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Herd-level factors associated with isolation of *Salmonella* in a multi-state study of conventional and organic dairy farms I. *Salmonella* shedding in cows

C.P. Fossler [a,*], S.J. Wells [a], J.B. Kaneene [c], P.L. Ruegg [d], L.D. Warnick [e], J.B. Bender [a], L.E. Eberly [b], S.M. Godden [a], L.W. Halbert [c]

[a] *University of Minnesota, College of Veterinary Medicine, 1354 Eckles Avenue, St. Paul, MN 55108, USA*
[b] *University of Minnesota, Division of Biostatistics, School of Public Health, 420 Delaware St. SE, MMC 303, Minneapolis, MN 55455, USA*
[c] *The Population Medicine Center, A 109 VMC, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA*
[d] *University of Wisconsin, Department of Dairy Science, 1675 Observatory Dr., Madison, WI 53706, USA*
[e] *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*

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Abstract

The objective of this study was to evaluate associations between herd characteristics and the isolation of *Salmonella* from dairy cows in Minnesota, Wisconsin, Michigan, and New York. Study farms were 129 conventional and organic farms enrolled without regard to previous history of *Salmonella* infection. Herds were sampled at 2-month intervals over a 1-year period. This is the largest study to date on *Salmonella* shedding in dairy cows and the only study evaluating herd-

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* Corresponding author. Present address: National Surveillance Unit, Centers for Epidemiology and Animal Health, USDA, APHIS, VS, 2150 Centre Ave., Bldg. B, Mail Stop 2E7, Fort Collins, CO 80526-8117, USA. Tel.: +1 970 494 7595; fax: +1 970 494 7229.
E-mail address: charles.p.fossler@aphis.usda.gov (C.P. Fossler).

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level risk factors using longitudinal sampling to characterize *Salmonella* shedding on dairy farms. *Salmonella* was isolated in fecal samples from 1026 (4.9%) of 20,089 cows. Over the course of the study, 113 (87.6%) of 129 farms had at least one positive cow sample. Multi-variable logistic regression using the generalized estimating equations approach was used to test the association between herd-level risk factors and the dependent variable of within-herd prevalence by visit (number of *Salmonella*-positive cows/number of cows sampled) after adjustment for effects of herd size, season, state of origin, and the multiple sampling occasions per herd. Factors retained in the final model included lack of use of tiestall or stanchion facilities to house lactating cows (OR = 1.9; 95% CI: 1.1–3.3), not storing all purchased concentrate or protein feeds in an enclosed building (OR = 2.5; 95% CI: 1.3–4.9), not using monensin in weaned calf or bred heifer diets (OR = 3.2; 95% CI: 2.0–5.4), access of lactating or dry cows to surface water (e.g., lake, pond, river, or stream) (OR = 2.3; 95% CI: 1.3–3.9), disposal of manure in liquid form (slurry or irrigation, as opposed to disposal of manure by broadcast/solid spreader only) on owned or rented land (OR = 1.8; 95% CI: 1.3–3.9), and cows eating or grazing of roughage from fields where manure was applied in solid or liquid form and not plowed under during the same growing season (OR = 1.8; 95% CI: 1.0–3.0). A seasonal association was also present as cows were more likely to be *Salmonella*-positive in summer, spring, and fall compared to winter. Herd size was not associated with *Salmonella* shedding in the final multi-variable model. The herd-level risk factors identified in this study could potentially be implemented in *Salmonella* control programs on dairy farms.
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1. Introduction

Salmonella infection can cause clinical disease in cattle, leading to signs of fever, diarrhea, and sometimes death, among other manifestations (Smith, 2002). *Salmonella* outbreaks can be detrimental to dairy producers due to increased mortality and treatment costs in clinically infected cows. Cattle can shed the pathogen subclinically for extended periods after initial infection (Evans and Davies, 1996; Gay and Hunsaker, 1993), which is important in terms of food safety risks because cattle shedding this pathogen may not show any clinical signs at slaughter. *Salmonella* has the ability to not only survive but also multiply in the environment. *Salmonella* is also an important and costly cause of disease in humans, with 95% of cases estimated to be of foodborne origin (Mead et al., 1999). Public health consequences of *Salmonella* have stimulated interest in reduction of *Salmonella* in foods of animal origin, and in the U.S. a system is in place for monitoring contamination of meat with *Salmonella*. Greater public concern over food safety, coupled with ongoing monitoring of contamination of meat, has generated increased interest in identifying means to reduce *Salmonella* shedding in cattle on-farm.

Nearly all dairy farms are expected to be *Salmonella*-positive, but in terms of numbers of cattle shedding, a relatively small percentage of farms appear to account for most *Salmonella*-positive cattle on-farm (Fossler et al., 2004). Because of the large expense and time commitment involved with identification of herd-level risk factors for *Salmonella* infection on dairy farms, investigations to date have been limited to either a cross-

sectional design, where farms were sampled once and herd status was based on presence versus absence of the organism (Huston et al., 2002; Kabagambe et al., 2000), or a case-control design, where *Salmonella* status was determined retrospectively based on laboratory submissions (Anderson et al., 1997; Evans and Davies, 1996; Vaessen et al., 1998; Warnick et al., 2001). Thus, risk factors identified to date have been based on presence versus absence of *Salmonella* on farms, not on the numbers of *Salmonella*-positive cattle. Given that all farms can be expected to be *Salmonella*-positive at some point in time, and given, at least in swine that temporal variability of *Salmonella* prevalence can be substantial (Funk et al., 2001), identification of risk factors that account for the prevalence of *Salmonella* of farms, not its presence, would be beneficial for on-farm *Salmonella* control.

The most consistently identified factor associated with *Salmonella* presence on dairy farms has been increased herd size (Huston et al., 2002; Kabagambe et al., 2000; Vaessen et al., 1998; Warnick et al., 2001). Other herd-level factors that have been associated with *Salmonella* presence on dairy farms include the following: purchase of cattle from dealers, keeping cattle in housing, lack of isolation facilities for sick animals, and the presence of wild birds and cats (Evans and Davies, 1996), southern region in the U.S., use of flush water systems for handling manure, and feeding brewer's products to lactating cows (Kabagambe et al., 2000), exposure to wild geese, rodent activity in housing and feed areas, and spreading poultry manure on bordering property (Warnick et al., 2001), and purchase of livestock, liver fluke infection, and feeding only grass in the summer (Vaessen et al., 1998).

The purpose of this analysis was to build upon previous analyses by identifying herd-level risk factors for *Salmonella* for a large number of dairy farms sampled multiple times over the course of a year.

2. Materials and methods

2.1. Farm selection

A total of 129 farms were selected in four states: Michigan (MI), Minnesota (MN), New York (NY), and Wisconsin (WI). Herds were enrolled according to farm type (32 organic and 97 conventional farms) and by farm size (by number of cows, both milking and dry) as previously described (Fossler et al., 2004, 2005; Zwald et al., 2004). Predetermined numbers of farms in four-size strata (30–49, 50–99, 100–199, and $\geq$200 cows [both lactating and non-lactating]) were enrolled on the basis of ability to evaluate potential risk factors, as previously described (Fossler et al., 2004, 2005). Farms were not matched. For conventional farms, lists of farms were obtained from the respective State Departments of Agriculture, and herds within approximately 100 miles of the respective universities were randomly selected to receive a mailing describing the research project (500–571 herds per state for a total of 2102 letters). Farms were asked to indicate interest in participation by returning a postcard. The final list of farms was obtained by randomly selecting 97 farms (distributed among the four-size strata) from among the 295 respondents that indicated willingness to participate.

For organic farms, contact information was obtained through independent organic certifying agencies, organic milk cooperatives, and personal contacts. In some states, all known organic farms within approximately 150 miles of each of the participating universities were contacted to determine eligibility based on the selection criteria and their desire to participate, and all herds that met the selection criteria and indicated a willingness to participate were eligible for the random selection process. Organic farms were required to be certified as organic by a recognized organic certification agency. This study was conducted prior to the implementation of U.S. standards governing organic crop or livestock production, which occurred in October 2002. In the absence of national standards, the organic industry relied on third party certification by private or state organizations to assure consumer confidence in organically produced products. Although rules were very similar across states and certification organizations, there were slight variations. For instance, all the private or state certification agencies required that only organic feeds (grown without synthetic pesticides, herbicides, or fertilizer) be given to dairy cows, and that routine use of antibiotics or parasiticides was not allowed. However, there was some variability across certification agencies regarding occasional use, with some prohibiting such use entirely, while others allowed such use as long as withdrawal periods (sometimes lengthy) were observed.

For inclusion in the study, all herds (both conventional and organic) had to meet all of the following criteria: (1) at least 30 milking cows, (2) at least 90% of cows of Holstein breed, (3) raise their own calves for replacement cattle, (4) maintain a herd record system, including individual identification for cows and calves, and (5) ship milk all year. Briefly, the distribution of herds by farm size and type was as follows: 30–99 cows: 24 organic, 36 conventional; 100 or greater cows: 8 organic, 61 conventional.

2.2. Sample collection

Farms were sampled up to five times from August 2000 through October 2001. For 94% of the farms, the first visit was conducted between October 2000 and January 2001. Subsequent visits to each farm were conducted at approximate 2-month intervals following the first visit. The mean interval between sampling visits was 68.5 days (standard deviation of 19.9 days, range of 28–152 days). The mean number of visits to farms was 4.7 (standard deviation of 0.8, range of 1–5).

Cattle samples were collected by placing approximately 10 g of fecal material obtained by rectal retrieval into Whirl-Pak® bags (NASCO, Ft. Atkinson, WI). A separate glove was used for the collection of each sample. The number of fecal samples collected per visit from each herd and from specific cattle groups were calculated to provide a similar probability of detecting at least one positive animal across herd sizes given the same prevalence of fecal shedding of *Salmonella* spp. for all herds (Warnick et al., 2003a). An estimated prevalence of *Salmonella* spp. of 7.5% in target cattle (cows to be culled within 14 days; cows due to calve within 14 days or within 14 days after calving; and cows designated as sick by farm personnel), and 2.5% in other cows (i.e., healthy lactating cows) were used in the calculations. Estimates of the number of cattle that would be available at each 2-month interval in the target cattle and healthy lactating cattle groups were made on the basis of the following statistics: 35% cull rate, 13.5-month calving interval, and 5% sick

cattle at any time. Resulting numbers of samples were rounded off to multiples of five for ease of implementation of the sampling protocol. During each sampling visit, up to 4, 5, 10, and 10 target cattle fecal samples from herds with 30–49, 50–99, 100–199, and ≥200 cows, respectively, were collected. On smaller farms, the designated number of target cattle was not always available at each sampling visit; therefore, fecal samples from as many target cattle as possible were collected. For target cattle groups, equal numbers (when available) of fecal samples from sick, periparturient, and to-be-culled cows were collected at each visit, and systematic sampling within groups was performed when greater than the targeted number of cattle were available. Also collected at each sampling visit were 20, 25, 30, and 30 healthy cattle fecal samples from herds with 30–49, 50–99, 100–199, and ≥200 cows, respectively. For healthy cows, systematic sampling was used to collect a representative number of fecal samples from cattle in each pen on the basis of total herd size and numbers in each pen. No effort was made to collect samples from the same cattle at subsequent herd visits.

2.3. Shipment and isolation

After collection, samples were either taken to the laboratory (Michigan) or shipped via overnight delivery in styrofoam boxes with ice packs (Minnesota, New York, Wisconsin). Samples were usually shipped the same day as collection but in some cases were kept in a refrigerator for 12–36 h before shipping. Laboratory methods for isolation were performed as previously described (Fossler et al., 2004).

2.4. Data collection

Information on potential risk factors was collected through the use of pre-tested questionnaires administered through personal interviews. An initial questionnaire collected detailed information about herd management practices (available upon request), and at each sampling visit a shorter questionnaire was administered to capture information more likely to change over time, such as herd additions or changes in rations or feeding practices.

2.5. Data analysis

Logistic regression methods were used to test the association between herd-level risk factors and the dependent variable of within-herd prevalence by visit (number of *Salmonella*-positive cows/number of cows sampled). The generalized estimating equations (GEE) approach was used to adjust for correlation of the multiple sampling occasions for each herd (SAS Version 8.2; SAS Institute Inc., Cary, NC) using ‘‘herd’’ as the subject effect in the repeated statement and within-herd ‘‘visit number’’ as the within-subject effect. A working independence correlation structure was chosen because the model included time-varying covariates (Pepe and Anderson, 1994). GEE models adjust for herd effects and give robust standard errors when using a working independence correlation structure. Associations between within-herd *Salmonella* prevalence and factors proposed to be important in *Salmonella* introduction and transmission were initially tested in

individual GEE models. Herd size (≥100 cows versus <100 cows), season (January–March = Winter; April–June = Spring; July–September = Summer; October–December = Fall), and state (WI, MN, MI, NY) were forced into the initial screening models and the final models because these factors were considered to be important potential confounding variables. Variables with $p < 0.2$ based on the GEE Parameter Estimate (*t*-test) became candidates for the multi-variable models. If a management characteristic was not present in at least 10% of study herds, that variable was excluded from the multi-variable model.

A backward stepwise process was used to build the final model. Variables were removed in a stepwise fashion, beginning with those with the highest *p*-value based on the GEE parameter estimate (*t*-test), until all variables left in the model had $p < 0.05$. Interactions and confounding variables were assessed on the basis of plausible biological relationships. The independent variables of interest in this analysis were measured at the herd-level, but interpretation of final odds ratios is done at the animal level since the proportion of *Salmonella*-positive animals was the dependent variable. Thus, final odds ratios can be interpreted as the odds for a cow to be *Salmonella*-positive on farms on which the factor of interest is present.

Population attributable risk was calculated for all variables remaining in the final multi-variable model using methodology described by Bruzzi et al. (1985) and in other studies (Kabagambe et al., 2000; Smith et al., 1998; Wells et al., 1996, 1999). Population attributable risk can be interpreted here as the proportion of *Salmonella*-positive cattle that could be eliminated if the effect of the variable of interest is removed. Thus, it provides an indication of the importance of each variable in the final model. Briefly, the formula used was $\mathrm{PAR} = 1 - \Sigma(\rho_j/R_j)$, where ρ_j is the proportion of all *Salmonella*-positive cows in stratum *j* and R_j is the relative risk for stratum *j*, which was estimated by odds ratios from the multi-variable models. The lower and upper confidence limits of the PAR were estimated using the 95% confidence intervals for the odds ratios from the multi-variable models.

3. Results

3.1. Descriptive statistics

Ninety-seven conventional and 32 organic farms within four different herd size categories were sampled. Approximately equal numbers of herds were enrolled in each state. Few organic farms with more than 100 cows were enrolled because of lack of availability of those farms within the four states. Of the 129 farms that participated in the study, nine reported a prior history of confirmed *Salmonella* infections in cattle within 2 years of study onset. Management characteristics and occurrence of *Salmonella* shedding in the enrolled herds has been previously described (Fossler et al., 2004; Zwald et al., 2004). *Salmonella* was isolated in fecal samples from 1026 (4.9%) of 20,089 cows, with 113 (87.6%) of 129 farms having at least one positive cow sample. For the 129 herds included in this analysis, herd size characteristics (total dairy cows, milking and dry) were as follows: median = 103, range = 26–875.

3.2. Univariable analysis of risk factors for Salmonella shedding in cows

Management practices that were not associated with *Salmonella* shedding in cows at $p < 0.20$ after adjustment for effects of season, state, herd size, and the multiple sampling occasions per herd included farm type (organic versus conventional), the percent of cows born off the farm, addition of cows, heifers, or weaned calves or any dairy cattle within the year preceding or during the study period, type of milking facilities, presence of pigs or wild geese on the operation, physical contact of cats with dairy cattle or their feed or water supply, lactating cow access to pasture, use of organic bedding, feeding of whole soybeans or soybean meal to lactating cows, using maternity housing as a hospital area for sick cows, placement of sick cattle in a pen separate from other lactating cows, milk production level of herd, or routine vaccination of cows with J5 vaccine (Appendix A). When use of either J5 or Endovac-Bovi® vaccines were collapsed into a summary measure, there was also a lack of association with this practice and *Salmonella* shedding in cows (data not shown). Only six farms reported routine use of Endovac-Bovi®, which was fewer than the 10% required for the variable to be analyzed separately. Prior history of confirmed *Salmonella* infection in dairy cattle within 2 years of study onset was not associated with *Salmonella* shedding in cows (data not shown). Fewer than 10% of farms reported this characteristic, which would have excluded it from the risk factor analysis, but it was examined because it was deemed important. Seven of the nine herds that reported prior *Salmonella* infections had a median within-herd cow prevalence over four to five visits of 5.0% or lower, so these farms did not appear to have high *Salmonella* prevalence levels in cows.

Variables (not including herd size, season, and state, which were forced into the model) eligible for inclusion in a multi-variable model were presence of domestic poultry on the operation, feeding of animal by-products (meat & bone, blood, fish or feather meal, or tallow/animal fat) to lactating cows, lactating cow housing type, type of maternity facility, use of inorganic bedding for lactating cows, feeding of cottonseed or brewer's products to lactating cows, storage practices for purchased concentrates or protein feeds, use of monensin or decoquinate in weaned calves or heifers, access of cows to surface water, use of chlorinated water for dairy cattle, average herd milk somatic cell count, use of chemicals, bait, or traps for rodent control, application of liquid manure to land on operation, grazing or harvesting of forage on ground where manure was applied during the same growing season, and using a loader bucket to move feed and manure (but not necessarily the same bucket for both). From the results of univariable analysis (after adjustment for herd, herd size, season, and state), *Salmonella* shedding in cows was significantly less likely on farms on which domestic poultry was present. The survey instrument used for this analysis did not distinguish type of poultry operation present (i.e., a few free-range chickens versus large poultry operations). Because the risk of transmission of *Salmonella* from small numbers of free-range poultry may be different from the risk of having large numbers of commercially raised poultry on the operation, presence of domestic poultry was excluded from further analysis. Herd size, state, and season were all significantly associated with *Salmonella* shedding in cows in a model containing only these three variables after adjustment for effects of the multiple sampling occasions per herd.

3.3. Multi-variable analysis of risk factors for Salmonella shedding in cows

Of the 16 independent variables examined in the multi-variable model for cows, 10 were removed during the model-building process. After adjustment for effects of herd size, state, season, and the multiple sampling occasions per herd, the following were associated with an increased odds of *Salmonella* shedding in cows: lack of use of tiestall or stanchion facilities to house lactating cows (versus any use of tiestall or stanchion facilities to house

Table 1
Factors associated with *Salmonella* shedding in cows in a multi-variable logistic regression (GEE) model (herd size, season, and state forced into the model)

Variable[a]	Level	Odds ratio	95% confidence interval	
			Lower bound	Upper bound
Season	Spring	2.3	1.5	3.7
	Summer	2.7	1.6	4.6
	Fall	2.1	1.2	4.0
	Winter	1.0		
State	MI	2.3	1.1	4.8
	MN	2.8	1.2	6.2
	WI	2.6	1.6	4.4
	NY	1.0		
Lack of tiestall/stanchion to house lactating cows on operation (all lactating cows not necessarily housed this way)	Yes	1.9	1.1	3.3
	No	1.0		
Purchased concentrates and protein feeds stored in an enclosed building	None or some of these feeds	2.5	1.3	4.9
	All of these feeds	1.0		
Lack of monensin use in weaned calves or bred heifers	Yes	3.2	2.0	5.4
	No	1.0		
Lactating or dry cow access to surface water (e.g., lake, pond, stream)	Yes	2.3	1.3	3.9
	No	1.0		
Manure disposal on owned or rented land by irrigation or application of slurry (surface or subsurface)	Yes	1.8	1.3	3.9
	No	1.0		
Cows eat or graze on roughage from fields where manure in solid or liquid form was applied to the surface but not plowed under during the same growing season	Yes	1.8	1.0	3.0
	No	1.0		

[a] All variables left in the model had $p < 0.05$ based on GEE parameter estimate (t-test) with the exception of herd size, which was forced into the model and was insignificant. Within-herd cow prevalence by visit (number of *Salmonella*-positive cows/number of cows sampled for a single visit, with 608 total visits) was the dependent variable, adjusting for correlation of observations within herd using a working independence correlation structure.

lactating cows, regardless of whether this was the only housing used), not storing all purchased concentrate or protein feeds in an enclosed building, not using monensin for weaned calves or bred heifers, access of lactating or dry cows to surface water (e.g., lake, pond, river or stream), disposal of manure in liquid form (slurry or irrigation) on owned or rented land by surface or subsurface application (as opposed to disposal of manure by broadcast/solid spreader only), and cows eating or grazing of roughage from fields where manure was applied in solid or liquid form and not plowed under during the same growing season (Table 1). Season, state, and farm size were forced into the model, and of these, only season and state remained associated with shedding in the final model. Compared to winter (January–March), *Salmonella* shedding was more likely in the summer period (July–September), followed by spring (April–June) and Fall (October–December). Cows from the three Midwestern states (Minnesota, Michigan, and Wisconsin) were more likely to be *Salmonella*-positive compared to New York.

3.4. Population attributable risks

Variables with the highest PAR included lack of use of monensin in weaned calf or bred heifer diets, season, and state of origin, all of which had a PAR above 0.50 (Table 2). Only those significant variables that could feasibly be suggested as *Salmonella* control options were included in the summary PAR calculations, so season and state were excluded.

4. Discussion

Particular strengths of this study included the large number of herds, the diversity of farm sizes sampled, and the longitudinal sampling approach spanning all months of the year. With over 20,000 samples from 129 herds in four states, this study represents the

Table 2
Individual variable and summary (all variables combined) population attributable risks for factors associated with *Salmonella* shedding in cows

Variable	PAR	95% CI
Season	0.52	0.26, 0.67
State	0.54	0.19, 0.72
Lack of use of tiestall/stanchion housing for lactating cows on operation (all lactating cows not necessarily housed this way)	0.39	0.09, 0.56
All purchased concentrates and protein feeds not stored in an enclosed building	0.20	0.08, 0.26
Lack of use of monensin in weaned calves or bred heifers	0.57	0.40, 0.67
Lactating or dry cow access to surface water	0.23	0.11, 0.29
Manure disposal on owned or rented land by irrigation or application of slurry (surface or subsurface)	0.46	0.20, 0.60
Cows eat or graze on roughage from fields where manure in solid or liquid form was applied to the surface but not plowed under during the same growing season	0.25	0.01, 0.38
All variables combined[a]	0.95	0.80, 0.98

[a] Summary PAR was calculated for only those significant variables that can be considered controllable (i.e., excluding season and state).

largest study to date on *Salmonella* shedding in dairy cows and the only study evaluating herd-level risk factors using longitudinal sampling to determine *Salmonella* status. In addition, conventional herds were randomly selected among those eligible for inclusion so that participating herds represented a diversity of management characteristics of herds within the sampling frame. A greater percentage of operations with over 100 cows and a lesser percentage of operations with fewer than 100 cows participated in this study compared to percentages of like-sized operations present within the respective four states. Thus, the farms selected for this study cannot be seen as representative of the underlying distribution of operations by herd sizes in the respective states. However, herd size was not significantly associated with *Salmonella* shedding in any of the final models, so these differences should not pose a problem with interpretation of results for each of the four states.

This study used the generalized estimating equations approach for analysis of risk factors, which is increasingly being used in epidemiology and clinical science for analysis of correlated data (Burton et al., 1998; Hanley et al., 2003). This approach allows for multi-variable analysis of longitudinal or other correlated data with binary outcomes and provides robust standard errors while adjusting for herd effects. One limitation of the GEE method is that it is not a likelihood-based test, and there are no widely accepted methods for assessing model fit. Significance of independent variables ($p < 0.05$ by GEE parameter estimate, i.e., *t*-test) was the only criteria used for variables to remain in the final model in this analysis. The purpose of this analysis, however, was to identify possible risk factors for *Salmonella* infections in dairy cows, which was accomplished using the methods described. Additional research would be necessary to indicate whether implementation of any of the possible control measures identified in this study could result in reduction of risk of *Salmonella* infection on dairy farms.

Herd size was not associated with *Salmonella* shedding, which is quite notable because herd size has been the variable most consistently identified across previous studies examining herd-level risk factors for *Salmonella* shedding in dairy cattle (Huston et al., 2002; Kabagambe et al., 2000; Vaessen et al., 1998; Warnick et al., 2001, 2003b). Median number of cows in these studies ranged from 58 to 370 (Huston et al., 2002; Kabagambe et al., 2000; Vaessen et al., 1998; Warnick et al., 2003b). The median number of cows of 103 from this analysis was comparable to the largest previous study (91 herds, median number of cows = 116) involving on-farm sampling of dairy cows (Kabagambe et al., 2000). Although four different size categories were used for enrollment of herds, it was decided to dichotomize herd size by <100 cows versus ≥100 cows in the analysis, as previously described (Fossler et al., 2005). There was no herd size association in the final model after categorization into the following four levels by number of milking and dry cows: 200 or more, 100–199, 50–99, and 30–49 (data not shown). It is possible the difference between this and other studies with respect to herd size resulted from important risk factors associated with herd size being included in this analysis (thus making herd size not significant) but not in the other studies. The two studies by Warnick et al. (2001, 2003b) focused on herds experiencing outbreaks of clinical disease, and it is possible that herd size could play a more important role in within-herd transmission of more virulent strains. A likely contributing factor is the

larger sample size used in this analysis, with 129 herds sampled up to five times over the course of a year, which provided more power to detect associations aside from herd size. Considering current U.S. dairy industry trends, reducing herd size is not a feasible approach to *Salmonella* control, and the identification of specific variables other than herd size offers promise for the prospects of on-farm *Salmonella* control. Although it was not significant in the final multi-variable model, herd size was forced into the model because it was a selection criteria for herds and can serve as a proxy for herd management information that was not collected, thus controlling for potential confounding effects (Gardner et al., 2002).

The association of state of origin with *Salmonella* shedding was unexpected. It is important to note that study herds were limited to regions located proximally to each university, so these herds cannot necessarily be considered as representative of the respective states. Laboratory personnel indicated that cattle samples from New York consistently contained less fecal material than the other three states. Since a greater quantity of feces increases test sensitivity for detection of *Salmonella* (Funk et al., 2000), it was expected that New York may have been less likely to have *Salmonella*-positive samples from cattle. However, samples from the farm environment were also collected in this study, as previously described (Fossler et al., 2005), and the procedure for collection of environmental samples was consistent across all four states. Because New York also was significantly less likely to have *Salmonella*-positive samples from the farm environment, it is possible that geographic differences in *Salmonella* shedding among the four states or regions may exist.

Farm type (organic versus conventional) was not associated with *Salmonella* shedding in any of the models examined. There is an absence of information in the scientific literature regarding differences between organic and conventional dairy farms with respect to fecal shedding of *Salmonella*. One might suspect that organic farms would have a higher prevalence of *Salmonella* because restrictions on their use of synthetic or petroleum-based fertilizers may make them more reliant on use of manure for fertilization of crops and pasture. In addition, because this study found that use of monensin, which is an antimicrobial, was associated with a decreased odds for *Salmonella* shedding, one may suspect that organic herds, in which antimicrobial use is avoided, would have an increased risk for *Salmonella* shedding. However, there are many more differences between conventional and organic farms than use of antimicrobials. For example, organic farms were significantly smaller and produced less milk than conventional herds in this study (Zwald et al., 2004). Organic farms also must feed only organically produced feed and allow access of cattle to the outdoors, among many other characteristics that distinguish them from conventional farms. This study was conducted prior to implementation of the USDA National Organic Program, but the aforementioned restrictions were in place at the time this study was conducted. It was apparent that the overall effect of being certified organic did not distinguish these farms from conventional operations in terms of *Salmonella* shedding after controlling for other significant factors.

Seasonal differences in *Salmonella* shedding in cows were observed. *Salmonella* shedding has been found to be more common in the summer months (Evans, 1996; Wells et al., 2001), or in the summer and fall seasons (Gibson, 1965), but a lack of seasonal

association has also been noted elsewhere (Huston et al., 2002). A possible explanation for the lack of a seasonal association in the study by Huston et al. may be that farms were only sampled once, and the outcome was herd-level *Salmonella* status (positive or negative). It is possible that positive farms were not as likely to have been sampled in warmer months in that study. Regardless, the approach used in this study (longitudinal sampling of farms across all seasons and using cattle-level results, adjusted for herd) provides strong evidence for a seasonal association with *Salmonella* shedding.

The following factors were not present in the final multi-variable model but were associated with an increased odds for *Salmonella* shedding in cows at $p < 0.05$ after adjustment for effects of herd size, season, state and the multiple sampling occasions per herd: not using a maternity pen (pen where cows calve) for individual cows only, lack of routine use of decoquinate in weaned calves or heifers after breeding, lack of chlorination of the primary water source for dairy cattle, using a loader bucket to move feed and manure (but not necessarily the same bucket for both), and lack of routine use of chemicals, bait, or traps for rodent control. The lack of presence of these variables in the final multi-variable model does not necessarily rule out their association with *Salmonella* shedding, especially since they were significant after adjustment for effects of herd size, state, season, and the multiple sampling occasions per herd. Rodents have been associated with infections of dairy cattle with *Salmonella* (Tablante and Lane, 1989), so lack of rodent control may be a factor in *Salmonella* shedding in cows. Lack of use of a maternity pen for individual cows has been demonstrated to be a risk factor for *Salmonella* shedding in calves (Losinger et al., 1995), so an association with shedding in cows is feasible. Use of chlorinated drinking water for dairy cattle was not associated with *Salmonella* shedding in a previous study (Kabagambe et al., 2000) but based on the results from this study it may deserve further analysis regarding its role in reduction of *Salmonella* shedding in cattle.

Cows on operations where tiestall or stanchion facilities were used for lactating cows were less likely to be shedding *Salmonella*. There were 70 farms that did not use tiestall or stanchion facilities, and all 70 of these farms used freestall housing, with 8 also making use of other group housing (e.g., loose housing in loafing sheds). It is difficult to separate effects of housing type from those of herd size, because the type of cattle housing is influenced by the size of the operation (i.e., number of cows), with tiestall or stanchion facilities being more common on smaller farms. However, there were 15 large farms with over 100 cows that used tiestall or stanchion facilities for lactating cows in this study, and the effect of housing type was significant even after adjustment for herd size. Because farms often used combinations of housing types, this analysis evaluated any use of tiestall or stanchion facilities and was not limited to their exclusive use. There were 13 farms that used both freestall and tiestall housing, and another 14 farms used a combination of tiestall and loose housing facilities for lactating cows. Huston et al. (2002) found use of freestalls to be associated with *Salmonella* shedding in univariable analyses but not in a multi-variable model. The lower risk of *Salmonella* shedding in cows housed in tiestalls or stanchions is understandable because of the nature of the housing situation: in tiestall or stanchion barns, cows are restrained with reduced potential for contact with fecal material from other cattle whereas in freestalls or loose housing facilities, freedom of movement allows

for more potential of contamination of feed and water with fecal material from other cows.

Cows on farms that stored all purchased concentrates and protein feeds in an enclosed building were less likely to be *Salmonella*-positive compared to farms in which all of these feeds were stored in an enclosed building (defined as covered on all sides and providing protection against birds, such as a round metal grain bin or a building with a roof and four walls where outside doors were kept shut on a routine basis). It cannot be determined from this analysis whether lack of protection from moisture or adverse weather, or whether lack of protection from birds, or some other unknown factor led to the higher risk of *Salmonella* shedding in cows. Evidence of access of wild birds to feed stores has been previously identified as a risk factor for *Salmonella* infections in cattle and feed contamination (Evans and Davies, 1996; Harris et al., 1997). Only storage facilities for purchased concentrates and protein feeds (and not all feeds) were assessed because it was hypothesized that the highest risk for *Salmonella* introduction would come from these feeds, which are more likely to originate off the farm. Occurrence of *Salmonella* in animal-derived feeds has been shown to be more common than in vegetable meal products, with over 50% of animal meal products found to be positive in an FDA survey (McChesney et al., 1995). Samples of bird droppings were obtained from cattle feeding or feed storage areas from this study, as previously described, with 4.3% being *Salmonella*-positive (Fossler et al., 2005). A recent study in which 892 birds were captured from nine dairies in California found 2.5% of birds to be *Salmonella*-positive, with bird prevalence by dairy ranging from 0.7 to 16.7% (Kirk et al., 2002). Although *Salmonella* prevalence in birds is relatively low (Kirk et al., 2002), access of birds or other wildlife to feed storage areas remains an opportunity by which cattle feeds can become *Salmonella*-contaminated. Since *Salmonella* can thrive in moist environments, protection of stored feed from rain or other inclement weather is important.

Lactating or dry cow access to surface water (e.g., lake, pond, river, or stream) resulted in a 2.4 times greater odds for *Salmonella* shedding in cows. Vaessen et al. (1998) found that increased water surface area to which cattle had access was associated with increased odds for infection with *Salmonella enterica* serotype dublin. *Salmonella* has often been reported in samples from rivers and streams (Wray and Davies, 2000), and surface water should generally be considered a risk factor for *Salmonella* infections because of the potential for contamination of surface water from runoff from manure-fertilized fields or from animals defecating into the water (Pelzer, 1989). Compared to disposal of manure by a broadcast spreader only, disposal of manure on owned or rented land by irrigation or application of slurry (by surface or subsurface application) was associated with a 2.1 times greater odds for *Salmonella* shedding in cows. Eating or grazing of forage by cows from fields where manure in solid or liquid form was applied to the surface but not plowed under during the same growing season was also associated with a 1.8 times greater odds for *Salmonella* shedding in the multi-variable model. Spreading of slurry on pastures has been linked previously to salmonellosis in cattle (Jack and Hepper, 1969), and *Salmonella* has been isolated from corn stalks 2, 4, and 6 feet above ground level on land irrigated with dairy lagoon water (Anderson et al., 2001). A study from the 1970's suggested that herds with slurry storage systems

were *Salmonella*-infected to a significantly greater extent than those with solid manure systems (Thunegard, 1975). It has been recommended that slurry be stored for at least a month followed by a month after spreading during which pasture should not be grazed (Jones, 1976), and survival of *Salmonella* in slurry is further dependent on pH, temperature, and storage time (Kearney et al., 1993). In this analysis, information was collected only on the methods used to spread manure on owned or rented land, not on the length of time manure was stored prior to spreading. Adherence to recommended methods to reduce the presence of pathogenic bacteria in manure slurry may be effective in reducing or eliminating the risk of *Salmonella* infection tied to spreading of slurry on fields. There was no significant difference in *Salmonella* shedding in cows between farms that grazed or harvested from land within 30 days from the time manure was applied but not plowed under versus farms that waited longer than 30 days. Results from this analysis suggest that more attention may need to be paid to methods by which farms store manure and apply it onto fields that will be grazed and harvested.

Cows were more likely to be *Salmonella*-positive on farms that did not use monensin in weaned and bred heifers. This result was unexpected, but the odds ratio for not using monensin (OR = 3.2, 95% CI: 2.0–5.4) was higher than any others in the multi-variable model, suggesting a real association. However, it is also possible that monensin use could be functioning as a proxy for some other farm characteristic on which information was not collected, thus representing a spurious finding. Monensin is an ionophore antibiotic derived from *Streptomyces* and is used in livestock production for its growth promotion and anticoccidial properties (Prescott, 2000). Its antimicrobial activity is primarily against Gram-positive bacteria, and when fed to ruminants, the rumen microflora becomes more Gram-negative (Prescott, 2000). Because of its spectrum of activity, it would seem that monensin would make the ruminant more prone to *Salmonella* infections, but perhaps use of monensin leads to increased levels of other Gram-negative bacteria, which competitively inhibit *Salmonella* colonization. Use of lasalocid, which is also an ionophore antibiotic, was not associated with *Salmonella* shedding, so the effect shown here by monensin use is paradoxical, as is the means by which monensin use in heifer diets could affect *Salmonella* shedding in lactating dairy cows. A previous study showed no association of use of monensin or lasalocid with *Salmonella* infections in feedlot cattle (Losinger et al., 1997), and studies of monensin use in chickens have also shown no effect on naturally acquired subclinical *Salmonella* infections (Hinton et al., 1986; Linton et al., 1985). In contrast, in other studies on experimentally infected chickens and naturally infected turkeys, there has been some indication that use of monensin may reduce *Salmonella* shedding (Cox et al., 2003; Holmberg et al., 1984), and monensin use was also shown to reduce the incidence of scours in sheep compared to laidlomycin proprionate and bambermycin (Edrington et al., 2003).

Population attributable risk can be interpreted here as the proportion of *Salmonella*-positive cattle that could be eliminated if the effect of the variable of interest is removed. The factors from the cow multi-variable model that could feasibly be changed (or altered in some way to decrease risk) on a dairy farm and their associated population attributable risks include lack of monensin use in heifer diets (57%), disposal of manure in liquid

form on owned or rented land (46%), harvesting or grazing roughage from fields where manure was applied during the same growing season (25%), access of cows to surface water (23%), and lack of storage of purchased concentrates and protein feeds in an enclosed building (20%). The PAR for individual risk factors should be regarded cautiously, for they do not add up to the summary PAR for all factors acting simultaneously, indicating that these factors are not mutually exclusive and the same factor may be necessary in more than one causal pathway (Bruzzi et al., 1985). Many of these factors are relatively widespread in the dairy industry. According to the NAHMS Dairy 2002 report, 35.1% of operations allowed cow access to surface water, 30.1% of operations applied liquid manure by surface application, with 86.1% of operations never or sometimes incorporating manure into soil within 24 h of application, and 55.6% of operations applied manure to pasture or hay during the growing season (USDA, 2003).

5. Conclusion

The relatively large number of herds sampled longitudinally in this study led to the ability to detect more factors associated with *Salmonella* shedding in cattle than have previously been identified. Specifically, factors associated with *Salmonella* shedding in cows included season of the year, state, lack of use of tiestall or stanchion facilities for lactating cows, not storing all purchased concentrates or purchased feeds in an enclosed building, lack of monensin use in heifer rations, access of cows to surface water, disposal of manure in liquid form to owned or rented land, and grazing or harvesting roughage from fields on which manure was applied during the growing season. While the factors found in this study show a statistical association with *Salmonella* shedding, this does not necessarily imply a direct causal link. Intervention trials or other studies are necessary to indicate that implementation of any of the control measures identified in this study could result in reduction of risk of *Salmonella* infection on dairy farms. This study is significant in that a number of factors were identified other than herd size that could realistically be implemented on dairy farms and potentially used for on-farm *Salmonella* control, which may also offer the potential to reduce transmission or introduction of other manure-borne pathogens as well. Additional research is needed to test the effectiveness of these factors in more controlled studies to assess their effect on *Salmonella* shedding in cattle.

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Appendix A

Results of univariable analysis of risk factors for *Salmonella* shedding in cows (herd size, season, and state were forced into each model). Variables with $p < 0.2$ were eligible for inclusion in the multi-variable model.

Variable	Level of variable	Number of herds	Number of cows positive/number of cows tested (%)	*p*-Value[a]
Number of cows in herd (both milking and dry)	<100	60	209/7460 (2.8%)	0.002
	≥100	69	817/12629 (6.5%)	
Farm type	Conventional	97	817/16000 (5.1%)	0.358
	Organic	32	209/4089 (5.1%)	
State	Michigan	32	297/5442 (5.5%)	0.011
	Minnesota	32	279/4639 (6.0%)	0.014
	New York	34	104/5256 (2.0%)	<0.001
	Wisconsin	31	346/4752 (7.3%)	
Season	Spring	181[b]	330/6044 (5.5%)	0.003
	Summer	158[b]	332/5200 (6.4%)	<0.001
	Fall	139[b]	252/4593 (5.5%)	0.012
	Winter	130[b]	112/4252 (2.6%)	
Presence of chickens, turkeys, domestic geese, or other poultry on operation	Yes	29	85/4102 (2.1%)	0.015
	No	100	941/15987 (5.9%)	
Presence of pigs on operation	Yes	13	120/17939 (0.7%)	0.948
	No	116	906/17939 (5.1%)	
Percent of cows born off the farm	Continuous (range: 0.0–83.3%; mean = 15.9%; median = 7.9%			0.774
Wild geese present on operation	Yes	53	302/7990 (3. 8%)	0.588
	No	76	724/12099 (6.0%)	
Cats have physical contact with dairy cows or heifers or their feed or water supply	Yes	121	963/18749 (5.1%)	0.740
	No	8	63/1340 (4.7%)	

Any weaned calves, heifers, or cows brought onto operation from outside sources within year preceding study or throughout duration of study	Yes	67	718/11149 (6.4%)	0.223
	No	62	308/8940 (3.5%)	
Any dairy cattle brought onto operation from outside sources within year preceding study or throughout duration of study	Yes	84	786/13444 (5.8%)	0.499
	No	45	240/6645 (3.6%)	
Primary type of milking facilities	Tiestall or stanchion	57	258/7552 (3.4%)	0.447
	Parlor	72	768/12537 (6.1%)	
Lack of tiestall/stanchion to house lactating cows on operation (all lactating cows not necessarily housed this way)	Yes	70	819/12046 (6.8%)	0.025
	No	59	207/8043 (2.6%)	
Maternity pen (pen where cows normally calve) for individual cow use only[c]	Yes	36	150/5233 (2.9%)	0.025
	No	93	876/14856 (5.9%)	
Lactating cow access to pasture (at any time of the year)[d]	Yes	53	247/7158 (3.5%)	0.758
	No	76	779/12931 (6.0%)	
Use of any organic bedding (e.g., straw, sawdust) for lactating cows[e]	Yes	94	635/14105 (4.5%)	0.742
	No	35	391/5984 (6.5%)	
Use of any inorganic bedding (e.g., sand) for lactating cows[e]	Yes	43	561/7148 (7.8%)	0.125
	No	86	465/12941 (3.6%)	
Lactating cows fed whole cottonseed or hulls	Yes	55	368/9567 (3.9%)	0.076
	No	74	658/10522 (6.3%)	
Lactating cows fed whole soybeans or soybean meal	Yes	108	669/13144 (5.1%)	0.751
	No	18	357/6945 (5.1%)	
Lactating cows fed brewer's by-products (includes distiller's grains, wet/dry not specified)	Yes	57	321/6785 (4.7%)	0.138
	No	72	705/13304 (5.3%)	
Lactating cows fed blood meal, meat & bone meal, fish/feather meal, or tallow/animal fat	Yes	71	459/9881 (4.6%)	0.170
	No	58	567/10208 (5.6%)	
Purchased concentrates and protein feeds stored in a enclosed building[f]	All of these feeds stored in enclosed building	68	334/10503 (3.2%)	0.021

Appendix A (*Continued*)

Variable	Level of variable	Number of herds	Number of cows positive/number of cows tested (%)	*p*-Value[a]
	Some or none of these feeds stored in enclosed building	61	692/9586 (7.2%)	
Lack of routine Monensin use in weaned calves up to breeding or heifers after breeding	Yes	96	848/14373 (5.9%)	<0.0001
	No	33	178/5716 (3.1%)	
Lack of routine Lasalocid use in weaned calves up to breeding or heifers after breeding	Yes	74	561/11252 (5.0%)	0.765
	No	55	465/8837 (5.3%)	
Lactating or dry cow access to surface water (e.g., lake, pond, stream)	Yes	36	404/5233 (7.7%)	0.011
	No	93	622/14856 (4.2%)	
Primary water source for dairy cattle is chlorinated	Yes	13	81/2250 (3.6%)	0.037
	No	116	945/17839 (5.3%)	
How often maternity housing used as a hospital area for sick cows	>Once a month	19	176/3226 (5.5%)	0.884
	<Once a month, or maternity housing not used	110	850/16863 (5.0%)	
Average bulk tank somatic cell count[g]	<300,000	89	627/14099 (4.5%)	0.145
	≥300,000 or greater	40	399/5990 (6.7%)	
Rolling herd average milk production[g]	<8165 kg	35	189/4357 (4.3%)	0.724
	≥8165 kg	94	837/15732 (5.3%)	
Sick cattle are placed in pen separate from other lactating cows	Yes	51	471/8692 (5.4%)	0.765
	No	78	555/11397 (4.9%)	
Routinely vaccinate cows with J5 vaccine	Yes	50	547/8873 (6.2%)	0.818
	No	79	479/11216 (4.3%)	
Routinely use chemicals, bait, or traps for rodent control	Yes	89	641/14045 (4.6%)	0.040

	No	40	385/6044 (6.4%)	
Manure disposal on owned or rented land by irrigation or application of slurry (surface or subsurface application)[h]	Yes	67	834/11564 (7.2%)	0.001
	No	62	192/8525 (2.3%)	
Cows eat or graze on roughage[i] obtained from fields where manure in solid or liquid form was applied to the surface but not plowed under during the same growing season	Yes	58	584/8985 (6.5%)	0.043
	No	71	442/11104 (4.0%)	
Use loader bucket to move feed and manure (could be same or different buckets)[j]	Yes	108	977/17300 (5.6%)	0.092
	No	21	49/2789 (1.8%)	

[a] *p*-Values are from the GEE parameter estimate (*t*-test) using PROC GENMOD in separate models containing each independent variable of interest and adjusting for effects of season, state, herd size, and the multiple sampling occasions per herd. Within-herd cow prevalence by visit (number of *Salmonella*-positive cows/number of cows sampled for a single visit, with 608 total visits) was the dependent variable, and a working independence correlation structure was used to adjust for correlation of observations within herd.

[b] Refers to number of herd visits (out of 608 total herd visits), not number of herds.

[c] Information on frequency of between-use cleaning not collected.

[d] "Pasture" could have provided $\geq$90% or <90% of roughage in cow ration but was distinguished from drylot access.

[e] Information on types of bedding typically used for lactating cows was collected. A farm could have used both organic and inorganic bedding (e.g., using sand part of the year and sawdust for the rest of the year, using an organic bedding type in one building and an inorganic bedding in another building, etc.).

[f] "Enclosed" was defined as covered on all sides and providing protection against birds such as a round metal grain bin or a building with a roof and four walls where outside doors are kept shut on a routine basis.

[g] Refers to farm personnel assessment of average value for the 6 months prior to first sampling visit.

[h] Farms that answered "no" used only broadcast/solid spreader methods to dispose of manure on owned or rented land. All 129 farms disposed of manure on owned or rented land.

[i] "Roughage" was defined as hay, fresh chop forage, or pasture that dairy animals may eat or graze.

[j] "No" responses here meant the farm either did not use a loader bucket to move feed (19 farms) or did not use a loader bucket to move manure (2 farms).

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