Long-term persistence of multi–drug-resistant *Salmonella enterica* serovar Newport in two dairy herds

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Objective—To evaluate the association between maintaining joint hospital and maternity pens and persistence of multi–drug-resistant (MDR) *Salmonella enterica* serovar Newport on 2 dairy farms.

Design—Observational study.

Sample Population—Feces and environmental samples from 2 dairy herds.

Procedure—Herds were monitored for fecal shedding of *S enterica* Newport after outbreaks of clinical disease. Fecal and environmental samples were collected approximately monthly from pens housing sick cows and calving cows and from pens containing lactating cows. Cattle shedding the organism were tested serially on subsequent visits to determine carrier status. One farm was resampled after initiation of interventional procedures, including separation of hospital and maternity pens. Isolates were characterized via serotyping, determination of antimicrobial resistance phenotype, detection of the *CMY-2* gene, and DNA fingerprinting.

Results—The prevalence (32.4% and 33.3% on farms A and B, respectively) of isolating *Salmonella* from samples from joint hospital-maternity pens was significantly higher than the prevalence in samples from pens housing preparturient cows (0.8%, both farms) and postparturient cows on Farm B (8.8%). Multi–drug-resistant *Salmonella* Newport was isolated in high numbers from bedding material, feed refusals, lagoon slurry, and milk filters. One cow excreted the organism for 190 days. Interventional procedures yielded significant reductions in the prevalences of isolating the organism from fecal and environmental samples. Most isolates were of the C2 serogroup and were resistant to third-generation cephalosporins.

Conclusions and Clinical Relevance—Management practices may be effective at reducing the persistence of MDR *Salmonella* spp in dairy herds, thus mitigating animal and public health risk. (*J Am Vet Med Assoc* 2006;228:585–591)

Salmonellosis is an important disease of cattle and humans. Exposure to contaminated meat and milk is a known mode of transmission of zoonotic strains of *Salmonella enterica* to humans.[1–5] Salmonellosis is one of the most important food safety threats in the United States and is estimated to be responsible for 1.4 million cases of disease and 500 deaths each year.[6] A worrisome development is the emergence of MDR (ie, reduced susceptibility to ≥ 5 antimicrobials) strains of *Salmonella* spp. Although multiple *Salmonella* serovars are MDR, 2 in particular have become widely recognized as agents of morbidity in humans and bovines. Multi–drug-resistant *Salmonella* Typhimurium definitive phage type 104 emerged in the mid-1980s and appears to have undergone clonal expansion on a global basis.[6–9] Recent emergence in North America of MDR *Salmonella* Newport has been associated with an increase in the frequency of isolation of *Salmonella* Newport from cases of human salmonellosis; Newport was the third most frequently isolated serovar during the past 3 years.[5,10] Proportional isolation rates for serovar Newport have concomitantly increased among clinical bovine isolates, notably in the Pacific northwest region of the United States.[11,a]

Multi–drug-resistant strains of *Salmonella* spp share clonal epidemiologic features in that most isolates are genotypically and phenotypically similar and appear to have become disseminated via expansion of a common and recently emerged ancestor strain.[9,12] Isolates typically have a penta-resistance phenotype (ACSSuT; ie, resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) and also frequently carry the plasmid-mediated AmpC–like β-lactamase gene *CMY-2*, which confers the trait of decreased susceptibility to cephamycins and third-generation cephalosporins.[5,12,13] Strains with those resistance phenotypes greatly complicate treatment and control of clinical disease in humans and bovines.[14] Because many resistance genes are located on mobile genetic elements such as plasmids and transposons, persistence of these strains in an environment constitutes a potential reservoir of antimicrobial resistance genes that may be transferred to other bacteria.[1,12,15] The use of antimicrobial drugs in livestock production is considered to have the potential to drive selection for antimicrobial resistance among bacteria, whether by promoting emergence of novel resistant strains or via enhanced dissemination of selected clones of resistant bacteria.[7,16] Because of these epidemiologic features, effective control of salmonellosis on dairy farms is more likely to be achieved through prevention of herd exposure and infection rather than through improved

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The authors thank Blanca Lopez and Russell McClanahan for technical assistance.

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MDR	Multi–drug-resistant
PFGE	Pulsed-field gel electrophoresis

diagnosis and treatment of individual cattle.[7,8] However, many features of the organism's on-farm epidemiology must be better defined to elucidate improved prevention strategies.[9,14]

Prevention of exposure of cattle to MDR *Salmonella* strains requires reducing the frequency with which new strains are introduced onto farms. However, factors that mitigate maintenance of MDR *Salmonella* strains in herds once introduced must also be considered because those factors decrease the length of the time during which a herd will constitute a source of potential transmission of infection to humans or other farms. Although results of broad-scale epidemiologic studies[17–23] suggest that there are multiple risk factors for establishment of *Salmonella* infections in dairy herds, few studies have examined in detail risk factors pertaining specifically to infection by MDR *Salmonella* spp. We hypothesized that the use of shared hospital-maternity pens, as opposed to maintaining separate pens for sick versus periparturient cattle, was a risk factor for persistence of MDR *Salmonella* Newport on dairy farms. The principal aims of our study were to estimate prevalence rates for MDR *Salmonella* Newport in cattle before, during, and after they were housed in joint hospital-maternity pens so that the nidus population of infected cows could be determined. Because salmonellae are known to survive outside of the gastrointestinal tract for prolonged periods of time, we also aimed to determine whether certain farm environments served as reservoirs of MDR *Salmonella* Newport. Longitudinal sampling of cattle with MDR *Salmonella* Newport was undertaken to better evaluate potential long-term carriage of this serovar in cattle. Finally, we aimed to determine the effects of introducing interventions specifically designed (on the basis of findings from aims 1, 2, and 3) to decrease endemic maintenance of MDR *Salmonella* Newport.

Materials and Methods

Study population—Two herds in the central part of the Columbia Basin in the state of Washington were sampled. Both farms were drylot dairy operations that milked > 1,000 cows and followed production and animal-management practices typical for dairies of this size in the region. Lactating cows were housed in free stalls with recycled sand for bedding. Nonlactating cows and heifers were housed in a drylot pen. Both dairies routinely introduced new stock onto the property and had purchased new heifers around the time of the initiating outbreaks of salmonellosis. Herds were selected for sampling on the basis of reported outbreaks of MDR and ceftiofur-resistant *S enterica* serovar Newport strains, as per information from the referring herd veterinarians.[b] Salmonellosis had been diagnosed previously in both herds, but none of those cases had involved the MDR *Salmonella* Newport strain. Both dairies used shared hospital-maternity pens in which clinically ill cattle were housed with healthy cows and heifers that were near parturition. Farm A had a second hospital pen that housed cattle with less severe or chronic types of disease (eg, mastitis or lameness). Farm B had 2 hospital pens, one of which housed sick or periparturient cows and the other which housed sick or periparturient heifers.

Sampling strategy—Farm A was initially investigated on July 29, 2002, and Farm B was initially investigated on September 3, 2002. Pertinent information relating to the outbreak was collected at the time of initial investigations. Farms were resampled 4 times after the initial outbreak investigations for a total of 5 sampling visits/farm. Sampling was performed approximately monthly (median number of days between each sampling, 43). Types and numbers of samples collected at each visit, including at the initial outbreak investigation, were summarized (**Appendix**). Randomly selected fecal pats were sampled in the preparturient (within 21 days of calving) and postparturient (within 21 days of calving) pens to estimate group prevalence rates for *Salmonella* Newport. Cattle in the hospital-maternity pens were sampled individually when possible, and samples from randomly selected manure pats were also collected to increase sample size. Various environments on the farms were sampled, with types and numbers of samples collected dependent on availability. Most feed, feed refusals, and bedding samples were taken from the hospital-maternity pen, although postparturient and preparturient cow pens were also sampled. Cows and heifers found to be shedding *Salmonella* on any sampling visit were resampled on subsequent visits (if still in the herd) to evaluate long-term carriage of MDR *Salmonella* Newport. After the last of the 4 follow-up visits, Farm B introduced interventions to decrease maintenance of *Salmonella* Newport in the herd. To examine the effect of these interventions, Farm B was sampled once monthly for 4 additional samplings beginning 15 weeks after the introduction of the interventions.

***Salmonella* spp isolation**—Approximately 10 g of feces was collected from each cow or manure pat. Solid samples were collected in sterile bags,[c] and liquid samples were collected in sterile polypropylene bottles. Equipment was sampled by swabbing surfaces and by rinsing with sterile buffered peptone water[d] and collecting the rinsate or swab in a sterile bag.[c] Samples were transported to the laboratory at 4°C. Five grams of each fecal sample was added to 45 mL of tetrathionate broth[e] and enriched for 48 hours. Twenty-five grams of solid samples was added to 225 mL of buffered peptone water, mixed thoroughly, and pre-enriched for 24 hours prior to being subaliquoted into tetrathionate broth and re-enriched for 24 hours. Parallel enrichments were created by subculturing the tetrathionate broth enrichment in Rappaport Vasilliadis broth[f] and enriching for 24 hours (ie, tetrathionate broth enriched for a total of 48 hours). Liquid samples (60 to 80 mL) were combined with an equal volume of double-concentration selenite-F broth[g] and enriched for 24 hours. All enrichments were performed at 37°C. Enrichments were streaked for isolation on 100-mm XLT4 agar plates[e] and incubated at 37°C for 24 to 48 hours. Five suspect colonies from each plate were confirmed as *Salmonella* on the basis of morphology on lysine iron agar, triple-sugar iron, and urea agar slants.[h] Isolates were stored in 30% glycerol at –80°C and lyophilized for future reference. Human *Salmonella* Newport strains were provided by the Washington State Department of Health Public Health Laboratory as part of concurrent research and surveillance collaboration.[i] Isolates were transported on solid media slants at ambient temperatures and stored in 30% glycerol at –80°C after arrival. Other bovine *Salmonella* Newport strains used for comparison were derived from the Field Disease Investigation Unit *Salmonella* culture collection and were isolated by use of similar methods as those described.

Isolate characterization—*Salmonella* isolates were grouped by use of a commercial slide agglutination method,[d] and representative isolates were sent to the National Veterinary Services Laboratories for serotyping. Antimicrobial resistance phenotype was determined for the following antimicrobials with the Kirby-Bauer disk diffusion method according to NCCLS[24] standards: ampicillin (A), ceftazidime (Caz), chloramphenicol (C), ciprofloxacin (Cip), nitrofuran-

toin (N), gentamicin (G), kanamycin (K), streptomycin (S), tetracycline (T), trimethoprim (Tm), triple sulfa (Su), and trimethoprim-sulfamethoxazole (Sxt). Detection of the *CMY-2* gene sequence in select isolates was performed by use of PCR assay.[2] Pulsed-field gel electrophoresis was performed by use of *Xba*1 restriction enzyme according to described methods[25] augmented with standard marker strains and restriction parameters.[j] Bands were resolved[k] and gel images were stored digitally by use of a bioimaging system.[l]

Statistical analysis—Descriptive statistics were calculated with commercially available software.[m] Prevalence data for *Salmonella* were compared with the χ^2 or Fisher exact test where appropriate.[n] Profiles derived via PFGE were analyzed with a software program.[o] Cluster analyses were performed and dendrograms created by means of an unweighted pair group method using arithmetic means with the Dice similarity coefficient. Optimization and position tolerance were set at 1.0%. Values of $P < 0.05$ were considered significant.

Results

Prevalence rates for isolation of *Salmonella* spp varied significantly ($P < 0.001$) by group (**Figure 1**). The prevalence of *Salmonella* detection on each farm was highest in cows and manure pats in the joint hospital-maternity pens, with approximately one third of samples from those pens having positive results. That value was significantly ($P < 0.001$ for both farms) higher than the prevalence detected in the preparturient pens, in which < 1% of samples had positive results. Prevalence values in samples from postparturient cows were also lower than those for cows in the hospital-maternity pens ($P = 0.09$ and $P < 0.001$ for Farms A and B, respectively) and were significantly ($P < 0.01$, both farms) higher than prevalence rates for preparturient cattle. A large proportion of the environmental samples contained *Salmonella* spp. Bedding samples had the highest prevalence of isolation, with 15 of 17 samples having positive culture results. Feed refusals (7/9 samples), lagoon slurry (8/11 samples), and milk filters (3/5 samples) also frequently contained *Salmonella* spp. Five of 20 water samples, 2 of 11 feed samples, and 3 of 23 equipment samples had positive culture results. Thirty-one cows were sampled again after being confirmed to be shedding MDR *Salmonella* Newport. Only 1 cow (on Farm A) was shedding *Salmonella* on 2 or more consecutive occasions. That cow was found to be shedding *Salmonella* on 4 consecutive samplings and was presumed to also be shedding between the sample periods; the organism was isolated from that cow for 190 days. The cow was initially recognized to be excreting MDR *Salmonella* Newport while in the hospital-maternity pen and continued to have positive culture results after being returned to the general milking herd.

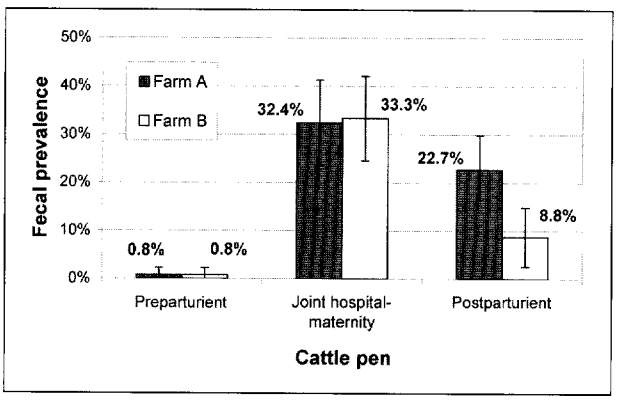
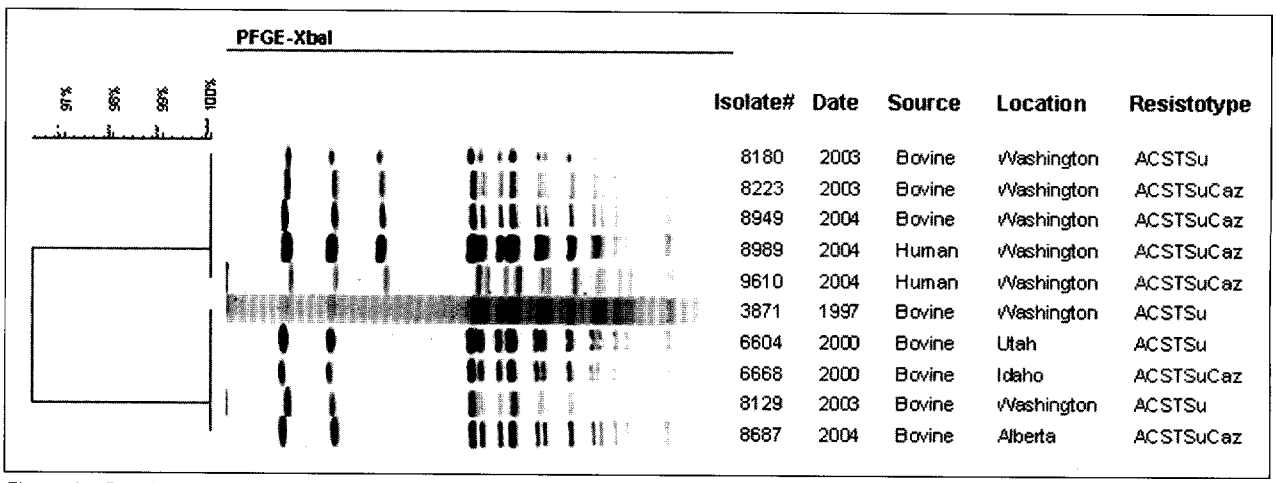


Figure 1—Prevalence of isolation of MDR *Salmonella enterica* serovar Newport from cattle in pre- and postparturient pens and in joint hospital-maternity pens on 2 dairy farms.

Salmonellae were isolated in 201 instances during the course of the study. Most (93%; 186/201) were of serogroup C2. All C2 isolates serotyped (n = 74) were identified as *Salmonella* Newport. Other serotypes detected were Bardo, Mbandaka, and Senftenberg. The predominant resistance phenotype among isolates was ACSSuTCaz (182/201). Most C2 (177/186) and *Salmonella* Newport (73/74) isolates had the ACSSuT resistance phenotype and low susceptibility to ceftazidime. Other C2 resistance phenotypes detected included A, AST, CSSuTCaz, and ACSSuTCazSxtTm. Only 6 isolates, including 3 C2 salmonellae, were susceptible to all antimicrobials tested. All isolates except one had identical restriction patterns. The predominant PFGE pattern (represented by isolate #8223; **Figure 2**) was identical to or



Isolate#	Date	Source	Location	Resistotype
8180	2003	Bovine	Washington	ACSTSu
8223	2003	Bovine	Washington	ACSTSuCaz
8949	2004	Bovine	Washington	ACSTSuCaz
8989	2004	Human	Washington	ACSTSuCaz
9610	2004	Human	Washington	ACSTSuCaz
3871	1997	Bovine	Washington	ACSTSu
6604	2000	Bovine	Utah	ACSTSu
6668	2000	Bovine	Idaho	ACSTSuCaz
8129	2003	Bovine	Washington	ACSTSu
8687	2004	Bovine	Alberta	ACSTSuCaz

Figure 2—Dendrogram of *Xba*1 restriction patterns for MDR *Salmonella* Newport isolates representative of bovine and human strains isolated between 1997 and 2004 from various locations in the northwest portion of the United States. *Isolate #8223 was obtained during the present study.

closely matched other bovine MDR *Salmonella* Newport strains from various locations in the Pacific northwest that had been isolated before and after the present study. There were also close similarities in restriction patterns with isolates obtained from human samples. All 38 MDR *Salmonella* C2 isolates that were subjected to PCR assay for CMY-2 contained the gene.

Operators on farm B introduced management interventions to reduce maintenance of *Salmonella* in the herd. Most important among these was separation of sick and parturient cattle into hospital and maternity pens, respectively. Cows and heifers were maintained in the maternity pen for 4 to 5 days after calving, after which they were released into the postparturient cow pen. Rectal temperatures were taken daily for each cow or heifer, and cattle were moved to the hospital pen for treatment if they developed pyrexia or diarrhea. Other biosecurity measures to reduce transmission of *Salmonella* between sick and recently calved cows included switching from use of a phenolic compound to a peroxygen biocide[P] for equipment disinfection, use of footbaths for personnel in the hospital-maternity pen section, strict protocols for disinfection of hands and equipment between handling of each sick or periparturient cow, strict separation of feeds allocated for the hospital versus maternity pens, and use of fresh sand for bedding in the hospital and maternity pens. The overall prevalence for *Salmonella* shedding among cattle decreased significantly (from 20% to 7.2%; $P < 0.001$) after initiation of these interventions; the decrease was observed in both fecal samples (from 14% to 3.7%; $P < 0.001$) and environmental samples (from 61% to 33%; $P = 0.01$). The prevalences of *Salmonella* shedding for each cattle group and for environmental samples before and after initiation of the interventions were summarized (**Table 1**). Decreases in the prevalence of detecting the organism in fecal samples were significant ($P < 0.05$) for cattle in postparturient and maternity pens.

Table 1—Prevalence of isolation of *Salmonella* (number of samples with positive culture results/total number of samples [percentage]) from cattle in each group and from samples collected from various areas of the dairy farm environment before and after separation of hospital and maternity pens on Farm B.

Sample type	Before intervention	After intervention
Hospital pen	37/111 (33%)	7/32 (22%)*
Maternity pen	—	4/92 (4%)*
Preparturient pen	1/132 (1%)	0/87 (0%)
Postparturient pen	7/80 (9%)	0/85 (0%)
Bedding	10/10 (100%)	4/6 (67%)
Equipment	0/5 (0%)	1/11 (9%)
Feed	0/2 (0%)	2/6 (33%)
Water	4/10 (40%)	1/6 (17%)
Lagoon	4/5 (80%)	1/3 (33%)
Feed refusals	7/9 (78%)	2/2 (100%)
Milk filter	3/5 (60%)	2/5 (40%)

*Prevalence data for isolating *Salmonella* from the hospital and maternity pens after pen separation were compared with the prevalence data for the joint hospital-maternity pen before initiation of interventions.

Discussion

Multiple researchers have examined risk factors for the presence of *Salmonella* on dairy farms. Some investigators reported on development of clinical salmonellosis in dairy herds,[21–23] whereas others examined shedding of *Salmonella* by cows, irrespective of expression of clinical disease.[17–20] In most of those studies, a large number of potential risk factors were evaluated via hypothesis-generating methods. Cross-sectional or case-control designs were used in most of the studies, a study design that prevents discrimination between factors involved in dissemination of *Salmonella* to a herd and the duration of the organism's persistence in the herd. Most included analysis of *Salmonella* serotypes that are uncommon among clinical diagnostic laboratory isolates from humans or domestic animals and hence may have had differing epidemiologic features.[26,27] Data from our study add new information as a result of the longitudinal study design, the study's focus on an epidemic strain of *Salmonella*, and the targeting of a specific hypothesis that a common hospital-maternity pen provided a niche where a given *Salmonella* strain could persist on a dairy farm for protracted periods of time. That hypothesis was made on the basis of previous reports[3,20,21,26] in which exposure of susceptible cattle to sick cattle was a risk factor for development of salmonellosis. The focus of our study on strains of the bacterium known to have public health implications is also important because few studies have addressed on-farm epidemiologic features of zoonotic *Salmonella* spp.[3]

At least 3 potentially interacting elements are proposed to be necessary for long-term persistence of a *Salmonella* strain on a given dairy farm: carrier animals, chain infections, and persistence of the organism in the environment. Results of earlier studies[4,22,28] revealed that there was prolonged maintenance of *Salmonella* within cattle herds, although that finding has only rarely been reported for individual cattle and is often associated with persistent mammary gland infections rather than fecal shedding. Although a true carrier state has been reported[26,27] for more host-specific serovars of *S enterica* subsp *enterica* (eg, Dublin), less is known about chronic carriage of classic zoonotic strains by cattle. Chronic shedding of zoonotic *Salmonella* spp by carrier animals is known to occur among livestock.[29] However, the sequential nature of data collected in the present study suggests that the distribution of carriers of non-Typhi *Salmonella* serovars in bovine populations is more similar to the distribution that exists among human populations, in which few individuals persistently excrete the organism.[30] These data, along with those from studies indicating that *Salmonella* strains may persist in cattle operations for longer periods of time than any individual cow remains in the population,[31] indicate that persistence on cattle farms is not primarily a function of the presence in the herd of individual long-term carriers.

Sustained infections may develop when infectious animals have sufficient contact with susceptible animals to maintain the basic reproductive number (R_0; number of new infections per existing infection in a given population of animals) higher than 1.[32] The cohousing of sick cows (including those with clinical salmonellosis) and periparturient cows, which enter and leave the pen rapid-

closely matched other bovine MDR *Salmonella* Newport strains from various locations in the Pacific northwest that had been isolated before and after the present study. There were also close similarities in restriction patterns with isolates obtained from human samples. All 38 MDR *Salmonella* C2 isolates that were subjected to PCR assay for *CMY-2* contained the gene.

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ly and are continually replaced with new cows, is a common dairy practice and tends to maintain $R_0 > 1$. The reduced prevalence of *Salmonella* spp detected in feces after separation of maternity and hospital pens on Farm B supports this view. Periparturient cows appear to be the adult cattle group most susceptible to *Salmonella* infection,[3,20,26] a finding that may be related to the influence of immunosuppression during late gestation, parturition, and the early stage of lactation. Suppression of the innate, nonspecific immune mechanisms, especially the responses of neutrophils and macrophages, has been reported[33–35] in periparturient cows challenged orally with *Salmonella*. Higher rates of antimicrobial use in this group of cattle may also contribute to greater fecal shedding and susceptibility to *Salmonella* spp.[21] Alternatively, it is possible that numerous cattle on the farms had latent infections but no detectable fecal excretion and that metabolic and immunologic changes associated with parturition promoted recrudescence of shedding. However, that scenario seems less likely given the low fecal isolation rates from preparturient cattle and the decrease in fecal samples containing the organism that were observed at Farm B after cows in the hospital and maternity pens were separated.

Generalizations that can be drawn from results of the present study were limited by the low number of farms studied and the fact that farm size and management particulars were not representative of dairy herds in all regions. The dynamics of *Salmonella* infection in given populations of cattle should be investigated for disease modeling and formulation of effective control strategies. Authors of a recent study[36] attempted to mathematically model the dynamics of *Salmonella* dispersion and maintenance in dairy herds with an emphasis on factors determining persistence. Although certain subgroups of cows were included in the model used in that study, a common hospital-maternity pen was not included.

Numerous studies[14,20,27,28,31] have yielded evidence that farm environmental niches are important sites of *Salmonella* persistence and exposure for cattle. In the present study, widespread environmental contamination by MDR *Salmonella* Newport was evident on both farms. Pen bedding appeared to be a particularly important *Salmonella* reservoir. Although material such as sand would presumably be an unlikely matrix for bacterial persistence, *Salmonella* spp can survive and multiply in seemingly hostile environments.[27] To reduce persistence of the organism in a herd, methods of disinfection and infection control must be devised for bedding materials, feeds, and feed refusals. Equipment and personnel who handle cattle with clinical illness should also be targets for disease-control measures. Certain farm environments from which the organism is consistently cultured, including lagoon or surface waters and milk filters, represent important exposure sources for humans. The absence of a more substantial decrease in prevalence of *Salmonella* contamination in environmental samples after the pens were separated on Farm B may reflect long-term survival of *Salmonella* in the environment or may indicate that only a few infectious cows can cause substantial environmental contamination.

Analysis of farm isolates of salmonellae via PFGE reveals their highly clonal nature. The predominant clone from Farms A and B appeared to be widespread, being isolated from multiple states and 1 Canadian province in the Pacific northwest region. The fact that the contemporary strain matched isolates from 1997 suggested that this clone has been in stable existence for some years. The existence of identical strains among bovine and human isolates supports that there is zoonotic transmission of these strains or a strong association between *Salmonella* populations in humans and temporo-spatially matched cattle.[1,12] Most *Salmonella* Newport isolates from the present study had the classic penta-resistant phenotype and reduced susceptibility to ceftazidime (as confirmed by detection of the *CMY-2* gene) that are associated with MDR *Salmonella* Newport clones. *Salmonella* serovar Bardo, with the ACSSuTCaz resistance phenotype, was detected among farm environment isolates. That serotype differs from *Salmonella* Newport only in lacking a factor-6 somatic antigen and may represent a rough strain or other modified form of the predominant *Salmonella* Newport strain.[q]

Although the use of antimicrobial drugs during the preslaughter phase of food-animal production is likely associated with development of resistant bacterial strains, other farm management factors also play a role. For disseminated clones of antimicrobial resistant bacteria, infection control is critically important in minimizing the initial degree of herd exposure and ongoing maintenance or persistence of infection in the herd.[8,9] Critical on-farm control points may represent opportunities for reducing transmission. In the current study, physical and operational separation of sick versus calving cattle decreased the prevalence of fecal shedding of MDR *Salmonella* Newport by dairy cattle. Separation of pens should be combined with measures to decrease the persistence of *Salmonella* in farm environmental niches and implementation of general infection control procedures.[3,4,8] Apart from being an important means of resolving existing *Salmonella* outbreaks (clinical or subclinical), institution of such measures may help prevent emergence of future strains of MDR *Salmonella* and other pathogens of bovine and public health importance.

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e. Remel, Lenexa, Kan.
f. Becton-Dickinson, Sparks, Md.
g. BBL, Cockeysville, Md.
h. Oxoid, Ogdensburg, NY.
i. Courtesy of Ravi Pallipamu and Donna Greene, Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman, Wash.
j. The National Molecular Subtyping Network for Foodborne Disease Surveillance, CDC, Atlanta, Ga.
k. CHEF-II, Bio-Rad Laboratories, Hercules, Calif.
l. Synoptics, Cambridge, England.
m. Microsoft Corp, Redmond, Wash.
n. EpiTable 6.0, Division of Public Health Surveillance and Informatics, CDC, Atlanta, Ga.
o. Applied Maths, Sint-Martens-Latem, Belgium.
p. VirkonS, Antec International, Sudbury, Suffolk, England.
q. Ferris K, National Veterinary Services Laboratory, Animal and Plant Health Inspection Service, USDA: Personal communication, 2005.

Appendix

Types and numbers of samples collected on visits to 2 dairy farms during a study of persistent MDR *Salmonella* Newport infection.

Site where sample was collected	Description	Collection method	No. collected/visit
Hospital pen	Fecal samples from cattle in the joint hospital-maternity pen; pen contained cattle hospitalized for acute or severe disease as well as cows and heifers that were calving, had calved in the last 24 to 96 hours, or were due to calve within 24 hours	Rectal palpation and manure pat	4 to 24 samples collected via rectal palpation; 0 to 21 samples collected from manure pats; 11 to 39 samples collected in total
Preparturient pen	Cows and heifers within 21 days of calving due date	Manure pat	20
Postparturient pen	Cows and heifers in the milking herd that had calved in the last 21 days	Manure pat	20
Equipment	Balling-drench guns, rectal thermometers, and disinfectant baths	Buffered peptone water sponge and rinsate	1 to 5
Feed	Hays (alfalfa and grass), silages (triticale and corn), cannery waste	Sterile bag[c]	1 to 2
Water	Drinking trough, surface waters	Sterile bottle	1 to 4
Lagoon	Slurry lagoon	Sterile bottle	1
Bedding	Recycled sand bedding	Sterile bag[c]	1 to 2
Feed refusals	Uneaten or spilled feed detritus	Sterile bag[c]	1 to 2
Milk filters	Removed shortly after milking, prior to line flush	Sterile bag[c]	2

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