The role of animal movement, including off-farm rearing of heifers, in the interherd transmission of multidrug-resistant *Salmonella*

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ABSTRACT

Fifty-nine commercial dairy farms were sampled 7 times over 15 to 21 mo to determine the role of animal movement, including off-farm rearing of heifers, in the interherd transmission of multidrug-resistant (MDR) *Salmonella* spp. Farm management data were collected by on-site inspections and questionnaires on herd management practices before and after the study. Forty-four percent (26/59) of herds did not acquire any new MDR *Salmonella* strains. The number of newly introduced MDR *Salmonella* strains acquired by the remaining 56% (33/59) of herds ranged from 1 to 8. Logistic regression models indicated that off-farm heifer raising, including contract heifer raising where heifers commingle with cattle from other farms [commingled heifers, odds ratio (OR) = 8.9, 95% confidence interval (CI): 2.4, 32.8], and herd size per 100-animal increment (herd size, OR = 1.04, 95% CI, 1.01, 1.05) were significantly associated with the introduction of new MDR *Salmonella* strains. The negative binomial regression similarly revealed that commingled heifers [relative risk (RR) = 2.3, 95% CI: 1.1, 4.7], herd size per 100 animals (RR = 1.02, 95% CI, 1.01, 1.03), and a history of clinical salmonellosis diagnosed before the study (RR = 2.5, 95% CI, 1.3, 5.0) were significantly associated with the number of new MDR *Salmonella* strains that were introduced. Factors not associated with the introduction of new MDR *Salmonella* strains were housing of heifers and cows in the same close-up pen, a common hospital-maternity pen, and the number of purchased cattle. This study highlights the role of animal movement in the interherd transmission of MDR *Salmonella* spp.

Key words: multidrug-resistant *Salmonella*, dairy farm, herd-level risk factor

INTRODUCTION

An estimated 1.4 million human salmonellosis cases and over 500 associated deaths occur annually in the United States (Mead et al., 1999; ERS/USDA, 2001). *Salmonella enterica* is often found in livestock and poultry, including dairy cattle. Nontyphoidal salmonellosis can result from infection by numerous *Salmonella* serovars, which are often transmitted via contaminated meat and milk (Mazurek et al., 2004; CDC, 2006; Van Kessel et al., 2007) or through direct contact with infected dairy cattle (Wall et al., 1994; Besser et al., 1997; Calvert et al., 1998). Because most infections result in asymptomatic or self-limiting diarrhea, antibiotics should be used only in immunocompromised patients and cases of systemic salmonellosis (Ruiz et al., 2004). Multidrug-resistant (MDR) *Salmonella* strains can further complicate the treatment of human cases of salmonellosis (Butaye et al., 2006).

Increasing travel and global trade may facilitate the introduction of new *Salmonella* serovars into importing countries. Reports of emerging MDR *Salmonella* strains and global spread have been documented (Hancock et al., 2000; Davis et al., 2007). In particular, inter-farm transmission may be a key element in the epidemiology of MDR *Salmonella*. Some investigators have suggested that the spread of emerging MDR *Salmonella* between regions is due to international travel and trade (Davis et al., 1999) and is facilitated by the use of antimicrobials. Transmission of non-foodborne *Salmonella* infection may be attributed to contact with animals, contaminated water, or the environment (Wall et al., 1995; Besser et al., 2000; Fey et al., 2000; Rice et al., 2003; Hendriksen et al., 2004). Previous studies indicated that wild birds and hedgehogs can play a role in the transmission of salmonellosis (Søbstad et al., 2000; Handeland et al., 2002; Boqvist and Vågsholm, 2005) but little is known about the extent of their influence on Washington State dairies. Risk factors have been identified in the transmission of MDR *Salmonella* between farms. Introduction of newly purchased cattle to

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Table 1. Management-related data from participating farms

Questions related to farm management	
1.	Numbers of cattle in the following categories: Lactating cows Dry cows Calves <3 mo of age raised on premises Calves <3 mo of age raised off premises Heifers 3–20 mo raised on premises Heifers 3–20 mo raised off premises Heifers 20 mo to calving raised on premises
2.	How many cattle of following types were purchased during the study period? Number of cows (lactating and dry) Number of bulls Number of calves Number of heifers
3.	Did any off-farm raised calves and heifers commingle with cattle from other sources?
4.	Have any salmonellosis cases been diagnosed during the 3 yr before the start of the study?
5.	Are heifers and cows in the same close-up pen?
6.	Where do cows calve?
7.	Are any other cows in contact with maternity cows in this location (e.g., sick cows)?
8.	Where are cows moved after calving?
9.	Are any other cows in contact with postpartum cows at this location (e.g., sick cows)?

the farm has been reported as one of the primary routes of *Salmonella* transmission (Evans, 1996; Zansky et al., 2002; Vanselow et al., 2007). Some evidence suggests that off-farm raising of heifer calves presents a similar risk of introduction (Hegde et al., 2005). Approximately 12.5% of US dairy farms send their heifer calves off-farm to be reared at dedicated contracted calf ranches where the heifers are commingled with other animals from source facilities (Wolf, 2003; USDA, 2007).

During the last decade there has been an alarming increase in the appearance of antibiotic-resistant *Salmonella* that may be a consequence of selective pressure associated with the use of antimicrobial agents in food animals (Varma et al., 2005). Increased morbidity and mortality may result in an inability to treat salmonellosis due to antimicrobial resistance. Investigators have also reported that antimicrobial-resistant strains may be more virulent and cause more prolonged or more severe illness than antimicrobial-susceptible strains (Travers and Barza, 2002). The purpose of this study was to determine the role of animal movement, including off-farm rearing of heifers, in the interherd transmission of MDR *Salmonella* spp.

MATERIALS AND METHODS

Study Farms

Sixty commercial dairy farms in Washington State were enrolled into the study using the following selection criteria: farm located within Washington State and operated as a commercial dairy farm. According to the Dairy Farmers of Washington (2007; <http://www.havemilk.com>), there were 496 dairy farms and 238,000

milk cows in Washington. Sample size calculation was performed as described previously (Christensen and Gardner, 2000) with consideration of the herd-level sensitivity (0.95), expected disease prevalence (8% or more), and expected test sensitivity (0.5). These farms represented 173,253 dairy cattle in Washington State. Dairy farms were selected across the 3 major dairy areas (western, central, and south-central) of the state, proportional to the total dairy farms in that region. Sixty-six farms were randomly selected alphabetically from a list of dairies, and contact was made through telephone and herd veterinarian to explain the project. Sixty farms were enrolled and 1 farm went out of business during the study, leaving us with 59 farms for study; 6 declined participation. Management-related information was collected via a standardized questionnaire and by on-site inspections (Table 1).

In Washington State, herd veterinarians regularly submit fecal samples to the Washington Disease Diagnostic Laboratory (WADDL, Pullman, WA) for suspected cases of salmonellosis. Information about historical cases of salmonellosis (within 3 yr of the start of the current study) and isolates were obtained from WADDL.

Sampling Plan

Each farm was visited 7 times at intervals of 2 to 4 mo over a period of 15 to 21 mo to collect samples. All samplings occurred between August 2005 and December 2007. A total of 7,009 pooled fecal samples were collected from the participating herds. Each fecal pool consisted of approximately 5 g of feces randomly collected from 10 individual, undisturbed fresh fecal pats

from the ground in the pen using sterile tongue depressors. An average of 16 fecal pools was collected per farm visit from different groups of cattle. These fecal samples were collected from lactating cows (3–9 pools), 2 to 3 dry cows (2–3 pools), close-up dry cows (2–3 pools), heifers (1–2 pools), cows in the maternity/hospital area (1–2 pools), and calves (1 pool). The number of fecal pools was adjusted according to the number of strings per group of animals. The more strings per group of target animals (e.g., lactating cows), the greater the number of fecal pools were collected. This sampling method was estimated to provide at least 95% probability of detecting a positive herd if the within-herd prevalence was 8% or more (Jordan, 2005). The sensitivity was likely to be higher than the computed number because more samples were collected from target populations (calves, lactating cows, maternity/hospital cows) reported to be at increased risk of infection (Hancock, 1996; Warnick et al., 2003a). A slurry sample of 50 mL from slurry pond was collected from each dairy farm.

Salmonella Culture and Identification

For isolation of *Salmonella* spp. from the feces, 12.5 g of each fecal sample was enriched in 112.5 mL of tetrathionate broth (Hardy Diagnostics, Santa Maria, CA) and 112.5 mL of Rappaport-Vassiliadis broth (Hardy Diagnostics). The cultures were incubated for 48 h at 42°C. Each enriched sample was plated onto xylose lysine tergitol-4 (XLT-4, Hardy Diagnostics) agar and MacConkey (Mac, Hardy Diagnostics) agar supplemented with ampicillin (256 µg/mL), chloramphenicol (8 µg/mL), and streptomycin (32 µg/mL; Sigma-Aldrich, Dallas, TX). The plates were incubated for 24 h at 37°C. In this method, 2 XLT-4 and 2 Mac plates per sample were used. All the Mac plates contained antibiotics as described above, whereas XLT-4 plates did not contain any antibiotics. For isolation of *Salmonella* spp. from the slurry, 25 mL of slurry sample was pre-enriched in 225 mL of buffered peptone water (BPW; Hardy Diagnostics) and incubated for 24 h at 37°C. Pre-enrichment steps were followed by enrichment steps by transferring 1 mL of BPW to 9 mL of tetrathionate broth and 100 µL of BPW to 10 mL of Rappaport-Vassiliadis broth and incubating for 48 h at 42°C. The enriched sample was plated onto XLT-4 and Mac agar plates and incubated for 24 h at 37°C. For confirmation of suspected *Salmonella*, 3 colonies from XLT-4 and Mac plates 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 and were incubated for 18 to 24 h at 37°C. One isolate per sample was further screened with agglutina-

tion 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 for the same sample on 2 different culture media) was selected for further characterization by antimicrobial susceptibility testing. Isolates showing antimicrobial resistance to 2 or more antimicrobials belonging to distinct classes of antimicrobial agents were designated as MDR isolates. One MDR isolate (except for isolate with multiple serogroups) per sample per visit was serotyped at the National Veterinary Service Laboratory (Ames, IA).

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 drug tested by the threshold zone size for resistance, as recommended by Clinical and Laboratory Standards Institute (formerly NCCLS) 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). These antimicrobial agents are the most relevant to both human and veterinary medicine. All *Salmonella* isolates were screened for antimicrobial susceptibility phenotypes using 10 different antimicrobial agents. Isolates classified as being MDR were further examined by serotyping and pulsed-field gel electrophoresis (PFGE).

PFGE

All 411 MDR *Salmonella* isolates from fecal samples were analyzed by PFGE following *Xba*I restriction digestion based on the PulseNet protocol for *Salmonella* using *Salmonella* Braenderup H9812 as a size standard (Hunter et al., 2005). The PFGE images were analyzed using BioNumerics v. 3.5 software (Applied Maths, Sint-Martens-Latem, Belgium). Independent strains were defined by PFGE profiles that differed in at least 2 bands with a position tolerance of 1.5%.

New MDR Salmonella Strain Introduction

Isolates from the first farm sampling or from pre-study diagnostic samples submitted to WADDL during

the previous 3 yr were considered pre-existing strains. For the purpose of this article, a strain found on the farm was considered a newly introduced strain (based on 2 or more PFGE band differences) if the strain was isolated at the second or later sampling visit and had not been observed on that farm in the first sampling visit or in diagnostic samples from suspected clinical cases for at least 3 or more years before the commencement of the study.

Data Analysis

Univariate analysis was performed to evaluate the unadjusted association between individual variables and the introduction of any new MDR *Salmonella* strains. Variables with P -values < 0.25 were entered into multivariable models in a stepwise manner. This was a preselection of variables to enter into the multivariable model. Logistic regression analysis was used to evaluate the association between potential herd-level factors and the probability of whether any new MDR strain had been introduced during the study period. Negative binomial regression was used to test the same factors for association with the number of new MDR *Salmonella* strains introduced during the study period. The main effects as well as interaction terms were retained in the final multivariate model if they were significant at a P -value < 0.05 . The number of months during which a herd was sampled was forced in the model to examine its potential confounding effect. All analyses were performed with SAS v.9.1 software (SAS Institute Inc., Cary, NC) and Excel (Microsoft, Redmond, WA).

RESULTS

A total of 1,113 *Salmonella* spp. were isolated from 59 farms. One farm that had an incomplete data set (the farm went out of business) was excluded in this analysis. Of the 1,113 isolates, 385 were multidrug-resistant. Multidrug-resistant *Salmonella* spp. were isolated from 66% (39/59) of dairy herds (Table 2). Forty-six percent (27/59) of herds did not acquire any new MDR *Salmonella* strains, whereas the number of new MDR *Salmonella* strains acquired by the remaining 54% (32/59) of herds ranged from 1 to 8 (Table 3). Seventeen herds were positive for a single newly acquired strain, whereas 2 of the 33 herds each acquired 8 MDR *Salmonella* strains.

Historical information on clinical *Salmonella* infection from participating herds (Table 4) indicated that 13 of 59 herds were diagnosed with clinical salmonellosis before the study based on *Salmonella*-positive clinical sample submissions to WADDL. Twenty-six MDR *Salmonella enterica* isolates were detected on clinical

submission samples. Historical MDR *Salmonella* strains listed in Table 2 were not isolated in the current study. All 26 isolates were resistant to ampicillin, followed by 25 isolates resistant to triple-sulfa, streptomycin, and tetracycline, 16 isolates resistant to chloramphenicol, 14 isolates resistant to ceftazidime, 13 isolates resistant to kanamycin, 4 isolates resistant to trimethoprim-sulfamethoxazole, and 2 isolates resistant to gentamicin. All isolates were resistant to at least 5 antimicrobials, and 5 (19%) were resistant to 7 or more antimicrobials. *Salmonella* Typhimurium was the most (12/26) commonly identified serovar followed by *Salmonella* Newport (9/26).

Table 5 summarizes the distribution and PFGE profiles of newly introduced MDR *Salmonella* serovars isolated from commercial dairy herds enrolled in the study between January 2006 and December 2007. Of the 59 herds, new strains of serovar Typhimurium were acquired by 20 (34%) herds, serovar Dublin by 9 (15%) herds, serovar Newport by 5 (8%) herds, and serovars Infantis, Montevideo, and Uganda by 3 herds each. Among the newly introduced strains of *Salmonella*, 13, 10, and 9 strains belonged to serovars Typhimurium, Newport, and Dublin, respectively. The strain designated T5 was the most frequently introduced strain of serovar Typhimurium isolated in several dairy herds. Other new strains include Hadar; Ohio; Oranienburg; I 4,12:Nonmotile; I Rough: 6,7:-; I Rough: 6,8:-; I 9,12:Nonmotile; I Rough: 9,12:-; I Rough:-; and I Rough: 6,8:-. A high diversity of antimicrobial resistance was observed within serovars and PFGE subtypes.

Three variables were found to be significantly associated with new MDR *Salmonella* strain introduction in univariate analyses (Table 6): heifer off-farm raising, including contract heifer raising with commingling of cattle from other sources [unadjusted odds ratio (OR) = 6.6]; salmonellosis diagnosed in the herd in the last 3 yr (unadjusted OR = 5.4); and herd size in 100-animal increments (OR = 1.015). These variables were also found to be significantly associated with the number of new MDR *Salmonella* strains introduced in participating herds (unadjusted OR = 2.0, 3.3, and 1.02, respectively).

The multivariable logistic regression model showed that the practice of raising heifers off-farm in situations where the heifers were commingled with cattle from other sources [commingled heifers, adjusted OR = 8.9, 95% confidence interval (CI), 2.4–32.8], and increasing herd size in 100-animal increments (herd size, adjusted OR = 1.04, 95% CI, 1.01–1.05) were significantly associated with MDR *Salmonella* strain introductions into dairies (Table 7). Multivariable negative binomial regression also showed that commingled heifers and increasing herd size were significantly associated with the

Table 2. *Salmonella* spp. isolation from pooled fecal samples in commercial dairies in the northwestern United States

Farm ¹	Pooled sample, n	Non-MDR ² <i>Salmonella</i>	MDR <i>Salmonella</i>	Farm	Pooled sample, n	Non-MDR <i>Salmonella</i>	MDR <i>Salmonella</i>
1	158	6	0	32	131	5	9
2	150	6	14	33	128	16	61
3	147	2	4	35	132	1	3
4	128	9	0	36	75	5	1
5	161	3	2	37	115	19	0
6	121	0	0	38	113	3	1
7	109	0	2	39	129	3	20
8	110	14	1	40	93	1	0
9	100	0	1	41	85	2	0
10	100	1	16	42	117	22	2
11	120	8	0	44	120	2	3
12	113	0	80	45	127	18	7
13	99	0	1	46	124	14	24
14	115	0	0	47	135	5	0
15	117	4	0	48	123	3	3
16	74	0	0	49	121	19	6
17	123	54	0	50	75	0	0
18	131	28	1	51	126	3	23
19	180	1	1	52	111	25	1
20	133	17	0	53	124	37	15
21	116	26	0	54	120	7	0
22	126	52	2	55	113	0	2
23	189	51	2	56	110	0	0
25	124	43	1	57	117	60	6
26	119	2	0	58	94	1	4
27	85	0	0	59	106	22	11
28	81	0	9	60	126	6	13
29	131	14	8	61	129	2	20
30	107	2	0	62	108	30	1
31	115	56	2				

¹Two feed mills numbered as farm 23 and 43. Sixty farms and 2 feed mills were numbered consecutively while enrolling farms and feed mills. However, feed mills are not included in this table. Farm 43 went out of business and its data were not included in the analysis.

²MDR = multidrug resistant.

number of new MDR *Salmonella* strain introductions [adjusted relative risk (**RR**) = 2.3, 95% CI, 1.1–4.7 and adjusted RR = 1.02, 95% CI, 1.01–1.03, respectively; Table 8]. In addition, clinical salmonellosis diagnosed in the herd in the 3 yr before the start of the study was also significantly associated with the number of new MDR *Salmonella* strain introductions (adjusted RR = 2.5, 95% CI, 1.3–5.0). No interaction terms were found to be significant.

DISCUSSION

In the present study, we investigated the role of animal movement in the interherd transmission of MDR *Salmonella* spp. because the transport of *Salmonella*-infected animals onto farms has been recognized as a primary route of *Salmonella* introduction on farms (Evans, 1996; Zansky et al., 2002; Vanselow et al., 2007). Our data indicate that 49% of the dairy farms raised heifers off-farm in ranches where they commingled with cattle from several sources. A recent study in the

northeastern United States reported a similarly high percentage of dairy farms that sent calves to a custom raiser (Fulwider et al., 2008).

We found that 27 (46%) of the herds did not acquire any new MDR *Salmonella* strains, whereas 32 (54%) of herds acquired one or more new MDR *Salmonella* strains during the study period. The presence of heifers raised off-farm on ranches where heifers commingle with cattle from several sources was found to be a significant risk factor for introduction of new MDR

Table 3. Introduction of new multidrug-resistant (MDR) *Salmonella* strains into 59 commercial dairies in the northwestern United States

New MDR <i>Salmonella</i> strains introduced, n	Farms, n (%)
0	27 (46)
1	17 (29)
2	5 (8)
3	7 (12)
6	1 (2)
8	2 (3)

Table 4. Historical information on previous clinical multidrug-resistant *Salmonella* spp. isolates from the dairy farms enrolled in the study

Farm	Serovar	PFGE type ¹	Antimicrobial resistance type ²	Isolates, n
1	Typhimurium	TYP004	ACSTSu	1
2	Newport	NEW005	ACSTSuCaz	1
	Typhimurium	wTYP041	ACKSxtSTSu	1
8	Newport	NEW013	ASTSuCaz	2
14	Heidelberg	wHEI006	ACGKSTSuCaz	1
18	Newport	NEW005	ACSTSuCaz	1
20	Typhimurium	TYP035	AKSTSu	1
32	Dublin	wDUB022	ACGKSxtSTSuCaz	1
	Typhimurium	TYP021	AKSTSu	3
		wTYP115	AKSTSu	1
33	Bardo	NEW005	ACSTSuCaz	1
	Newport	NEW005	ACSTSuCaz	3
37	Meleagridis	wMEL003	AAmcCaz	1
	Newport	NEW005	ACSTSuCaz	1
			ACSTSuCaz	1
	Typhimurium	TYP035	AKSTSu	1
55	Typhimurium	TYP018	ACKSxtSTSu	1
60	9,12:Nonmotile	w9_12_N004	ACKSTSu	1
	Typhimurium	TYP035	ACKSxtSTSuCaz	1
		w4_12_001	AKSTSu	1
62	Typhimurium	TYP004	ACSTSu	1

¹Pulsed-field gel electrophoresis 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.

Salmonella strains into commercial dairy herds. This finding indicates that off-farm heifer raising operations where calves commingled with cattle from other sources was significantly associated with an increased risk of negative dairy herds becoming positive for *Salmonella*. The results of this study corroborate a finding of a previous study (Hegde et al., 2005), in which the authors reported that the heifer-raising operation could serve as a clearinghouse for *Salmonella* spp. A recent study, however, reported that the risk of *Salmonella* transmission from the heifer feedlot back to the dairy was low (Edrington et al., 2008). The latter study, however, used only a limited number of dairy herds and no statistical analysis was performed.

Previous studies reported that purchase of cattle from other sources presents important risks of *Salmonella* transmission between herds (Wray et al., 1990; Evans and Davies, 1996; Zansky et al., 2002). The present study failed to find a significant effect of cattle purchases (cow, bulls, calves, and heifers) on introduction of new MDR *Salmonella* strains into commercial dairy herds. A recent study found that introduction of new cattle was not associated with *Salmonella* status dairy herds (Huston et al., 2002). It is not known why the present study did not find cattle purchase as a risk factor for interherd transmission of *Salmonella*; however, it is possible that the high rate of off-farm heifer raising

(nearly 50% of the farms in the present study raised heifers off-farm, where they commingled with cattle from other sources, or replaced stock through contract heifer raising) mitigated the effects of cattle purchases in the present study. As a farm introduced more heifers as replacement stock through contract heifer-raising, they purchased fewer heifers directly from other farms.

Farms with a history of clinical salmonellosis were at increased risk of acquiring new *Salmonella enterica* strains. Although a previous study identified a significant association between recent clinical salmonellosis and detection of *Salmonella* in fecal samples from the same herd for up to 8 mo (Warnick et al., 2001), we did not find the same serovar in herds that had a history of clinical salmonellosis. It is unclear why background strains of *Salmonella* spp. did not persist in the present study herds, but this may be due to intermittent shedding patterns of *Salmonella* in cattle, environmental conditions, and other unknown factors. The discrepancy between our result and that of the Warnick et al. (2001) study could be related to the observations that clinical salmonellosis occurred in some of the participating herds in the present study more than a year before the start of the study, and herds with clinical salmonellosis were likely managed differently than noninfected herds. Given that the association was still significant after adjustment for other variables, this finding suggests

Table 5. Pulsed field gel electrophoresis (PFGE) profiles and antimicrobial resistance phenotypes of newly introduced multidrug-resistant *Salmonella* strains isolated from the study herds

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

¹PFGE 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.

³Popoff (2001).

Table 6. Univariate analyses of herd-level factors associated with introduction of one or more new multidrug-resistant (MDR) *Salmonella* strains into dairy herds in the northwestern United States

Variable	Level	Any new MDR <i>Salmonella</i> introduction		Number of new MDR <i>Salmonella</i> introductions	
		<i>P</i> -value	Odds ratio	<i>P</i> -value	Relative risk
Any cows purchased	Yes	0.86	0.88	0.65	1.3
	No		1		1
Any bulls purchased	Yes	0.66	1.28	0.45	0.7
	No		1		1
Any calves purchased	Yes	0.81	0.71	0.83	0.8
	No		1		1
Any heifers purchased	Yes	0.1	0.36	0.99	1
	No		1		1
Any cattle purchased	Yes	0.38	0.63	0.31	0.69
	No		1		1
Herd size	Per 100 animals	0.24	1	0.02	1
Number of cows purchased ¹	Number	0.54	0.92	0.52	1
Number of bulls purchased ¹	Number	0.07	0.88	0.46	0.99
Number of calves purchased ¹	Number	0.75	1.07	0.42	0.98
Number of heifers purchased ¹	Number	0.92	0.99	0.94	1
Total cattle purchased	Number	0.51	0.99	0.84	1
Common hospital-maternity pen	Yes	0.77	0.84	0.97	1
	No		1		1
History of clinical salmonellosis	Yes	0.01	5.4	<0.01	3.3
	No		1		1
Heifers commingled ²	Yes	<0.01	6.6	0.05	2
	No		1		1
Heifers and cows in the same close-up pen	Yes	0.54	0.7	0.57	1.2
	No		1		1

¹Numbers in log₁₀ scale.²Off-farm raised heifers including contract-raised heifers commingled with cattle from other sources.

the existence of important risk factors for the introduction of new strains that were not found in the present study. Previous studies suggest that recent antimicrobial treatment can increase the probability of isolating *Salmonella* in calves, heifers, and cows (Warnick et al., 2003b; Berge et al., 2006). It is possible that antimicrobial use at the farm level increases the susceptibility of dairy herds to acquire new strains.

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

farms was based on more intensive sampling around dairy farms. 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* prevalences have been demonstrated to vary significantly between these groups (Warnick et al., 2003a; Cobbald et al., 2006). It is possible that a pre-existing strain was missed, but the chance of this occurring was minimized through the sampling and microbiological methods employed.

The results of our study indicate that increased herd size was significantly and positively associated with

Table 7. Multivariable logistic regression model for examination of the management variable as a risk factor for the introduction of multidrug-resistant *Salmonella* strains into dairy herds

Variable	Coding	Odds ratio	χ^2	95% confidence interval ¹		<i>P</i> -value
				Lower bound	Upper bound	
Off-farm commingled ²	Yes	8.9	10.7	2.4	32.8	0.001
	No	1				
Herd size	Per 100 increase	1.04	4.7	1.01	1.05	Reference 0.031

¹Maximum likelihood approximation²Off-farm raised heifers including contract-raised heifers commingled with cattle from other sources.

Table 8. Multivariable negative binomial regression model for examination of management variables as a risk factor for the introduction of multiple new multidrug-resistant *Salmonella* strains into dairy herds

Variable	Coding	Relative risk	χ^2	95% confidence interval ¹		P-value
				Lower bound	Upper bound	
Off-farm commingled ²	Yes	2.3	5.5	1.1	4.7	0.019
	No	1				Reference
Salmonellosis in last 3 yr ³	Yes	2.5	6.8	1.3	5	0.009
	No	1				Reference
Herd size	Per 100 increase	1.02	9.3	1.01	1.03	0.002

¹Maximum likelihood approximation.²Off-farm raised heifers including contract-raised heifers commingled with cattle from other sources.³Clinical salmonellosis diagnosed in the 3-yr period preceding the study period.

increased risk of introduction of new MDR *Salmonella* strains and with increased numbers of new MDR *Salmonella* introductions. This finding corroborates that of another study, which found that increases in herd size and the number of cattle purchased from test-positive herds in the previous year-quarter were significantly and positively associated with an increased risk of negative herds becoming positive for *Salmonella* (Nielsen et al., 2007). Several previous studies also found an association between herd size and *Salmonella* prevalence, which might be affected by the *Salmonella* introduction rate (Kabagambe et al., 2000; Huston et al., 2002; Warnick et al., 2003b).

In conclusion, the findings of the present study strongly implicate off-farm rearing of heifers in the transmission of MDR *Salmonella* strains between farms. Other factors, including increased herd size and a history of clinical salmonellosis, were also associated with the introduction of MDR *Salmonella* into dairy herds. For future studies, other important risk factors associated with interherd transmission of *Salmonella* should be investigated. Studies are needed to clarify whether and how off-farm rearing of heifers can be done without increasing the risk of *Salmonella* introduction.

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