PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 35, No. 4, p.280-289

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Prevalence and Antimicrobial Resistance of *Salmonella enterica* Shed from Range and Feedlot Cattle from Post-weaning to Slaughter

ABSTRACT

The objectives of this study were to (1) estimate the prevalence of *Salmonella* in beef cows and steers at postweaning, finishing and slaughter, and (2) determine antimicrobial resistance of isolates, and (3) assess the association between resistance and presence of class 1 integrons. Fecal samples were collected from 48 cows and 48 steers at multiple sampling periods, and mid-line sponge samples collected from steer hides before slaughter. Bacteriological culture, antimicrobial resistance tests, and polymerase chain reaction testing were performed. *Salmonella* prevalence varied from 8% (3/38) to 92% (35/38) in cows and from 28% (13/47) to 100% (24/24) in steers, with higher estimates at postweaning than at finishing and slaughter. Of the 200 isolates recovered, the majority (56%) were resistant to 2 or more antimicrobials. Class 1 integrons were detected in 98 isolates, of which 88 (90%) carried the conserved sequence for *aadA* resistance gene encoding for streptomycin/spectinomycin resistance. Study findings revealed that most steers shed *Salmonella* at postweaning, indicating widespread exposure either before or after weaning. The recovery of multidrug resistant isolates and presence of class 1 integrons carrying the *aadA* resistance gene further underscores the dilemma and public health significance associated with veterinary use of antibiotics such as streptomycin in beef cattle.

INTRODUCTION

Nontyphoidal *Salmonella* spp. are considered one of the most important foodborne pathogens that affect humans. In the United States (U.S.), each year, foodborne pathogens cause an estimated 9.4 million episodes of illness, 55,961 hospitalizations, and 1,351 deaths. Nontyphoidal *Salmonella* spp. cause 11% of the illnesses and are the leading cause of hospitalizations (35%) and death (28%) *(35)*. One of the reasons *Salmonella enterica* (*Salmonella*) is widely associated with foodborne illnesses is its prevalence in beef cattle. The occurrence of *Salmonella* in U.S. beef cattle is well documented *(1, 8, 13, 14, 16, 23, 26, 28)*. *Salmonella* present in feces and on the hides of beef cattle during harvest has been linked to beef carcass contamination *(3, 14, 23, 26, 28)*. Several *Salmonella*

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outbreaks in humans have been associated with the consumption of undercooked ground beef *(4, 6, 15, 34, 36)* and other beef products *(5)*.

Information on the prevalence of *Salmonella* fecal shedding at post-weaning, finishing and slaughter can be used to identify pre-harvest interventions that reduce *Salmonella* contamination of beef. Previous studies that investigated the prevalence of *Salmonella* fecal shedding in feedlot cattle focused mostly on the finishing and slaughter stages, at feedlot entry and at slaughter. Reported prevalence estimates at feedlot entry varied from 0.7% to 64.7% *(10, 16, 22, 38)*, 5.6% to 13% *(21)* at finishing, and 0% to 72.6% at slaughter *(10, 16, 22, 38)*. Additional studies are needed to identify critical control points in the production chain (i.e., post-weaning, finishing, and slaughter) that can be targeted in pre-harvest interventions to reduce pathogen loads.

The detection of antimicrobial resistant *Salmonella* in beef cattle is a major public health concern. *Salmonella* strains resistant to a wide range of antimicrobial agents have been isolated from beef cattle *(8, 19, 32)*, and resistance to antibiotics commonly used in treatment of human diseases has been reported for amoxicilin, chloramphenicol, ampicillin, streptomycin, and tetracycline *(8, 19, 32)*. A substantial number of nontyphoidal *Salmonella* strains isolated from humans are resistant to antibiotics *(7, 27, 29)*. Human infection with antimicrobial-resistant *Salmonella* is associated with increased risk of severe infection, hospitalization, and death *(40)*.

The ability of microorganisms to evade or to become resistant to antibiotics can be attributed to several mechanisms, including mobile DNA elements such as integron gene cassettes, transposons, and plasmids. Mobile DNA elements facilitate the transfer of antibiotic resistance genes between different species of bacteria *(17)* and are an efficient route of acquisition and vertical and horizontal dissemination of resistance determinants *(9, 24, 25, 30, 37)*. Four classes of integrons have been described *(20, 30)*, and class 1 integrons are the most frequent in clinical strains of different organisms *(19)*. Class 1 integrons have been reported in resistant *Salmonella* isolates obtained from human clinical strains *(19)*, poultry *(17)*, and animals or their environment *(11)*. Although integrons have been reported in resistant *Salmonella* isolates, few studies have evaluated the relationship between the presence of class 1 integrons and antimicrobial resistance in *Salmonella* isolated from beef cattle. Information on antimicrobial resistance patterns, distribution of class 1 integrons, and the relationship between antimicrobial resistance and presence of class 1 integrons is essential in understanding antimicrobial resistance of *Salmonella* isolated from beef cattle.

The objectives of this study were (1) to estimate the prevalence of *Salmonella* in beef cows and steer calves at postweaning (off the range or pasture), at finishing and slaughter, and (2) to determine the antimicrobial resistance patterns of the isolates, and (3) to assess the association between antimicrobial resistance and presence of class 1 integrons.

MATERIALS AND METHODS

The study protocol was approved by the Institute for Animal Care and Use Committee at North Dakota State University (approved protocol #A0813). Part of the methods herein (study design, housing and feeding of cattle, sampling design, and sampling procedure) have been described elsewhere *(12)*.

Study design

This longitudinal study involved 48 cows and 48 spring-born calves reared in a semi-arid region of western North Dakota and was conducted over a 9-month sampling period (September 2008 to June 2009). This study was superimposed on a foundational study designed to investigate the effect of weaning date on postweaning steer growth and economic performance through the growing and finishing period to final harvest. The natural range grazing phase of the foundational study was conducted at the North Dakota State University Dickinson Research Extension Center (DREC) ranch headquarters located north of Dickinson. In the foundational study, the cows and their steer calves were randomly assigned to two weaning date treatments (Early Wean [EW] and Normal Wean [NW]) based on steer calf birth date.

Cattle management

The 48 cows and their 48 steer calves were managed at the DREC at the onset of the study. The cows remained at the DREC throughout the study period; however, after postweaning corn grazing, the calves were transferred to the University of Nebraska Panhandle Feed Lot (NPF) in Scottsbluff for growing and finishing. The cows grazed native pasture from the beginning of the study (September 2008) until 3 November 2008; then through 30 April 2009, cows were fed an alfalfa-grass mixed diet while housed in a winter dry lot. The 48 calves were assigned to treatments (EW and NW) based on birth date, weighed, and processed, which included branding and vaccination on April 29 and 30, 2008. The EW steer calves (n = 24) were weaned on August 13, 2008, and the NW steer calves (n = 24) were weaned on November 3, 2008. After each weaning date, the steer calves were held in a feedlot pen at the DREC, where they were fed an alfalfa plus brome grass (*Medicago sativa* and *Bromus inermis*) mixed hay (10% crude protein) for 9 to 14 days while they recovered from weaning and then placed in 1.82 ha fields of unharvested corn. The 24 EW steers grazed unharvested corn from August 27 to October 14, 2008, when fecal samples were

collected from each steer before steers were shipped to the NPF on October 15. The 24 NW steers were weaned on November 3, 2008, held in a drylot until November 12, and grazed unharvested corn from November 12 to December 22, 2008, when fecal samples were collected and steers were shipped to the NPF. The steers were kept at the feed lot until they reached final weight (approximately 601 kg) and were then slaughtered at the Cargill Meat Solutions packing plant (Fort Morgan, CO).

Sampling

For each cow, samples (fecal grab) were collected at four different periods *(Table 1)* following procedures described previously elsewhere *(12)*. At sampling periods 3 and 4, only 38 of the 48 cows were sampled, because 10 cows had been sold.

For each steer, samples were collected at five different periods *(Table 1)*. At sampling periods 4 and 5, only 47 of the 48 steers were sampled because one steer could not be restrained to collect the samples. The final sampling (sampling period 5) for steers occurred over a 2-month period when each steer was at proper weight for slaughter. Both fecal and sponge hide samples were collected at this period, whereas only fecal grab samples were collected for the first four collection periods. Sampling period 1 was synchronized for both the steers and cows, but the other sampling periods were different because of sampling difficulties in the field.

For steers, the total sampling period (September 2008 through June 2009) wasdivided into three periods: postweaning, finishing, and slaughter. Postweaning was defined as September through December 2008 (sampling period 1 through 3) while steers were at the DREC (before moving to the NPF). Finishing was defined as the period at the feedlot: October or December 2008 through May 2009 (sampling period 4). Slaughter was defined as late May and early June 2009 (sampling period 5).

Bacteriological culture

Fecal and sponge samples were collected and cultured as previously described *(22, 28)*. Briefly, each fecal sample was enriched overnight and then incubated with immunomagnetic beads (Dynabeads anti-*Salmonella*, Dyna Biotech, Inc., Lake Suceess, NY), following the manufacturer's instructions. Biochemical testing was performed by stabbing a single colony on triple iron sugar (Becton Dickinson, Sparks, MD) slants, and identifying colonies displaying hydrogen sulfide production, using API20E strips (bioMérieux, France). Serotype data for the *Salmonella* isolates were not obtained because of funding limitations.

Each sponge sample was transferred to a sterile stomacher bag containing 100 ml of buffered peptone water, and the sample was homogenized in a stomacher for 90 sec. Following homogenization, 10 ml of homogenate was added to an equal volume of 2× buffered peptone water and incubated overnight at 37°C. Following incubation, the previously described procedure of isolation, selection, and verification was completed.

Table 1. *Salmonella* prevalence in steers and cows at the different sampling periods

Sampling Period	Sampling Date(s)	Sample Type	Number of Positives No. (%)
Steers			
1	Sept. 22, 2008	Fecal grab	23/48 (48)
2	Oct. 15, 2008	Fecal grab	13/24 (54)
3	Dec. 22, 2008 Feb. 19, 2009	Fecal grab Fecal grab	24/24 (100)
4	May 28, 2009	Fecal grab	22/47 (47)
5	to June 7, 2009	Sponge	22/48 (46)
Cows			
1	Sept. 22, 2008	Fecal grab	27/48 (56)
2	Nov. 3, 2008	Fecal grab	18/48 (38)
3	Feb. 7, 2009	Fecal grab	3/38 (8)
4	May 7, 2009	Fecal grab	35/38 (92)

Antimicrobial resistance testing

Antimicrobial resistance testing was performed using the National Antimicrobial Resistance Monitoring System CMV1AGNF panel according to the manufacturers' instructions (Sensititre, Trek Diagnostics, West Lake, OH, USA). Each isolate was screened for resistance using full range minimum inhibitory concentrations (MICs). The 15 different antimicrobials tested and the corresponding MICs and breakpoints are shown in *Table 2*.

DNA extraction

Bacterial DNA was extracted from the *Salmonella* isolates using the single cell lysing buffer (SCLB) protocol, as described previously *(27)*. After the final step, samples were stored at -20°C until further analysis.

Class 1 Integron detection

PCR amplification of the Integrase (IntI) gene was performed as previously described *(17, 21)*, using the primer sequences: *Int1* – Forward: 5′– TCT CGG GTA

Table 2. Antimicrobial drugs tested, corresponding MICs, breakpoints, number of resistant isolates and percent resistance

Antimicrobial Drug	MICs* (µg/ml)	Number of Resistant Isolates n = 200 (%)
Amikacin	0.5 – 64	0
Amoxicillin/ Clavulanic acid	1/0.5 – 32/16	93 (47)
Ampicillin	2 – 32	110 (55)
Cefoxitin	0.5 – 32	
Ceftiofur	0.12 – 8	1 (1)
Ceftriaxone	0.25 – 64	0
Chloramphenicol	2 – 32	114 (57)
Ciprofloxacin	0.015 – 4	0
Gentamicin	0.25 – 16	0
Kanamycin	6 – 64	0
Nalidixic acid	0.5 – 32	0
Streptomycin	32 – 64	111 (56)
Sulfizoxazole	16 – 512	111 (56)
Tetracycline	4 – 32	111 (56)
Trimethoprim-sulfamethoxazole	0.12/2.38 – 4/76	0

*Breakpoints were the same as the upper range of MICs for all antibiotics with the exception of tetracycline, which had a breakpoint of 16 µg/ml.

ACA TCA AGG-3′ and *Int1*-Reverse: 5′-AGG AGA TCC GAA GAC CTC-3 *(17)*. The PCR mastermix contained the following concentration: 5X PCR buffer, 10 pmol/L of each primer, 0.2 mM dNTPs, and 2.5 U Taq Polymerase (Promega, Madison, WI). For each sample, 25 µl of mastermix was placed in a sterile PCR tube and 2 µl template DNA added. Amplification parameters were: 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. Following amplification, horizontal electrophoresis was performed. All PCR reactions included both positive and negative controls.

Conserved sequence detection

Salmonella isolates determined to contain the Integrase 1 gene were tested for presence of the conserved sequence of integron 1, using the primer sequences: *Int1* 5'CS: 5' GGCATCCAAGCAGCAAG 3' and 3'CS 5' AAGCAGACTTGACCTGA 3' *(2)*. The PCR protocol described above was used. PCR amplicons were visualized by horizontal gel electrophoresis. Amplicons were then purified by use of a Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI). After purification, DNA was stored at -20°C until the purified product was sent for sequencing at Macrogen (Rockville, MD). A representative sample of 8 PCR products/amplicons were prepared and sent to Macrogen (Rockville, MD) for nucleotide sequencing. Upon receiving the sequencing results, a BLAST analysis was completed to identify the target gene.

Data analysis

Prevalence estimates of *Salmonella* shedding at different production stages (post weaning, finishing, and slaughter) were determined by calculating proportions of steers and cows that tested positive at the different sampling times. Antimicrobial resistance patterns of isolates were recorded and frequencies of resistant isolates determined. The association between presence of integron 1 gene and antimicrobial resistance was assessed using Fishers' Exact test and the Chi-square test. Statistical significance was set at $P < 0.05$.

RESULTS

Prevalence of *Salmonella* in steers and cows

A total of 362 fecal samples were collected over the multiple sampling periods; 190 samples from steers and 172 from cows. Fifty percent (95/190) of samples from steers and 48% (83/172) of samples from cows tested positive for *Salmonella*.

In steers, prevalence in fecal samples varied from 28% to 100% over the five sampling periods *(Table 1)*. Prevalence was higher at post-weaning (sampling times 1 to 3; 63%) than at finishing (sampling time 4; 28%; $P = < 0.001$) and slaughter (sampling time 5; 47%; $P = 0.07$). Prevalence was also higher when steers were housed on pasture (postweaning) compared to the feedlot (finishing and slaughter). A total of 48 sponge samples were collected from hides of steers at slaughter (sampling time 5) and 46% tested positive for *Salmonella*. In cows, prevalence varied from 8% to 92% over the four sampling periods *(Table 1)*. A higher prevalence was observed in cows during the warmer months of May (sampling time 4; 92%) and September (sampling time 1; 56%) than during the cooler months of November (sampling time 2; 38%) and February (sampling time 3; 8%).

Antimicrobial resistance of *Salmonella* isolates

A total of 200 *Salmonella* isolates were recovered, 95 isolates from steer fecal samples, 83 from cow fecal samples, and 22 from steer hide samples. All 200 isolates were examined for antimicrobial susceptibility, and 42% were susceptible to all antimicrobials tested *(Table 3)*. Fifty-six percent were resistant to 2 or more antimicrobials and 3% displayed resistance to only one antimicrobial. The most frequent resistance observed was to chloramphenicol (57%), followed by tetracycline (56%), sulfizoxazole (56%), streptomycin (56%), ampicillin (55%), amoxicilin/clavulanic acid (47%), and ceftiofur (1%) *(Table 2)*. No resistance was observed for amikacin, cefoxitin, ceftriaxone, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, and trimethoprim-sulfamethoxazole.

Class 1 Integron analysis

Forty-nine percent (98/200) of the *Salmonella* isolates contained of the integrase 1 gene. Association between presence of the integrase 1 gene and resistance to the different antimicrobial drugs was evaluated *(Table 4)*. In isolates recovered from steers, a significant association was observed between the presence of the integrase 1 gene and antimicrobial resistance to amoxicilin/clavulanic acid, chloramphenicol, ampicillin, streptomycin, sulfizoxazole, and tetracycline. For example, in the case of ampicillin, the odds (OR) of *Salmonella* isolates being resistant to ampicillin were 16.9 times higher if they possessed the integrase 1 gene than if the integrase gene was absent *(Table 4)*. Moreover, when *Salmonella* isolates possessed the integrase 1 gene, the risk of showing resistance to ampicillin was 5-fold greater than for isolates without the integrase 1 gene. Therefore, among *Salmonella* isolates resistant to ampicillin, 80.6% of the resistance was associated with presence of the integrase 1 gene. Similarly significant associations between presence of the integrase 1 gene and resistance to other antimicrobial drugs (amoxicillin/clavulanic, chloramphenicol, streptomycin, sulfizoxazole, and tetracycline) were also found, whereas a negative association was observed between presence of the integrase 1 gene and resistance to cefoxitin *(Table 4)*. In isolates recovered from cows, significant associations were found between presence of integrase 1 gene and antimicrobial

Table 3. Antimicrobial resistance patterns of *Salmonella* isolates recovered from steers and cows

Resistance Pattern[a]	*Salmonella* isolates No. (%)		
	Steer n = 117*	Cow n = 83	Total n = 200
Susceptible	48 (41)	36 (43)	84 (42)
Tet, Sulfiz, Strep, Chlor, Amp	9 (7.7)	9 (11)	18 (9)
Tet, Sulfiz, Strep, Chlor, Amp, Amox/Clav	55 (47)	38 (45.7)	93 (46)
Chlor	4 (3.4)	NA	4 (2)
Cef	1 (0.9)	NA	1 (0.5)

[a]Amk, Amikacin; Amox/Clav, Amoxicillin/Clavulanic acid; Amp, Ampicillin; Cef, Cefoxitin; Ceft, Ceftiofur; Ceftx, Ceftriaxone; Chlor, Chloramphenicol; Cipro, Ciprofloxacin; Gent, Gentamicin; Kana, Kanamycin; Nal, Nalidixic acid; Strep, Streptomycin, Sulfiz, Sulfizoxazole; Tet, Tetracycline; Trim-Sulf, Trimethoprim-Sulfamethoxazole.

*Total number of isolates collected from steers (n = 117) includes 95 isolates from fecal samples and 22 isolates from sponge samples.

Table 4. Association between antimicrobial drug resistance and presence of integrase 1 gene in *Salmonella* isolates recovered from steers

Antimicrobial Drug	Integrase-1 Positive % Resistant	X^2	OR (95% CI)	*P*-value	Relative Risk	Attributable Fraction
Amox_CLA	79.0%	38.2	13.5 (5.8, 32.7)	< 0.0001	3.46	71.0%
Chloramphenicol	86.0%	40.1	16.7 (6.4, 43.9)	< 0.0001	5.63	82.1%
Cefoxitin	1.6%	0.9	0 (Undefined)	0.5202	0.98	-2.0%
Ampicillin	84.9%	41.7	16.9 (6.6, 43.2)	< 0.0001	5.16	80.6%
Streptomycin	84.9%	41.7	16.9 (6.6, 43.2)	< 0.0001	5.16	80.6%
Sulfizoxazole	84.9%	41.7	16.9 (6.6, 43.2)	< 0.0001	5.16	80.6%
Tetracycline	84.9%	41.7	16.9 (6.6, 43.2)	< 0.0001	5.16	80.6%

Integrase-1 positive % resistant = percentage of resistant isolates that possessed the integrase 1 gene and were resistant to the particular antimicrobial, X^2 = Chi-square value, OR = Odds ratio, CI = confidence interval.

resistance to amoxicilin/clavulanic acid, chloramphenicol, ampicillin, streptomycin, sulfizoxazole, and tetracycline *(Table 5)*.

Conserved sequence detection

Testing of 98 isolates for presence of the conserved sequence of class I integrons, showed that 88 (90%) isolates possessed the conserved sequence. All 88 isolates had product size of 1000 bp for the conserved sequence. A representative sample of 8 PCR products was sent for nucleotide sequencing, and BLAST results indicated the presence of the *aadA* resistance gene in all 8 representative samples.

Table 5. Association between antimicrobial drug resistance and presence of integrase 1 gene in *Salmonella* isolates recovered from cows

Antimicrobial Drug	Integrase-1 Positive % Resistant	X^2	OR (95% CI)	*P*-value	Relative Risk	Attributable Fraction
Amox_CIA	77.8%	31.7	18.7 (6.1, 57.2)	< 0.0001	3.6	72.2%
Ampicillin	91.7%	45.4	53.6 (15.2, 218.7)	< 0.0001	11.3	91.1%
Chloramphenicol	91.7%	45.4	53.6 (15.2, 218.7)	< 0.0001	11.3	91.1%
Streptomycin	91.7%	45.4	53.6 (15.2, 218.7)	< 0.0001	11.3	91.1%
Sulfizoxazole	91.7%	45.4	53.6 (15.2, 218.7)	< 0.0001	11.3	91.1%
Tetracycline	91.7%	45.4	53.6 (15.2, 218.7)	< 0.0001	11.3	91.1%
Cefoxitin	0.0%	NA	NA	NA	NA	NA

Integrase-1 positive % resistant = percentage of resistant isolates that possessed the integrase 1 gene and were resistant to the particular antimicrobial, X^2 = Chi-square value, OR = Odds ratio, CI = confidence interval.

DISCUSSION

Prevalence of *Salmonella* in steers

Prevalence of *Salmonella* shedding in steers varied over the five sampling periods, with a higher prevalence observed at postweaning than at finishing and slaughter. Previous studies have reported varying prevalence estimates in feedlot cattle during finishing and at slaughter. In one study, a decrease in *Salmonella* shedding over time was reported; prevalence estimates were 40% at entry in feedlot, 9% at day 30, < 1% at day 60, and 0% at slaughter (day 120–150) *(16)*. In contrast, in a second study, an increase in *Salmonella* shedding over time was reported; prevalence estimates were 0.7% on arrival at feedlot, 5.6% on a second sampling, 13% on a third sampling, and 62% on a fourth sampling *(22)*. In a third study, prevalence estimates at feedlot entry and within 24 h of harvest, using fecal samples collected from pen floors of cohorts of feedlot cattle, were reported as 64.7% and 72.6%, respectively *(10)*. A fourth study reported prevalence of 1.9% and 0.2% in fecal samples collected from beef cattle at arrival at a feedlot and at slaughter, respectively *(38)*. In general, it is difficult to make comparisons of prevalence estimates between the present and previous studies because of differences in factors such as study populations, geographical locations, and laboratory methods. Findings from the present study suggest that *Salmonella* fecal shedding at postweaning, before entry into feedlots, was high, and that shedding continued through the finishing and slaughter stages.

Prevalence of *Salmonella* fecal shedding was higher when steers were housed on pasture (at postweaning) rather than in the feedlot (finishing and slaughter). The difference in prevalence may be explained by factors such as the environment in which the steers were kept and duration of exposure to that environment. Before weaning, steers were kept on pasture together with cows (cow–calf). Cows or their environment may be a direct or indirect source of infection for naïve calves. Transmission of *Salmonella* to nursing calves may occur through the fecal-oral route, during activities such as suckling of teats contaminated with infected fecal material from the dam, or other cattle in the herd. In the present study, the magnitude of exposure to *Salmonella* may have been high before weaning (while with cows) and at postweaning. The change in environment (from postweaning on pasture to finishing at a feed lot) may have contributed to the observed differences in the prevalence of *Salmonella* shedding. This finding suggests that the postweaning stage should be considered in pre-harvest interventions aimed at reducing *Salmonella* shedding in beef cattle.

The prevalence of *Salmonella* in hide samples collected from steers before slaughter was relatively high. This finding is comparable to results of previous studies that investigated the prevalence of *Salmonella* on hides of feedlot cattle before slaughter. In one study, the proportion of samples that tested positive for *Salmonella* was higher for hide samples collected at the abattoir after exsanguination (80%) than for hide samples collected from the same steers at the feedlot before shipment (37%) *(14)*, and in another study, a prevalence of 70% was reported for hide samples collected from harvest-ready cattle *(23)*. In the present

study, prevalence estimates in hide and fecal samples before slaughter were the same (47%). This finding differs from results of previous studies that reported greater prevalence estimates on hides than in feces *(23, 26)*. The detection of *Salmonella* on hides of steers before slaughter is a major food safety risk because hides are a potential source of indirect carcass contamination during hide removal *(33)*.

Prevalence of *Salmonella* in cows

In cows, the prevalence of *Salmonella* shedding varied over the four sampling periods, and no clear trends in prevalence were observed over time, or during the period when cows were housed on pasture (sampling time 1) and winter dry lot (sampling times 2, 3 and 4). In contrast, in a study that investigated the prevalence of *Salmonella* fecal shedding in free-range pregnant beef cows, no *Salmonella* were detected in fecal samples from breeding cows *(38)*. Findings from the present study revealed that *Salmonella* fecal shedding occurs in beef cows during the weaning period and continues for prolonged periods after weaning, while on range and in a winter dry lot.

Seasonal variation in prevalence of *Salmonella* shedding was observed in cows but not in steers. A higher prevalence was observed in cows during the warmer months of May and September, than in the cooler months of November and February. Previous studies reported variable findings on seasonal variation of prevalence of *Salmonella* shedding. In a U.S. study, no seasonal variation was observed following testing of hide swabs at the abattoirs and feces from pen floors of feedlots during winter, spring, and summer *(23)*. In other two U.S. studies, prevalence estimates of 5.5% and 6.3% were reported for pen floor samples collected from feedlots during fall (October and September) *(8, 13)*. Findings from the present study on seasonal variation of *Salmonella* shedding should, however, be interpreted with caution, because the length of the study period (9 months) was not adequate to assess seasonality. Another longitudinal study with a longer period of study and with different cohorts of feedlot cattle is warranted to provide definitive data on seasonality of *Salmonella* shedding by beef cattle.

Antimicrobial resistance of *Salmonella* isolates

Overall, 58% of the *Salmonella* isolates were resistant to at least one antimicrobial drug. The most frequently observed resistance was to chloramphenicol, tetracycline, sulfizoxazole, streptomycin, ampicillin and amoxicilin/clavulanic acid. Similar to our study, a previous U.S. study in beef heifers and steers reported frequent resistance to chloramphenicol, tetracycline and ampicillin, in addition to sulfadiazine, streptomycin and spectinomycin *(18)*. In another U.S. study, frequent resistance to sulfamethoxazole and streptomycin was reported for *Salmonella* isolated from beef cattle feces, hides, and carcasses *(14)*. In the present study, two multiple drug resistance (resistance to 2 or more antimicrobials) profiles were observed. One profile combination was tetracycline, sulfizoxazole, streptomycin, chloramphenicol and ampicillin, and the second combination was tetracycline, sulfizoxazole, streptomycin, chloramphenicol, ampicillin and amoxicilin/clavulanic. A previous study reported combined resistance to sulfamethoxazole and streptomycin for *Salmonella* isolated from beef cattle feces, hides, and carcasses *(14)*. Because of differences in antimicrobial panels used, it is difficult to make comparisons of antimicrobial resistance between our results and results of previous studies. Nonetheless, findings of the present study further document the occurrence of antimicrobial resistant *Salmonella* in beef cattle. The detection of resistant *Salmonella* in beef cattle is a major public health concern, because transmission of this pathogen to humans may occur through beef carcass contamination. Human infection with antimicrobial-resistant *Salmonella* is associated with increased risk of severe infection, hospitalization, and death *(40)*.

Class 1 Integron analysis

In the present study, class 1 integrons were present in almost half of the isolates. Previous studies investigated the distribution of class 1 integrons in *Salmonella* isolates obtained from other sources, but not beef cattle. A previous study reported class 1 integrons in 68/333 of *Salmonella* isolates obtained from clinical samples, pork, and sewage *(19)*. In another study that investigated the distribution of class 1 to 4 integrases among veterinary bacterial strains isolated from birds, poultry, and reptiles, class 1 integrases were the most commonly identified of the 4 classes, and 93/151 of the *Salmonella* isolates contained class 1 integrases *(17)*.

Further analysis of the *Salmonella* isolates with class 1 integrons revealed that 90% possessed the *aadA* resistance gene. The *aadA* resistance gene has been reported in *Salmonella (31, 39)*, and encodes for streptomycin/spectinomycin resistance *(19)*. This finding is not surprising, because 56% of the *Salmonella* isolates in the present study were resistant to streptomycin. A plausible explanation is that selection and dispersion of *aadA* resistance genes in class 1 integrons could be related to the extensive use of this antibiotic in veterinary treatments *(19)*. The *aadA* resistance gene in integrons has been reported in clinical isolates from humans, although streptomycin is seldom used in human medicine *(19)*. These findings highlight the potential public health significance associated with this type of antimicrobial resistance.

A significant association between the presence of integrase 1 gene and antimicrobial resistance to amoxicilin/clavulanic acid, chloramphenicol, ampicillin, streptomycin, sulfizoxazole, and tetracycline was observed in the present study. The observed antimicrobial resistance was attributed to the presence of the integrase 1 gene. These findings suggest that class 1 integrons may have the capability

to disseminate antimicrobial resistance genes within *Salmonella*. More conclusive studies that examine the distribution and role of other classes of integrons, and DNA mobile elements are needed to confirm these findings.

The present study had a number of limitations. First, because study samples were collected from only one cohort of cattle (cows and steers), extrapolations of these study findings should consider the potential differences in study populations, as well as differences in geographical locations and laboratory methods used. Second, serotype data for the *Salmonella* isolates in the present study were not determined. Therefore, it was not possible to conduct additional analyses, such as assessing the relationship between antimicrobial resistance and serotype, assessing the relationship between presence of integrons and serotype, and determining if serovars of the isolates were similar to the serovars most commonly associated with human illness. Third, only one isolate from each study sample was tested for antimicrobial resistance; as a result, the frequency of antimicrobial resistance may have been underestimated in some samples if the isolates that predominated on enrichment were susceptible to antimicrobials. A previous study reported that multiple indistinguishable pulsed-field gel electrophoresis (PFGE) subtypes and serotypes of *Salmonella* were observed in lymphnode, fecal and hide samples collected from a single beef carcass *(18)*. Finally, the effect of weaning date (early wean or normal wean) on *Salmonella* or antimicrobial resistance prevalence was not investigated in the present study. The objective of the present study was to estimate the prevalence of *Salmonella* (and antimicrobial resistance) in beef cows and steer calves, regardless of weaning date.

In conclusion, prevalence of *Salmonella* fecal shedding in beef cows and steers varied over time, and in steers shedding was highest at postweaning and continued through the finishing and slaughter stages. Fecal shedding in steers has important food safety implications, because detection of *Salmonella* from cattle ready for slaughter has been linked to beef carcass contamination. The present study also revealed that over half of the *Salmonella* isolates were resistant to at least one antimicrobial drug. The detection of resistant *Salmonella* in beef cattle is a major public health concern because of the potential transmission of such pathogens to humans through beef carcass contamination. Finally, the present study revealed that class 1 integrons were present in almost half of the *Salmonella* isolates. Class 1 integrons carry the *aadA* resistance gene that encodes for streptomycin/spectinomycin resistance, and may have the capability to disseminate antimicrobial resistance genes within *Salmonella*. This finding highlights the dilemma and public health significance associated with veterinary use of antibiotics such as streptomycin in beef cattle.

ACKNOWLEDGMENTS

The authors thank Catherine Logue for use of laboratory space and equipment and guidance throughout the project; Susan Olet for help with statistical analysis; Lisa Mowry and Michael Mahero for assistance with laboratory work; Charles Stoltenow and Neil Dyer for guidance throughout the project; and the Dickinson Research Extension Center (DREC) staff for their assistance in sample collection. Funding for this project was provided by United States Department of Agriculture, Animal and Plant Health Inspection (USDA APHIS), USDA Grant# 43500-7690-05348-FAR0012193.

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