poultry meat has been reported to be seasonal with higher prevalence in summer than other seasons (Wallace et al, 1997). Although *Salmonella* recovery was reported to be higher in spring than winter, the study was limited in that it spanned over a period of only 6 months so could not possibly provide us with the best estimates of seasonal occurrence of *Salmonella.*

While some previous researchers (Zhao et al, 2001) reported similar *Salmonella* prevalence (4.2%) to ours, others *(*Soultos et al, 2003*)* reported lower levels. Low *Salmonella* incidence rates in chicken of 1.5% were reported by Soultos et al *(2003)*. Another study (Zhao et al, 2006) of *Salmonella* from retail foods of animal origin reported a higher prevalence (6%) than what we observed. However, the *Salmonella* distribution within the meat products was similar to ours, with ground turkey and chicken having the highest *Salmonella* contamination rates; overall, six percent of 6,046 retail meat samples (n = 365) were contaminated with *Salmonella*, the bulk recovered from either ground turkey (52%) or chicken breast (39%). There are other studies that have reported higher *Salmonella* prevalence (16.4% to 35.8%) than reported here (Domínguez et al, 2002; Duffy et al, 199; Mayrhofer et al, 2004, White et al, 2001). In one study (White et al, 2001), 200 meat samples were processed and 41 (20 percent) contained *Salmonella*, with a total of 13 serotypes. The majority of *Salmonella* isolates (61.5%) in the Khaitsa et al (2007b) study were recovered from ground turkey. In the study by Kegode et al (2008), *Salmonella* prevalence was 3% (13/ 456) of all retail meat samples. The *Salmonella* contamination rate for chicken was 4.1% (5/123), which is strikingly similar to what Zhao et al (2001) reported for grocery stores in the Washington, DC metropolitan area. In that study, *Salmonella* was isolated from 3.0% of the 825 meat samples, and chicken had a *Salmonella* contamination rate of 4.2%. Furthermore, the percentage of *Salmonella* recovered in the assorted turkey and chicken parts was similar to findings of the larger FoodNet study conducted in 2002 to 2003 (Zhao et al, 2006). Kegode et al (2008) did not report any *Salmonella* from beef and pork products tested.

Recovery of *Salmonella* from the retail meat products was not influenced by the store type (Khaitsa et al, 2007b). The possible explanations for this finding include; similar product batches within stores, the location of stores within one city, low number of stores sampled, short sampling time and the relatively smaller number of samples tested. It is possible that the relatively low prevalence of *Salmonella* recovered from our study hindered our ability to detect a significant difference among the stores. Also, the relatively smaller number of stores in our study (5 compared to 58 in that study (Zhao et al, 2001) may have explained the difference in results.

Khaitsa et al (2007b) reported the predominant *Salmonella* serotype in retail meats as *S. heidelberg* (30.8%) followed by *S. kentucky* (15.4%). Studies have reported different serotypes

and proportions recovered from meat products. One study found that *S. heidelberg* was predominant in chicken, *S. Montevideo* in beef, *S. hadar* in turkey and *S. derby* in pork (Schlosser et al, 2000). The three major *Salmonella* serotypes (Heidelberg, Typhimurium and Kentucky) reported by Kegode et al (2008) were similar to major serotypes reported by the larger studies conducted by FoodNet and others (Zhao et al, 2001; CDC, 2005; CDC, 2006). For example, in 2005, the *Salmonella* serotypes accounting for 56% of human infections included Typhimurium (20%), Enteritidis (15%), Newport (10%), Javiana (7%), and Heidelberg (5%) (CDC, 2006). Another study found the predominant serotype to be *S. typhimurium* var Copenhagen (Sorensen et al, 2002). Other studies have reported the predominant serotype to be *S. enteritidis* (Domínguezet al, 2002; Mayrhofer et, 2004), *S. bredeney* (Duffy et al, 1999) and *S. anatum* (Mrema et al, 2006). The different results may reflect the different meat types examined (meat cuts vs ground meat) or different geographic locations of sampling. Regional variation in predominant serotypes of bacterial foodborne pathogens has previously been reported (CDC, 1998).

In the study by Tumuhairwe et al, 2007) that investigated the temporal and spatial distribution of 1465 salmonellosis outbreaks involving 49/50 states in the US , overall, when the incidence rates were computed, the states with higher rates were not necessarily those with higher outbreak occurrences, an indication that these states probably had better reporting systems. Membership in FoodNet (US federal agency that actively monitors seven foodborne disease trends including *Salmonella*) may have explained the comparatively large number of reports originating from California, Maryland, and New York. The four major *Salmonella* serotypes commonly isolated in humans in the US are: S. Enteritidis, S. Typhimurium, S. Heidelberg and S. Newport; Three of these serotypes (S. Enteritidis, S. Heidelberg and S. Newport) were the most implicated in both TMAOs and SOOVs compared to the other serotypes. Additionally, S. Reading was frequently isolated in TMAOs in this study. This observation was in agreement with other studies (CDC, 2005; CDC, 2006) that have cited S. Reading as a common serotype in turkey meats. Also, it is interesting to note that S. Reading and S. Heidelberg were among the serotypes recovered from turkey farms and their environment, where S. Heidelberg was relatively more common in both humans and turkeys than S. Reading.

The Centers for Disease Control Foodborne Diseases Active Surveillance Network (FoodNet) data indicate that outbreaks and clusters of food-borne infections peak during the warmest months of the year (CDC, 2006). Additionally, some studies have shown that the rate of microbial contamination of food products follows the same trend (CDC, 2003; CDC, 2006). Since our study was conducted during the warmest months of the year, the prevalence estimates of the food-borne pathogens obtained should be fairly representative of their true estimate. One limitation of the study was that we could not evaluate the seasonality of microbial contamination of retail meats due to the short sampling period; the study was conducted only during one season (summer). It has been suggested that future food safety studies focusing on seasonality components of microbial contamination of retail meats may require larger sample sizes and longer analysis periods (Zhao et al, 2006. Also, the location of sampling, the relatively smaller number of samples tested and low number of stores sampled may have influenced the results of this study. S. Heidelberg was the predominant serotype identified (23%), followed by S. Saintpaul (12%), S. Typhimurium (11%), and S. Kentucky (10%). Overall, resistance was most often observed to tetracycline (40%), streptomycin (37%), ampicillin (26%), and sulfamethoxazole (25%). Twelve percent of isolates were resistant to cefoxitin and ceftiofur, though only one isolate was resistant to

ceftriaxone. All isolates were susceptible to amikacin and ciprofloxacin; however, 3% of isolates were resistant to nalidixic acid and were almost exclusive to ground turkey samples (n = 11/12). All Salmonella isolates were analyzed for genetic relatedness using pulsed-field gel electrophoresis (PFGE) patterns generated by digestion with Xba1 or Xba1 plus Bln1. PFGE fingerprinting profiles showed that Salmonella, in general, were genetically diverse with a total of 175 Xba1 PFGE profiles generated from the 365 isolates. PFGE profiles showed good correlation with serotypes and in some instances, antimicrobial resistance profiles. Results demonstrated a varied spectrum of antimicrobial resistance and PFGE patterns, including several multidrug resistant clonal groups among Salmonella isolates, and signify the importance of sustained surveillance of foodborne pathogens in retail meats. (Zhao et al, 2006).

4.3 *Salmonella* from clinical cases of animals and humans

In the study by Oloya et al (2007), more *Salmonella* isolates were recovered from feces of apparently healthy feedlot cattle (25.8%) than range or beef cattle (3.9%) or dairy (1.2%) cattle. A similar *Salmonella* prevalence in feedlot cattle had been reported before and been attributed to low hygiene in feedlots (Vanselow et al. 2007; Khaitsa et al. 2007a). Also, previous reports of *Salmonella* prevalence in range cattle (Ranta et al. 2005) and dairy cattle (Sorensen et al. 2003; Huston et al. 2002) have been comparable to what is reported by this study, and have been consistently lower than in feedlot cattle. However, the isolation of *Salmonella* in sick or dead cattle (13.6%) and sick humans (41.2%) was indicative of its increasing role in causing disease in both groups of hosts (Besser et al. 2000; Padungtod and Kaneene 2006). Previous studies have reported lower prevalence of salmonellosis in both humans and cattle in ND (Tumuhairwe et al. 2008) and the US (Tumuhairwe et al. 2007).

Human isolates were more diverse (32 different serotypes) than cattle (9 serotypes) or other domestic animal species with the following predominant serotypes; *S. Typhimurium* (cattle and man), S. *Newport* (cattle, man and turkey) and *S. Heidelberg* (man and turkey) (Oloya et al, 2007). The occurrence of *Salmonella* serovars; Agona, Anatum, Heidelberg, Newport, St. Paul and Typhimurium in turkey and man, Infantis, Mbandaka, Newport and Typhimurium in cattle and man and many other less frequently recovered serotypes in both domestic animals and man, highlights the scope and magnitude of risk of *Salmonella* infection from individual species of domestic animals to man (Besser et al. 2000; Gorman and Adley 2004; Oloya et al. 2007; Padungtod and Kaneene 2006). Previous studies had reported clonal relationships of *Salmonella* serovars from humans and non-animal and animal sources and products (Gorman and Adley 2004; Padungtod and Kaneene 2006; Zhao et al. 2003).

The PFGE results showed occurrence of similar genotypes of *Salmonella* isolates in both domestic animals and humans (Oloya et al, 2007). However, it was not possible to ascertain whether the transmission was from domestic animals to humans or either way. Previous studies (Besser et al. 2000; Gorman and Adley 2004) have provided incriminating evidence against food animals or their products as being responsible for transmission of *Salmonella* to humans. The most common PFGE fingerprint profiles I, II, III and IV had strong cattle and human involvement (Figure 2). Since *Salmonella* serovar Typhimurium was a major infection in both domestic animals and humans the isolation of *Salmonella* serotypes with similar PFGE fingerprints profiles in both groups confirms existence of common clones or genotypes between human and animal sources and suggests occurrence of an epidemic strain circulating between the two groups (Tsen et al. 2002). Interestingly, the isolation of serovars with the exact similar PFGE fingerprint patterns in cattle preceded those in

humans, suggesting a difference in timing of outbreak and possibly, the direction of infection from domestic animals to humans. Recent evidence of clustering of *S. Typhimurium* infection in domestic animals and correspondingly high case reports of the same serovars in humans in the same counties of ND (Oloya et al. 2007), concurs with an earlier observation that region and infection of domestic animals influence S*almonella* occurrence in humans (Torpdahl et al. 2006).

AMR profiles showed that most domestic animal strains were multidrug resistant (Oloya et al, 2007). Cattle isolates were resistant (>76.5%) to Amoxicillin/clavulanic acid, ampicillin, chloramphenicol, streptomycin and tetracycline, while human isolates were of comparatively lower resistance to the similar individual drugs (1.6-8.1%) or drug combinations. Only 1 human isolate with similar PFGE profile as the main group of cattle isolates, had similar range of multidrug resistance, providing a single evidence of a possible AMR transmission from cattle to humans. Whereas parallel development of resistance in humans as result of using antibiotics that are identical to those used in animals (Phillips et al. 2004; Tumuhairwe et al. 2007) could not be ruled out, this scenario is less likely. Various epidemiological studies (Besser et al. 2000; Padungtod and Kaneene 2006; Zhao et al. 2003) have provided insights into the roles of domestic animals or their products in the transmission of *Salmonella* and associated antimicrobial drug resistance to humans. Occurrence of serovars with similar PFGE profile may suggest that some cases of human salmonellosis are the results of the circulation of certain strains between animal and human hosts (Phillips et al. 2004). However, the occurrence of different AMR profiles within the similar PFGE patterns suggests fairly established strains in which the domestic animal isolates are more subjected to antimicrobial pressure in the production systems (Zhao et al. 2003), hence the higher resistance compared to the human isolates. If the widespread use of antimicrobial agents in animal husbandry is selecting for antimicrobial-resistant serotypes and there is transmission to humans, then these ought to be reflected in the resistance profiles of salmonella isolates from humans in the same period.

The presence of resistance to chloramphenicol or drug patterns; amoxicillin-ampicillin and chloramphenicol-kanamycin-tetracycline combinations in humans but not in domestic animals could have equally resulted from use of these antibiotic drugs in humans (Phillips et al. 2004). The fact that most isolates with multi-drug resistance were from cattle and only a single human case had the similar resistance profile suggests that *Salmonella* in cattle or predominantly food animals may not play a significant role in transmitting AMR to S*almonella* in humans. This observation may also support the argument that adequate cooking destroys bacteria in the food (Phillips et al. 2004) and could be that one important barrier to both human infection and AMR transfer. Evidence linking antimicrobial use in food animals to human health risk points to but does not prove a human health threat (Barza and Travers 2002). Attempts could also be made to explain this difference in light of the time lag between time of outbreaks in cattle and humans. Reduction in the antibiotic selection pressure from cattle to humans could result in loss of expression of specific resistance genes (Dowd et al. 2008) as well as loss of the mobile genetic elements responsible for resistance (Kang et al. 2006), but this is beyond the scope of this study.

The diverse *Salmonella* serotypes observed infecting man, suggests other possible sources of infection in human environment. Differences could also arise from the fact that not all infections arise directly from farm animals in contact with the farmers, but also from other sources such as pets and contaminated produce (Johnston et al. 2006) or water sources (Phillips et al. 2004) that may not have been captured in this study. In conclusion, this study

demonstrated that although there were similarities in *Salmonella* genotypes responsible for infection in both domestic animals and humans in the 2000-2005 period, both the AMR and multidrug resistance levels in animals were higher than in humans suggesting that resistance acquired in domestic animals did not translate directly into the burden of resistance in humans.

Greene et al (2008) conducted a nationwide study in the US to test for regional differences in risk factors for human infection with salmonellosis. The study analyzed distributions of the two most prevalent MDR Salmonella phenotypes in the United States, 2003-2005: (i) MDR-ACSSuT (resistant to at least ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) Typhimurium; (ii) MDR-AmpC (resistant to at least ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, amoxicillin/clavulanic acid, and ceftiofur, and with decreased susceptibility to ceftriaxone) Newport. Participating public health laboratories in all states forwarded every 20th Salmonella isolate from humans to the National Antimicrobial Resistance Monitoring System for Enteric Bacteria for antimicrobial susceptibility testing. Among the serotypes Typhimurium and Newport isolates submitted 2003-2005, pansusceptible, MDR-ACSSuT Typhimurium, and MDR-AmpC Newport were identified. Patterns of resistance, demographic factors, and cattle density were compared across regions. Of 1195 serotype Typhimurium isolates, 289 (24%) were MDR-ACSSuT. There were no significant differences in region, age, or sex distribution for pansusceptible versus MDR-ACSSuT Typhimurium. Of 612 serotype Newport isolates, 97 (16%) were MDR-AmpC, but the percentage of MDR-AmpC isolates varied significantly across regions: South 3%, Midwest 28%, West 32%, and Northeast 38% ($p < 0.0001$). The South had the lowest percentage of MDR-AmpC Newport isolates and also the lowest density of milk cows. More Newport isolates were MDR-AmpC in the 10 states with the highest milk cow density compared with the remaining states. Overall, 22% of pansusceptible Newport isolates but only 7% of MDR-AmpC Newport isolates were from patients <2 years of age. For both serotypes, MDR phenotypes had less seasonal variation than pansusceptible phenotypes. This was the first analysis of the distribution of clinically important MDR Salmonella isolates in the United States. MDR-ACSSuT Typhimurium was evenly distributed across regions. However, MDR-AmpC Newport was less common in the South and in children <2 years of age. Information on individuals' exposures was needed to fully explain the observed patterns. Moreover, another study (Nielsen, 2009) reported variation in antimicrobial resistance in sporadic and outbreak-related Salmonella enterica serovar Typhimurium from patients in Denmark. Variation in antimicrobial resistance and corresponding changes of SGI1 were shown among isolates from a foodborne outbreak (Nielsen, 2009).

5. Conclusion

The study on *Salmonella* occurrence from naturally infected feedlot cattle housed at the North Dakota State University cattle feedlot research facility highlighted the genotypic variation in *Salmonella* isolated in healthy feedlot steers and also supported previous reports that not all MDR *salmonella* Typhimurium do carry a wide variety of resistance genes, and also that isolates with the same resistance phenotype often have different resistance genotypes. Also the widespread AMR observed in the majority of *Salmonella* isolates was not matched with presence of integrons, an indication that besides integrons, AMR in *Salmonella* may be explained by other mechanisms that warrant further research. Prevalence

of *Salmonella* in grass fed cattle in ND was 7.1%, relatively higher than some studies have reported. *Salmonella* Typhimurium was the most common cause of salmonellosis in animals in North Dakota. *Salmonella* Typhimurium (Copenhagen) serotype was identified as the major serotype that was being shed by ranch beef cattle. The data show that multi-drug resistance was widespread among the *Salmonella* recovered from apparently healthy grass fed cattle. The emergence of multi-drug resistant *Salmonella* reduces the therapeutic options in cases of invasive infections and has been shown to be associated with an increased burden of illness.

The study of salmonella occurrence in dairy cattle demonstrated that a substantial percentage of cattle in this dairy was shedding *Salmonella* in the feces, and antimicrobial resistance among the five *Salmonella* isolates was widespread. It is possible that some management practices of dairies related to antimicrobial use may contribute to developing *Salmonella* serotypes that are resistant to antimicrobials. The study on *Salmonella* occurrence in a bison herd indicated that Salmonellae were shed in feces of bison at a comparable prevalence to that of cattle herds in the US, and that the isolates were multidrug resistant. The data contribute to risk assessment of *Salmonella* in bison and highlight the possible existence of antimicrobial resistance in bison. The multi-drug resistance reported among the *Salmonella* isolates warrants further study considering that the serotype *S.* Typhimurium is widely distributed and has the potential of greatly impacting human and animal health. The study on retail meats indicate that turkey meat products from retail stores may occasionally be contaminated with *Salmonella* possessing a varied spectrum of antimicrobial resistance. The contamination was dependent on the type of meat and the time of sampling. These data confirm that both raw and ready to eat retail turkey meat products may be vehicles for transmitting salmonellosis, some of which is resistant to antimicrobials justifying the need for sustained surveillance of foodborne pathogens in retail meats.

The study that compared *Salmonella* isolates from clinical cases of humans and animals reported that human isolates were more diverse than cattle or other domestic animal species. PFGE results confirmed occurrence of similar *Salmonella* genotypes in both domestic animals and humans, with the isolation in cattle preceding those in humans. This suggests a spread of infection from domestic animals to humans. AMR profiles showed that domestic animal strains were multidrug resistant. Only 1 human isolate had similar PFGE profile as cattle isolates with a similar range of multidrug resistance, providing a single evidence of a possible AMR transmission from cattle to humans. This study demonstrated that although there were similar *Salmonella* genotypes from domestic animals and humans, the AMR levels observed in domestic animal isolates was higher than in humans, implying that cattle or food animals may not play a significant role in transmitting AMR to S*almonella* in humans and that the occurrence of resistance in animal isolates may not translate directly into resistance in human isolates in this area.

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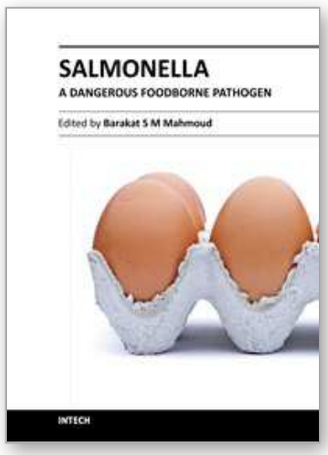

Salmonella - A Dangerous Foodborne Pathogen

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More than 2,500 serotypes of Salmonella exist. However, only some of these serotypes have been frequently associated with food-borne illnesses. Salmonella is the second most dominant bacterial cause of food-borne gastroenteritis worldwide. Often, most people who suffer from Salmonella infections have temporary gastroenteritis, which usually does not require treatment. However, when infection becomes invasive, antimicrobial treatment is mandatory. Symptoms generally occur 8 to 72 hours after ingestion of the pathogen and can last 3 to 5 days. Children, the elderly, and immunocompromised individuals are the most susceptible to salmonellosis infections. The annual economic cost due to food-borne Salmonella infections in the United States alone is estimated at $2.4 billion, with an estimated 1.4 million cases of salmonellosis and more than 500 deaths annually. This book contains nineteen chapters which cover a range of different topics, such as the role of foods in Salmonella infections, food-borne outbreaks caused by Salmonella, biofilm formation, antimicrobial drug resistance of Salmonella isolates, methods for controlling Salmonella in food, and Salmonella isolation and identification methods.

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