Introduction of new multidrug-resistant *Salmonella enterica* strains into commercial dairy herds

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ABSTRACT

A longitudinal observational study of 59 dairy herds was conducted in Washington State to estimate the rate of introduction of new multidrug-resistant (MDR) *Salmonella enterica* strains onto commercial dairy herds. Samples were collected on these herds over 7 visits separated by intervals of 2 to 4 mo over a period of 15 to 21 mo. Samples were cultured for *Salmonella* spp. and serogroup, serovar, and antimicrobial susceptibility patterns were identified for MDR *Salmonella* isolates. Fingerprinting generated by pulsed-field gel electrophoresis (PFGE) using *Xba*I restriction enzyme digestion generated genotyping profiles for all MDR isolates identified in the study. The rate of new MDR *Salmonella* strain introduction was 0.9 per herd-year (95% confidence interval: 0.6–1.4). The rates for the most commonly introduced MDR *Salmonella* serovars were 0.4/herd-year for Typhimurium, 1.2/herd-year for Newport, and 0.1/herd-year for Dublin. Thirty-three of 59 herds (56%) had at least one new MDR *Salmonella* introduction during the study period. The number of new MDR *Salmonella* strains acquired by dairy herds ranged from zero to 8. Thirteen of the 59 herds had a history of clinical salmonellosis. Among these 13 herds, 6 herds acquired new MDR *Salmonella* strains, although these strains were different than historical clinical strains. These data indicate that acquisition of new MDR *Salmonella* strains by dairy herds was a common event in participating herds, although the number of strains introduced varied greatly among herds.

Key words: multidrug-resistant *Salmonella*, dairy farm, incidence rate, introduction

INTRODUCTION

Salmonella enterica is a common zoonotic pathogen that is one of the leading causes of foodborne bacterial infections in the United States (CDC, 2005). An estimated 1.3 million human clinical salmonellosis cases and more than 500 human deaths associated with *Salmonella* may occur annually in the United States (Mead et al., 1999). Domestic livestock are considered the main reservoir for foodborne salmonellosis including strains with multidrug resistance (Sanchez et al., 2002).

Zoonotic bacteria demonstrating antimicrobial resistance are an emerging problem worldwide (FAO/WHO, 2007). Transmission of zoonotic MDR *Salmonella* is of particular concern because of the limited effectiveness of first-line antimicrobials when treating infections, particularly pediatric infections, and because drug-resistant strains may be more likely to cause prolonged or severe illnesses than drug-susceptible strains (Butaye et al., 2006). Publications of *Salmonella* transmission from animals to humans include meat or milk from cattle (Spika et al., 1987), meat from poultry (McPherson et al., 2006), and infection through direct contact with infected cattle (Bezanson et al., 1983; Fey et al., 2000). Multidrug resistance is a characteristic of many newly emerging strains that are believed to spread mainly by clonal expansion (Hancock et al., 2000; Davis et al., 2002, 2007a; Velge et al., 2005).

Several studies have been published on the epidemiology of *Salmonella* and risk factors associated with *Salmonella* transmission between cattle herds. A recent study in the southwestern United States reported a low level of risk of *Salmonella* transmission from the heifer feedlot back to the dairy herds (Edrington et al., 2008). In previous studies, the transport of infected cattle into cattle herds has been described as the primary route of interherd transmission of *Salmonella* spp. (Wray et al., 1990; Evans, 1996; Zansky et al., 2002). *Salmonella* prevalence in preweaned calves has been shown to be lower in herds that are closed (i.e., no cattle are introduced into the herd; Berge et al., 2006). Other studies

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Table 1. Attributes of 59 northwestern US commercial dairy herds enrolled in this study

Attribute	Value	Range
Median herd size (dairy cattle of all ages), n	1,800	231–17,775
Median number of lactating cows, n	750	100–7,150
Median number of dry cows, n	130	0–1,409
Median number of calves <3 mo on-farm, n	80	0–3,000
Median number of calves <3 mo off-farm, n	0	0–685
Median number of heifers from 3 to 20 mo on-farm, n	230	0–3,500
Median number of heifers from 3 to 20 mo off-farm, n	40	0–5,600
Median number of heifers from 20 mo to calving off-farm, n	100	0–700
Farms that introduced new animals during the study, ¹ %	53	—
Median number of cattle introduced, n	2	0–1,340
Farms that raised any cattle off-farm, %	47	—

¹New animal introduction into the farm was defined as new animal purchase or returning cattle from a contract-heifer raising operation or owned cattle raised offsite where cattle mingled with cattle from other sources during the study period.

have shown that *Salmonella* transmission can occur via movements of people, equipment, fomites, wild animals, and through environmental contamination (Langvad et al., 2006; Nielsen et al., 2007a). Direct cattle-to-cattle contact from other herds can also result in the introduction of *Salmonella* spp. into dairy farms (van Schaik et al., 2002). The rate of cattle introduction from test-positive herds has been shown to be associated with recipient herds changing from *Salmonella*-negative status to positive (Nielsen et al., 2007b). Another potential source of *Salmonella* transmission includes livestock feed (Anderson et al., 2001; Davis et al., 2003).

Relatively few studies have been performed to examine the rate at which *Salmonella* spp. are introduced onto commercial dairy herds. In the United Kingdom, a previous study reported an incidence rate of 0.43 cases of salmonellosis per farm-year at risk for any serovar of *Salmonella enterica* (Davison et al., 2006). No data are currently available on the incidence of MDR *Salmonella* strain introduction into dairy herds in North America. Such information is useful in undertaking risk assessments for human transmission (FAO/WHO, 2007). Therefore, the purpose of this study was to estimate the rate at which MDR *Salmonella* strains were introduced into commercial dairy herds in the northwestern United States.

MATERIALS AND METHODS

Study Herds

Fifty-nine commercial dairy herds ranging in size from 231 to 17,775 head (cattle of all ages) in Washington State were sampled during the period from August 2005 to December 2007. According to the Dairy Farmers of Washington State (<http://www.havemilk.com>; 2007), there were 496 dairy herds and 238,000 milk cows in Washington State. Dairy herds were selected

across the 3 major dairy areas (western, central, and south-central) of Washington State, proportional to the total dairy herds in that region. Sixty-six herds were randomly selected alphabetically from the list of herds, and contact was made through telephone and by herd veterinarians to explain the project. Sixty-six dairy herds were enrolled, 6 declined participation, and 1 farm went out of business during the study, leaving us with 59 herds for study. In Washington State, herd veterinarians regularly submit fecal samples to Washington State Disease Diagnostic Laboratory for suspected cases of salmonellosis. Management-related information was collected via a standardized questionnaire and by on-site inspection (Table 1).

Sampling Plan

Each farm was visited 7 times to collect samples at intervals of 2 to 4 mo over a period of 15 to 21 mo from August 2005 to December 2007. In total, 7,009 pooled fecal samples were collected from the 59 dairy herds (Table 2). Each fecal pool consisted of approximately 5 g of feces collected from 10 individual, undisturbed fresh fecal pats from the pen floor using sterile tongue depressors. An average of 16 fecal pools was collected per farm per visit from different groups of cattle in the farm. The number of pools collected from each group was proportional to the group size. Pooled fecal samples were obtained from hutch calves (1), heifers (1–2), lactating cows (3–9), dry cows (2–3), close-up dry cows (2–3), and cows in the maternity/hospital area (1–2). Certain groups of cattle (calves, lactating cows, maternity/hospital cows) were selectively sampled to increase sensitivity, as *Salmonella* prevalence is reported to be greater in these groups than in the general herd (Hancock, 1996; Warnick et al., 2003). This sampling method was estimated to provide at least a 95% probability of detecting a positive herd if the within-herd prevalence

Table 2. Number of farm visits, samples, and sample sources per farm visit from August 2005 through December 2007

Farm visit number ¹	Total farm visits, n	Samples, n				
		Pooled feces ²	Pooled feed ³	Milk filter ⁴	Slurry	Total
1	59	1,274	664	50	82	2,129
2	59	955	644	52	71	1,781
3	59	950	631	53	50	1,743
4	59	976	654	51	53	1,793
5	59	920	690	51	49	1,769
6	59	980	667	33	56	1,795
7	59	954	632	50	53	1,748
Total	413	7,009	4,582	340	414	12,758

¹The interval between visits ranged from 2 to 4 mo.

²Pooled fecal samples per farm ranged from 15 to 21.

³Feed collected from 5 different areas of particular feed type in each feed storage area.

⁴Some farms did not have a milk filter at time of visit.

was 8% or more (Jordan, 2005). Initial sample size estimates were based on recovery of *Salmonella* from a herd. The samples were thereafter targeted to high-risk commodities and animals to recover as many different types of *Salmonella* as could be present.

Samples of all available component feed types were collected from respective farms and from 2 feed mills that supplied feeds to most dairy herds enrolled in the study. Each pooled feed sample consisted of approximately 10 g of feed collected from 5 different areas of a particular feed type in each feed storage unit. On average, 10 feed pools were collected from each farm per visit. Feed samples were collected using sterile gloves after removing 2.5 to 5 cm of the top surface layer of feed. Samples were transported to the laboratory at 4 to 8°C and were processed within 24 h of collection.

Other samples included slurry and milk filters. Using sterile gloves, up to 2 slurry samples of 50 mL from the slurry pond, and up to 2 milk filters from the trash bin were collected from each dairy farm.

Salmonella Culture and Identification

For isolation of *Salmonella* spp., 12.5 g of each fecal sample was enriched in 112.5 mL of tetrathionate broth (TB, Hardy Diagnostics, Santa Maria, CA) and 112.5 mL of Rappaport-Vassiliadis broth (R10; Hardy Diagnostics) and incubated for 24 h at 37°C. Enriched samples were plated onto xylose lactose tergitol-4 agar (XLT-4, Hardy Diagnostics) and MacConkey (Mac, Hardy Diagnostics) agar plates supplemented with ampicillin (256 µg/mL), chloramphenicol (8 µg/mL), and streptomycin (32 µg/mL, Sigma-Aldrich, Dallas, TX) and incubated for 24 h at 37°C. Feed (25 g) or slurry (25 mL) samples were pre-enriched in 225 mL of buffered peptone water (BPW, Hardy Diagnostics) and incubated for 24 h at 37°C before performing paral-

lel enrichments by transferring 1 mL of BPW to 9 mL of TB and 100 µL of BPW to 10 mL of R10, incubating for 48 h at 42°C, and cross-plating onto XLT-4 and Mac media. The fecal *Salmonella* isolation protocol was used for isolation of *Salmonella* from milk filters. For confirmation of suspected *Salmonella*, 3 colonies from XLT-4 and Mac were inoculated onto lysine iron agar slants and incubated for 24 h at 37°C. *Salmonella* suspects from lysine iron agar were transferred to triple sugar iron agar slants and subsequently to urea agar slants incubated 18 to 24 h at 37°C. One isolate per sample was further screened with agglutination tests for serogroup identification using commercial polyvalent A-I and Vi antisera (Difco Laboratory, Detroit, MI). Isolates were then banked in brain-heart infusion broth containing 25 to 30% buffered glycerol and stored at 80°C. One isolate per sample (except when different serogroups were detected from the same sample either on single or 2 different culture media) was selected for further characterization with antimicrobial susceptibility testing.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method (Bauer et al., 1966). Each isolate was classified as resistant or susceptible to each antimicrobial tested by the threshold zone size for resistance as recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines (NCCLS, 2003a,b) using the following antimicrobial drugs (BD Diagnostics, Sparks, MD): ampicillin (10 µg), ceftazidime (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), amoxicillin-clavulanic acid (20/10 µg), kanamycin (30 µg), streptomycin (10 µg), tetracycline (30 µg), triple-sulfa (a combination of sulfadiazine, sulfamethazine, and sulfamerazine; 250 µg),

and trimethoprim-sulfamethoxazole (1.25/23.75 µg). Isolates showing antimicrobial resistance to 2 or more antimicrobials belonging to distinct classes of antimicrobial agents were designated as MDR isolates. A total of 385 MDR *Salmonella* isolates (one isolate per sample per visit except if more than one serogroups detected in a sample) from the current study and 26 historical clinical MDR *Salmonella* isolates collected before the study were serotyped at the National Veterinary Service Laboratory (Ames, IA). A 3-tube most-probable-number (MPN) method was used to determine the level of *Salmonella* contamination of positive feeds (Blodgett, 2006).

Pulsed-Field Gel Electrophoresis

All MDR *Salmonella* isolates from fecal samples were analyzed using pulsed-field gel electrophoresis (PFGE) following *Xba*I restriction digestion based on the PulseNet protocol for *Salmonella* and using *Salmonella* Braenderup H9812 as a size standard (Ribot et al., 2002). The PFGE images were analyzed using BioNumerics software (v. 3.5, Applied Maths, Sint-Martens-Latem, Belgium). Independent strains were defined by PFGE profiles that differed by at least 2 bands with a position tolerance of 1.5%.

Data Analysis

For the purpose of this study, a strain found on the farm was considered a newly introduced strain if the strain was isolated during the second through seventh sampling visits and had not been previously observed on that farm in the first sampling visit and in diagnostic samples from suspected clinical cases for at least 3 or more years before the commencement of the study.

Introduction of new strains was not based on serotypes, but on PFGE profiles, where we have designated a new strain for isolates from farm visits 2 to 7 that had a PFGE banding pattern different (based on at least 2 PFGE band differences) from historical and baseline isolates (visit 1). Herd classification at the start of the study (visit 1) was based on isolation of *Salmonella* from any source (pooled feces, feed, milk filters, or slurry). New introductions of *Salmonella* spp. were based purely on isolation of *Salmonella* from pooled feces specimens.

Incidence rates of introduction of new MDR *Salmonella* strain into the herds were calculated using Excel 2003 (Microsoft, Redmond, WA) and SAS version 9.1 (SAS Institute Inc., Cary, NC). Incidence rates for events, including 95% confidence intervals (CI), were calculated using a Poisson distribution and expressed as new strains per herd-year at risk. Overall MDR in-

cidence rate was calculated as the number of specific *Salmonella* serovar-negative herds at commencement of sampling that become positive, divided by the total observation time at risk. The incidence rates were calculated by using either the serovar-specific status of the herds or their overall new MDR *Salmonella* spp. status. A herd was considered at risk for new MDR *Salmonella* strains until the herd was positive for that respective MDR *Salmonella* strain.

RESULTS

Description of New Strain Introduction

Sixty-six dairy herds were enrolled, 6 declined participation, and 1 farm went out of business during study period. The study thereby included 59 herds containing 173,253 cattle with farm size and geographical distributions representative of Washington State. A total of 7,009 fecal and 4,582 feed pool samples were collected in these herds. The median total herd size in terms of dairy cattle of all ages and of lactating cows only were 1,800 (range 231–17,775) and 750 (range 100–7,150), respectively (Table 1). Fifty-three percent of the herds introduced new animals during the study period.

Historical information on clinical *Salmonella* infection from participating herds (Table 3) indicated that 13 of 59 herds were diagnosed with clinical salmonellosis before the study, based on *Salmonella*-positive clinical sample submissions to Washington State Disease Diagnostic Laboratory. A total of 26 MDR *Salmonella enterica* isolates were detected in historical clinical submission samples. Among the MDR strains, antimicrobial resistance was seen in all 26 isolates to ampicillin; followed by 25 isolates to triple-sulfa, streptomycin, and tetracycline; 16 isolates to chloramphenicol; 14 isolates to ceftazidime; 13 isolates to kanamycin; 4 isolates to trimethoprim-sulfamethoxazole; and 2 isolates to gentamicin. All MDR isolates were resistant to at least 5 antimicrobials, whereas 5 (19%) were resistant to 7 or more antimicrobials. Among the historic clinical MDR strains, *S. Typhimurium* was most common serovar (12/26) followed by *S. Newport* (9/26). None of the historical MDR *Salmonella* strains were isolated in the 7 farm visits. Among 13 herds positive for history of clinical salmonellosis before the study, 5 were positive for MDR *Salmonella* at the first farm visit, and 8 acquired new strains at farm visit 2 or later.

A total of 70 new introductions of MDR *Salmonella* strains were observed in 33 dairy herds (Table 4). The most common serovars identified among the new strains were Typhimurium (14 introductions), Newport (10), Dublin (8), Montevideo (3), and Uganda (2). Other serovars included Ohio, Oranienburg, Hadar, Infantis,

Table 3. Characteristics of *Salmonella* spp. isolated at farm visit 1 and records of historical clinical isolates from participating dairy herds in Washington State

Herd	Serovar ¹	Serogroup	PFGE type ²	Antimicrobial resistance ³	Clinical isolates	Isolates at farm visit 1
1	Typhimurium	B	TYP004	ACSTS _u	1	
2	Newport	C2	NEW005	ACSTS _u Caz	1	
	Typhimurium	B	wTYP041	ACKSxtSTS _u	1	
5	Lexington	E1	NEW005	AKSTS _u		1
8	Newport	C2	NEW013	ASTS _u Caz	2	
12	Bardo	C2	NEW005	ACSTAmcSuCaz		1
	ND	C2	NEW005	ACSTAmcSuCaz		18
	ND	C2	wNEW017	ACSTAmcSuCaz		1
	Newport	C2	NEW005	ACSTAmcSuCaz		4
	ND	C2	NEW005	ACSTAmcSuCaz		2
14	Heidelberg	B	wHEI006	ACGKSTS _u Caz	1	
18	Newport	C2	NEW005	ACSTS _u Caz	1	
20	Typhimurium	B	TYP035	AKSTS _u	1	
22	Typhimurium	B	wTYP001	ACSTAmcSu		2
28	ND	C2	NEW046	ASTAmcSuCaz		4
	Newport	C2	NEW046	ASTAmcSuCaz		1
	ND	C2	wNEW004	ACKSxtSTAmcSuCaz		1
29	Uganda	E1	wUGA001	CKSxtSTS _u		1
32	Dublin	D1	wDUB010	ACKSTAmcSuCaz		1
	ND	D1	wDUB022	ACGKSxtSTS _u Caz	1	
	Typhimurium	B	TYP021	AKSTS _u	3	
	ND	B	wTYP115	AKSTS _u	1	
33	Bardo	C3	NEW005	ACSTS _u Caz	1	
	Newport	C2	NEW005	ACSTS _u Caz	3	
	Typhimurium	B	TYP035	AKSTS _u		4
35	Anatum	E1	wANA003	Su	1	
	Idikan	G	wIDI001	Su	1	
37	Meleagridis	E1	wMEL003	NT	1	
	Newport	C2	NEW005	ACSTS _u Caz	2	
	Typhimurium	B	TYP035	AKSTS _u	1	
38	Typhimurium	B	TYP035	AKSTS _u		1
39	ND	C2	NEW005	ACSTAmcSuCaz		4
	Newport	C2	NEW005	ACSTAmcSuCaz		2
46	Bardo	C3	NEW024	ACSTAmcSuCaz		1
48	9,12:Nonmotile	UN	w9_12_N001	ACKSTS _u		1
51	Typhimurium	B	TYP035	ACKSTAmcSuCaz		1
55	Newport	C2	NEW051	ACSTAmcSu		1
	Typhimurium	B	TYP018	ACKSxtSTS _u	1	
58	ND	B	w4_12_001	AKSTS _u		1
	ND	B	wB_003	AKSTS _u		2
	Typhimurium	B	wB_003	AKSTS _u		1
59	ND	B	TYP035	AAmcCaz		1
	ND	C2	NEW005	ACSTAmcSuCaz		1
	Newport	C2	NEW005	ACSTAmcSuCaz		1
	Typhimurium	B	TYP035	AAmcCaz		1
		B	TYP187	AKSTAmcSuCaz		1
60	9,12:Nonmotile	D	w9_12_N004	ACKSTS _u	1	
	Newport	C2	wNEW001	ACKSTAmcSuCaz		1
	Typhimurium	B	TYP035	ACKSxtSTS _u Caz	1	
		UN	w4_12_001	AKSTS _u	1	
62	ND	C2	NEW005	ACSTAmcSu		6
	Newport	C2	NEW005	ACSTAmcSu		1
	ND	C2	NEW005	ACSTAmcSu		1
	ND	C2	NEW005	ACSTAmcSuCaz		1
	Typhimurium	B	TYP004	ACSTS _u	1	

¹ND = serotyping was not performed as the herds associated with these isolates never acquired new multidrug-resistant *Salmonella* strains with corresponding pulsed-field gel electrophoresis (PFGE) types, and research constraints.

²PFGE type as designated by Washington Department of Public Health with the exception of types starting with the letter w, which indicates a new PFGE type designated at Washington State University.

³A = ampicillin; C = chloramphenicol; G = gentamicin; K = kanamycin; S = streptomycin; Su = triple sulfa; T = tetracycline; Caz = ceftazidime; Amc = Amoxicillin-clavulanic acid; Sxt = trimethoprim-sulfamethoxazole; NT = not tested; UN = unknown.

Meleagridis, and nonserotypeable isolates. New strains T5, D1, I1, and N1 were acquired by 13 (22%), 3 (5%), 3 (5%), and 2 (3%) herds, respectively (Table 4). Fifty-eight (83%) strains were resistant to 5 or more antimicrobials tested including ceftazidime, a third-generation cephalosporin.

Twenty-six (44%) herds did not acquire any new introductions of MDR *Salmonella*, whereas 33 (56%) herds acquired at least one new introduction of MDR *Salmonella* during the study period. The number of new introductions of MDR *Salmonella* on these herds varied from zero to 8 (Figure 1). Eighteen herds acquired 1 new MDR *Salmonella* strain, 12 herds acquired 2 or 3 new MDR *Salmonella* strains, and 1 and 2 herds acquired 6 and 8 new introductions, respectively. The incidence rate of introduction of MDR *Salmonella* strains in Washington State commercial dairy herds was 0.9 per herd-year (95% CI: 0.6–1.4). The rates of introduction for the most commonly introduced MDR *Salmonella* serovars were 0.4/herd-year for Typhimurium, 1.2/herd-year for Newport, and 0.1/herd-year for Dublin.

Table 5 summarizes the temporal and geographical distributions of newly introduced MDR *Salmonella* serotypes isolated from the study herds. Of the 59 herds, new strains of serovar Typhimurium were acquired by 27 (39%) herds followed by Newport (17%), Dublin (16%), Infantis, Montevideo, and Uganda (4%). Newly introduced strains of serovar I 4,12:nonmotile, Infantis and Newport were isolated from herds in 4 counties of Washington State. Strains of serovars Typhimurium and Oranienburg were isolated from herds in 3 and 2 counties, respectively.

MDR *Salmonella* Contamination in Feed

A total of 4,582 pooled feed samples from the 59 herds and 665 feed samples from 2 feed mills were collected over the 7 sampling periods. Of those, 3.5% (158) of feeds were positive for any *Salmonella* spp. Of the feed source *Salmonella* spp., 83, 8, and 9% were pan-susceptible, resistant to 1 antimicrobial, and resistant to 2 or more antimicrobials (i.e., MDR), respectively. Overall, the prevalence of pan-susceptible and MDR *Salmonella* in feeds were 2.5 and 0.5%, respectively. Feeds from 12 (19%) herds yielded 12 MDR *Salmonella* during 413 farm visits (Table 6). Of 665 feed mill samples, only 1 sample (0.2%) was positive for MDR *Salmonella*. This MDR feed mill *Salmonella* Bardo isolate was from mixed grains and it was never isolated in study herds during the sampling period. Multidrug-resistant *Salmonella* Typhimurium were isolated from several feed types on farms, including straw, corn silage, calf grains, and haylage. Multidrug-resistant *S. Dublin* and *S. Newport* were isolated from calf grain, bakery

waste, and cannery waste at these farms, respectively. *Salmonella* Saint-Paul was the only serovar isolated from cottonseed at a single farm. The MDR *Salmonella* feed isolates exhibited resistance to 2 to 7 antimicrobials. The MDR *Salmonella* strains recovered from feed were not recovered from cattle fecal samples on the same farm. Quantitative analysis of feeds positive for MDR *Salmonella* showed that none of the samples were positive for *Salmonella* in triplicate MPN dilution series using inoculum quantities of 0.1, 0.01, and 0.001 g after 48-h enrichment in TB. Thus, the computed MPN concentration of MDR *Salmonella* for all types of feeds was less than 3 cfu/g. These feeds were positive only by the selective enrichment of 25 g of feed.

DISCUSSION

The rate of new MDR *Salmonella* strain introduction in 59 Washington State herds was 0.9 per herd-year. This rate of introduction is higher than 0.43 cases per farm-year at risk reported in England and Wales (Davison et al., 2005). The estimate from that study also included pan-sensitive *Salmonella* strains, which accounted for a majority of newly introduced strains. Hence, the rate of MDR *Salmonella* introduction (not computed separately) in the British study must have been at least 4 times lower than the rate in the present study. This difference is possibly because of differences in herd size, feeding, management, and environmental factors between British herds and western US herds. The most common *Salmonella* serovars found in the present study were Typhimurium, Newport, and Dublin, whereas in the British study, the most common serovars were Dublin, Agama, and Typhimurium (Davison et al., 2005).

Our finding of the most common *Salmonella* serovars in the order of Typhimurium, Newport, and Dublin is consistent with the emergence, re-emergence, and increasing isolation of these serovars in previous studies in the northwestern US commercial dairy herds (Berge et al., 2004, 2006; Velge et al., 2005; Davis et al., 2007a, b). The MDR *Salmonella* strain designated T5 in the present study was acquired by 13 herds. This strain belonged to *Salmonella* Typhimurium PFGE subtype WA-TYP035/187, which had previously been reported to have emerged and disseminated in dairy herds in the Pacific Northwest and has been seen increasingly in human clinical cases (Davis et al., 2007a). Our data reflect emerging serovars for the herds sampled, which in turn is an indicator of emergent serovars for the region. The concept of regional emergence of strains is exemplified across studies by the T5 strain, which was reported in all dairy herds in the Pacific Northwest. The main purpose of the current study was to describe

Table 4. Characteristics of newly introduced multidrug-resistant *Salmonella* strains isolated from fecal samples from 33 of 59 dairy herds from August 2005 through December 2007

Strain type designation	Serovar	PFGE type ¹	Antimicrobial resistant type ²	Farm, n	Farm ID	Farm visit number
A	I 4,12:Nonmotile	wTYP014	AKTSu	1	57	3
B	I Rough:6,7:- ³	wNEW012	AAmc	1	60	6
C	I Rough:6,8:- ³	wNEW035	AAmc	1	62	7
CC	I 9,12:Nonmotile	w9_12_N003	ACGKSTAmcSuCaz	1	23	5
E	I Rough:9,12:- ³	wD1_001	ACSxtSTAmcSu	1	52	7
F	I Rough:- ³	wINF001	ACKSTAmcSuCaz	1	51	6
D1	Dublin	w9,12:N002	ACSTAmcSuCaz	1	36	7
		wDUB002	ACSTSu	1	3	2
		wDUB002	AKAmcSuCaz	1	51	3
D2	Dublin	wDUB003	ACGKTAmcSuCaz	1	32	5
D3	Dublin	wDUB004	ACGKTAmcSuCaz	2	29, 44	6, 3
D4	Dublin	wDUB007	ACGKSTAmcSuCaz	1	9	6
D5	Dublin	wDUB008	AAmcSuCaz	1	3	2
D6	Dublin	wDUB009	ACSTSu	1	51	3
D7	Dublin	wDUB012	ACKTAmcSuCaz	1	32	4
D8	Dublin	wDUB016	ACGKSTAmcSuCaz	1	7	6
G1	Meleagridis	wMEL001	AAmcCaz	1	2	7
H1	Hadar	wHAD001	ST	1	8	5
H2	Hadar	wHAD004	ST	1	10	7
I1	Infantis	wINF001	ACSTAmcSuCaz	2	32, 33	5, 5
			ACSTAmcSuCaz	1	39	6
N1	Newport	NEW005	ACSTAmcSu	1	35	5
			ACSTAmcSuCaz	1	60	6
N2	Newport	NEW046	ASTAmcSuCaz	2	10, 18	3, 3
N3	Newport	NEW076	ASTAmcSuCaz	1	10	6
N4	Newport	wNEW005	ACSTAmcSuCaz	1	12	2
N5	Newport	wNEW010	ACSTAmcSuCaz	1	12	2
N6	Newport	wNEW013	ACSTAmcSuCaz	1	12	2
N7	Newport	wNEW015	ACSTAmcSuCaz	1	12	2
N8	Newport	wNEW016	ACSTAmcSuCaz	1	12	2
N9	Newport	wNEW018	ACSTAmcSuCaz	1	12	2
N10	Newport	wNEW036	ACSTAmcSuCaz	1	12	2
O	Ohio	wOHI001	TSu	1	33	2
R	Oranienburg	wORA001	AAmcCaz	1	25	4
T1	Typhimurium	TYP004	ACSTSu	1	46	2
T2	Typhimurium	TYP012	ASSu	1	33	3
T3	Typhimurium	TYP016	AKSTSu	1	53	5
T4	Typhimurium	TYP139	AKSTSu	1	33	4
T5	Typhimurium	TYP035/187	AKSTAmcSuCaz	7	2, 3, 5, 12, 39, 42, 57	2, 7, 3, 4, 2, 2, 2
	Typhimurium	TYP035/187	AKSTAmcSuCaz	1	48	6
	Typhimurium	TYP035/187	AKSTSu	3	23, 29, 49	5, 3, 6
	Typhimurium	TYP035/187	AKSTSuCaz	1	45	2
	Typhimurium	TYP035/187	AKSxtSTAmcSuCaz	1	44	6
T6	Typhimurium	TYP139	AKSTAmcSuCaz	1	51	6
T7	Typhimurium	wTYP002	ACSTSu	1	46	6
T8	Typhimurium	wTYP013	AKT	1	33	4
T9	Typhimurium	wTYP013a	KSTSu	1	31	6
T10	Typhimurium	wTYP062	AKSTSu	1	60	6
T11	Typhimurium	wTYP093	AKSTAmcSuCaz	1	51	7
T12	Typhimurium	wTYP108	ACKSTAmcSuCaz	1	51	6
T13	Typhimurium	wTYP113	AKSTAmcSuCaz	1	51	7
T14	Typhimurium	wTYP114	AKSTAmcSuCaz	1	51	6
U1	Uganda	wUGA001	CKSxtSTSu	2	19, 28	5, 4
U2	Uganda	wUGA010	CKSxtSTNaSul	1	39	6
V1	Montevideo	wMON006	ACSTAmcSuCaz	1	29	3
V2	Montevideo	wMON007	ACSxtSTAmcSu	1	31	7
V3	Montevideo	wMON022	ACGKSTAmcSuCaz	1	57	6

¹Pulsed-field gel electrophoresis (PFGE) type as designated by Washington Department of Public Health with the exception of types starting with the letter w, which indicates a new PFGE type designated at Washington State University.

²A = ampicillin; C = chloramphenicol; G = gentamicin; K = kanamycin; S = streptomycin; Su = triple sulfa; T = tetracycline; Caz = ceftazidime; Amc = Amoxicillin-clavulanic acid; Sxt = trimethoprim-sulfamethoxazole; NT = not tested; UN = unknown.

³Popoff (2001).

Table 5. Temporal and geographical distribution of newly introduced multidrug-resistant *Salmonella enterica* serovars isolated from 59 herds in different counties (n = 7) of Washington State

Serovar	Jan–Apr 2006	May–Aug 2006	Sep–Dec 2006	Jan–Apr 2007	May–Aug 2007	Sep–Dec 2007	Counties, n	Herds, n (%)
Typhimurium	1	2	7	6	4	7	3	27 (38.6)
Newport	7	2		1	2		4	12 (17.1)
Dublin	2			8		1	1	11 (15.7)
Infantis				2	1		4	3 (4.3)
Montevideo			1	1	1		1	3 (4.3)
Uganda			2		1		1	3 (4.3)
Hadar			1		1		1	2 (2.9)
I 9,12:Nonmotile				1			1	1 (1.4)
I 4,12:Nonmotile				1			4	1 (1.4)
I Rough:6,7:-					1		1	1 (1.4)
I Rough:6,8:-						1	1	1 (1.4)
I Rough:9,12:-						1	1	1 (1.4)
Meleagridis					1		1	1 (1.4)
Ohio		1					1	1 (1.4)
Orianenburg			1				2	1 (1.4)
I Rough:-						1	1	1 (1.4)

the introduction of new strains not seen before in particular dairy herds.

Although some studies have reported evidence of *Salmonella* introduction in cattle via feeds (Lindqvist et al., 1999; Davis et al., 2003), several other studies have failed to show direct evidence indicating feed as a potential source of *Salmonella* infection in cattle (Richardson, 1975; Williams, 1975; Wray and Sojka, 1977). In the current study, most (88%) of the feed *Salmonella* isolates were pan-susceptible, and only a very small proportion (0.5%) were contaminated with MDR *Salmonella*. The concentration of MDR *Salmonella* found in feeds was much lower than that likely to result in an infectious dose in healthy animals (Smith et al., 1979; Spier et al., 1991). Certain factors can reduce the

infectious dose in cattle (Chambers and Lysons, 1979; Mattila et al., 1988). Although MDR *Salmonella* were isolated from feed samples from different herds, we did not find MDR *Salmonella* feed strains introduced or acquired by dairy herds on the same farm. This does not mean, however, that higher contamination levels do not occur, as has been documented in some studies (Anderson et al., 1997). The rarity and low concentration of MDR *Salmonella* contamination of feed in the present study do indicate that introduction of MDR *Salmonella* infections into dairy herds via feed is unlikely to be common.

It was interesting to note that feed samples from feed mills before delivery to the farm were very rarely contaminated with *Salmonella*. Considering that 665 feed

Table 6. Distribution of multidrug-resistant *Salmonella* serovars cultured from different feed types from commercial herds (n = 59) in Washington State from August 2005 through December 2007

Farm	Serovar	PFGE subtype ¹	Feed source	Date collected	Antimicrobial resistant type ²	Farm visit number	Isolates, n
1	Dublin	DUB001	Calf grain	8/24/2005	ACSTAmcSu	1	1
6	Tennessee	ND	Cracked corn	9/19/2005	SSu	1	1
16	Typhimurium	TYP035/187	Bakery waste	3/6/2007	AKSTAmcSuCaz	6	1
33	Infantis	ND	Soybean meal	8/13/2007	ACSTAmcSu	7	1
33	Infantis	ND	Mixed grain	8/13/2007	ACSTAmcSuCaz	7	1
34	Bardo	NEW024	Mixed grain ³	12/5/2006	ASTAmcSuCaz	7	1
38	Saint-Paul	ND	Cottonseed	7/17/2006	ACSTAmcSuCaz	1	1
42	Oranienburg	wORA001	Mineral	10/17/2006	AAmcCaz	2	1
45	Typhimurium	TYP035/187	Straw	4/9/2007	AKSTAmcSuCaz	5	1
46	Typhimurium	wTYP112	Corn silage	8/27/2007	AKSTAmcSulCaz	6	1
48	Typhimurium	TYP004	Calf grain	10/23/2006	ACSTSu	2	1
51	Typhimurium	TYP004	Haylage	10/25/2006	ACSTSu	2	1
53	Typhimurium	TYP016	Straw	7/9/2007	AKSTSu	5	1
61	Newport	NEW005	Cannery waste	7/16/2007	ACSTAmcSu	5	1

¹PFGE = pulsed-field gel electrophoresis; ND = not done.

²See Table 2 for abbreviations.

³Feed mill samples before shipment to farms.

Figure 1. Distribution of 59 dairy herds based on the number of new multidrug-resistant *Salmonella* strains acquired over 15- to 21-mo sampling periods.

mill samples were analyzed, the power of the study was sufficient to detect *Salmonella*-positive feed. However, if low-level contamination is present in the feed mill samples and the regrowth of *Salmonella* only occurs at the farm, then it is possible that our sampling method was not sensitive enough to capture this low-level contamination. Another hypothesis is that the feed was contaminated at the farm. Great care was taken to take a sterile sample below the surface of the pile. There is, however, frequent movement of feed on farms. It may be that most feed contamination at the farm reflects the herd or farm infection rather than the feed being a primary cause of contamination. Although some farm feed was positive for MDR *Salmonella*, we never isolated feed-associated strains (based on PFGE profiles) from cattle feces on repeated samplings. Because of the low level of contamination and because strains in feed were not found in cattle, we believe that feed is not a major pathway for introduction of new strains in Washington State.

Diagnostic laboratory records indicated that 13 of 59 herds had a history of salmonellosis at some point before the commencement of the study. It is widely believed that *Salmonella* infections can be subclinical in cattle, and not all clinical cases of salmonellosis would have been submitted for diagnostic testing for *Salmonella*. Although it is possible that farm selection (based on voluntary participation) could have been influenced by known salmonellosis status, 22% of herds that had a history of clinical salmonellosis did not acquire any new MDR *Salmonella* strains in the present study compared with 78% of herds that had no evidence of previous cases. On the other hand, 70% (23/33) of herds that acquired new MDR *Salmonella* strains at farm visit 2 or later were negative for MDR *Salmonella* at farm visit 1, which indicates that herds that acquired new strains

were not likely to have MDR *Salmonella* at the start of the study.

Culture-dependent isolation of *Salmonella* spp. is naturally less than 100% sensitive. However, methods such as pooling of samples, targeted sampling, and sample pre-enrichment were used to improve the recovery of damaged cells and subsequent isolation rates. Initial assessment of *Salmonella* strains present in the dairy herds was based on more intensive sampling around herds. Certain sample types were targeted (e.g., pooled sampling, milk filters, slurry) that increase the chances of determining herd-wide status. Cattle sampling was divided between different groups of cattle on the farm (e.g., heifers, lactating, dry), as *Salmonella* and MDR-*Salmonella* prevalence have been demonstrated to vary significantly between these groups (Warnick et al., 2003; Cobbold et al., 2006). It is possible that a pre-existing strain was missed but that the chance of this occurring was minimized through the sampling and microbiological methods employed. Selecting multiple colonies per sample, use of multiple culture media, use of multiple enrichment broths, and samples from targeted cattle groups should maximize the isolation rate, which indirectly reduces the chance of missing a pre-existing strain.

Minor genetic events can change PFGE patterns. Therefore, the possibility exists for misclassification based on PFGE just as with any other typing method. However, PFGE has been found to be of significant value in identifying epidemiologically related isolates in many studies. For the purposes of our study, PFGE was judged to be a reasonable compromise between methods that are more insensitive to detecting different strains (e.g., serotypes, phage types) and those that are more likely to falsely detect new strains because of their high sensitivity to minor genetic changes (e.g., multiple-locus variable-number tandem repeat analysis, amplified fragment length polymorphisms).

In conclusion, our findings suggest that new MDR *Salmonella* strains are frequently introduced into Washington State commercial dairy herds. Feed probably plays a lesser role in *Salmonella* transmission to herds than other modes of introduction such as cattle introduction. The herds enrolled in the current study were considered representative of dairy herds in Washington State and may represent similarly managed herds in other regions of the United States. Herds sampled represented a wide range of production practices and herd management types, which in turn are representative of most large-scale and intensive dairying production practices outside the Pacific Northwest. The management factors associated with the rate of *Salmonella* introduction are described elsewhere (Adhikari et al., 2009). Further studies are needed to determine the

epidemiology of MDR *Salmonella* introduction, maintenance, and dissemination at the farm level and to examine whether the patterns of new strain introduction reported in the current study are similar to that in other US dairy herds.

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