A Cross-Sectional Study on the Prevalence of *Listeria monocytogenes* and *Salmonella* in New York Dairy Herds

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**ABSTRACT**

As part of our long-term objective of assessing risk for *Listeria monocytogenes* and *Salmonella* spp. in dairy herds, we carried out a cross-sectional study to determine the prevalence of the two organisms. The study population consisted of a sample of dairy herds enrolled in the Quality Milk Promotion Services at Cornell during the period of April 1998 to March 1999. The sample was stratified by geographical region to assure representation. Four hundred and four dairy farms were enrolled in the study. In-line milk filters were collected from each farm for bacteriological examination of *L. monocytogenes* and *Salmonella* spp. Four hypothesized risk factors were evaluated for their association with the likelihood of the presence of each of the two organisms using logistic regression analysis. *Listeria monocytogenes* was isolated from 51 (12.6%) of the milk filters. We found region-specific differences in the rate of farms with positive milk filters for this pathogen. *Salmonella* spp. were isolated from 6 (1.5%) milk filters. One isolate was confirmed as *Salmonella enteritica* Serotype Typhimurium DT 104. There was no significant association between any of the hypothetical risk factors and the likelihood of *Salmonella* spp. isolation. Our study demonstrated that both *L. monocytogenes* and *Salmonella* spp. were prevalent in milk filters in New York dairy herds and that *Salmonella* was isolated at a significantly lower rate then *L. monocytogenes*.

(Key words: prevalence, *Listeria monocytogenes*, *Salmonella* spp., New York State)

**INTRODUCTION**

*Listeria monocytogenes* and *Salmonella* spp. are two important pathogens for foodborne diseases [Centers for Disease Control and Prevention (CDC), 1985; CDC, 1998b]. The series of recent multistate outbreaks of listeriosis and salmonellosis have highlighted the concerns and anxiety over the safety of the public food supply (CDC, 1998a; Mahon et al., 1999). *Salmonella* is a prominent pathogen in the etiology of food- and nonfood-related illnesses in humans and many species of animals (Bean et al., 1990). This organism generally resides in the intestinal tract of animals but may also be found in other ecological niches (Al-Nakhli et al., 1999; Smith et al., 1994). Over 2384 different serotypes of *Salmonella* spp. (Vlaemynck, 1994) have been identified. Depending on the species of infecting *Salmonella* spp. a variety of clinical signs have been reported, including septicemia, abortion, and gastroenteritis (Vlaemynck, 1994).

In contrast to *Salmonella* spp., *L. monocytogenes* rarely causes disease in the majority of the population (Ryser and Marth, 1991), but it has devastating consequences in the immunocompromised individual (Ryser and Marth, 1991). *Listeria monocytogenes* is primarily a saprophytic bacterium that is ubiquitous in the environment. Twelve serotypes of *L. monocytogenes* have been identified (Ryser and Marth, 1991). Clinical manifestations for listeriosis in immunosuppressed populations include meningitis, septicemia, and abortion or severe neonatal infection in pregnant women (Lorber, 1990; Niels le Souef and Walters, 1981). Listeriosis has a high reported fatality rate, which explains the zero tolerance of the FDA for ready-to-consume food products (Food and Drug Administration, May 13, 1999).

Raw milk and unpasteurized or improperly pasteurized milk products are among the most common causes of sporadic illnesses by *Salmonella* and *Listeria* (Ryser and Marth, 1991; West et al., 1988). Several major outbreaks of listeriosis and salmonellosis have been linked to tainted milk and milk products (Altekruse et al., 1998; CDC, 1985; Dalton et al., 1997; Vought and Tatini, 1998). This has led to regulation for pathogen reduction before reaching the consumer. Consequently, it is
believed that control and effective reduction of the pathogen load should be implemented even at the farm level (Food Safety and Inspection Service, 1998; Trout and Osburn, 1997). However, to develop an effective preventive or control program, information related to the specific hazard is required at the farm level. Furthermore, factors that influence and contribute to that hazard in the farm population need to be described.

Information on the prevalence of *L. monocytogenes* and *Salmonella* spp. in New York State dairy herds is lacking. A first step toward a comprehensive risk assessment, our study was performed to establish the prevalence rate of *L. monocytogenes* and *Salmonella* spp. in New York State dairy herds, and to discuss the factors associated with the occurrence of these organisms.

**MATERIALS AND METHODS**

**Study Design**

A cross-sectional study for *L. monocytogenes* and *Salmonella* spp. was conducted between April 1998 and March 1999. In this design, a sample of dairy herds that were enrolled in the Quality Milk Promotion Services (QMPS) program was selected and sampled for the presence of these bacteria. At the time of sampling, data on the hypothesized risk factors were collected.

**Target and Study Population**

There are 8700 dairy herds in New York State (New York State Department of Agriculture and Markets, 1998). The number of dairy farms that were consulted for mastitis-related problems by QMPS between April 1998 and March 1999 was 2624 out of 8400 enrolled herds. The selection and enrollment of farms for the study were based solely on visitation by QMPS personnel. During farm visits, QMPS personnel delivered a solicitation letter to farmers inviting them to participate in this study. With due farmers’ consent, milk filters were collected immediately after milking for bacteriological culture. Information on farms census and demography, management, and milking practices were collected using a standardized QMPS questionnaire.

**Sampling**

We performed a stratified simple random sampling in our study design. This design used the convenience of the QMPS mutually exclusive administrative regions (central, east, north, and west) of New York State. A simple random sampling was done independently within each stratum to ensure representative sampling of the population. The number of farms to be sampled from the target population was computed using the following assumptions: 1) the expected prevalence of *Salmonella* spp. is 1.5%; 2) the level of error protection in the level of prevalence estimate is 95% (α = 0.05), and 3) the acceptable level of error in the prevalence estimate is 10%. The sample size calculation was computed using the following formula as suggested by Levy and Lemeshow (1980):

\[
 n = \frac{(Z^2)(N)(P_y)(1 - P_y)}{(Z^2)(P_y)(1 - P_y) + (N - 1)(\varepsilon^2)(P_y)}
\]

where \( n \) was the sample size, \( N \) was the size of the target population (8400), \( P_y \) was the expected prevalence of *Salmonella* spp., based on previous studies, \( Z \) is the Z-value for the selected \( \alpha \) level and \( \varepsilon \) was an estimated value by which the sample estimate should not depart from the true population prevalence (0.1). A similar calculation was performed for *L. monocytogenes*, with the expected prevalence of 5%. Based on the above formula, 361 and 348 farms were determined necessary to estimate the prevalence of *Salmonella* spp. and *L. monocytogenes*, respectively. However, a sample size of 400 farms was used to account for samples that were damaged or that had missing information. Therefore, 100 dairy farms in each of the four QMPS administrative regions in the state were sampled. The number of farms sampled was equally divided among the four seasons to account for potential seasonal variations. Thus, 25 farms were sampled from a region each season. Sampling was completed within an 8- to 10-wk period for each season.

**Sample Collection and Storage**

A fresh, unsqueezed milk filter was collected from each farm and placed in a sterile plastic resealable bag. The samples were packed into refrigerated coolers, transported to the respective regional QMPS laboratory, and immediately stored in freezers at −30°C. Once a week, each QMPS regional laboratory delivered the filters to the NY State Diagnostic Laboratory, College of Veterinary Medicine at Cornell University, for microbiological analysis.

**Data Collection and Management**

Data on the hypothetical risk factors were collected via a personal interview with the farm manager/owner by using a questionnaire. A QMPS field technician completed the questionnaire at the time of sample collection. We assessed the association between season of the year, region of the study population, herd size, and the level of herd bulk milk tank SCC (BTMSCC) level, for
the presence or absence of the pathogens under study. Missing data were later mailed to the farmers for completion. A coding system for the data set was developed from the questionnaire. Data were entered and managed in Microsoft Access.

**Microbiological Analysis**

*Listeria monocytogenes*. Twenty-five grams of milk filter sections, soaked with milk, was inoculated into 300 ml of enrichment media (EB) prepared as suggested by the Food and Drug Administration (FDA/BAM, 1995) in sterilized 500-ml Erlenmeyer flasks. Samples were initially incubated for 4 h at 30°C. Nalidixic acid 0.5% wt/vol (4 ml/500 ml of EB; Sigma Chemical Co.), acriflavine 0.5% wt/vol (0.455 ml/500 ml; Sigma) and cyclohexamide 1% vol/vol (2.56 ml/500 ml of EB; Sigma) were added and broth was incubated at 30°C for an additional 20 and 44 h before plated onto two selective media (Oxford and Palcam; Unipath Ltd, Basingtoke, Hampshire, U.K.). The agar plates were incubated at 30°C for 24 to 48 h. Three to five esculin-positive single colonies with typical *Listeria*-like morphology were selected and plated onto tryptic soy agar with 6% yeast extract (Difco Laboratory), a nonselective medium, and incubated at 30°C for 18 to 24 h. A single colony from each plate was then inoculated into 5 ml of either brain heart infusion (Difco) or tryptic soy broth with 6% yeast extract, incubated at 30°C for 8 to 12 h and used for DNA extraction. DNA was prepared by using the InstaGene Purification Matrix method following the manufacturer’s protocol. The isolates were confirmed as *L. monocytogenes* by an established PCR method (Bassler et al., 1995; Batt, 1997).

*Salmonella* spp. Twenty-five grams of milk and milk filter sections were transferred and mixed into 225 ml of tetraionate broth (Difco) which was incubated at 42°C for 18 to 24 h. Tetraionate broth was then streaked onto XLT-T and Brilliant Green (BBL Becton Dickinson and Co., Cockeysville, MA) agar, incubated for 24 ± 2 h at 37°C and examined for typical *Salmonella* spp. colonies. Up to three suspicious colonies were inoculated into Kliger Iron Slant (BBL Becton Dickinson and Co., Cockeysville, MA) from each plate and incubated at 37°C for 8 to 12 h. Suspect colonies were then tested for agglutination with *Salmonella* spp. polyvalent O antiserum followed by the identification using the AP80 panel in the sensitizer system (Trek Diagnostics, West Lake, OH). *Salmonella* were then sent to the USDA/APHIS, US National Veterinary Services Laboratories, Ames, Iowa, for a complete serotyping.

**Data Analysis**

The prevalence of *L. monocytogenes* positive herds in the target population during the period of sampling was calculated using the formula:

\[ P = \frac{\text{Number of herds positive for } L. \text{ monocytogenes}}{\text{Salmonella}} \]

\[ \frac{\text{Total number of herds during the study}}{\text{Total number of herds during the study}} \]

Data analysis was performed using the SAS statistical package (SAS Institute Inc., Cary, NC). We performed an initial descriptive analysis to examine the consistency of observations and to check for unlikely or missing values. The outcome in the statistical analysis was dichotomous, where herds were classified as positive or negative, depending on the presence or absence of the respective pathogens. The region-specific prevalence was computed as the proportion of samples that were positive in each region from all the herds sampled within that region. The bivariate association between regions, seasons, herd BTMSCC and herd size to a positive outcome was tested using chi-square analysis and evaluated for significance at \( P \leq 0.05 \). Stratified analysis by variable region and season was performed to investigate stratum-specific effects of herd size and BTMSCC to the probability of the outcome. Then, the bivariate association between the levels of each variable to the outcome was examined using simple logistic regression analysis. The significance of association was evaluated at \( \alpha = 0.05 \). Variables found to be significant in the bivariate analysis were further considered in multivariate logistic regression analysis using the stepwise selection procedure. This selection method uses forward algorithm and the significance was evaluated at \( \alpha = 0.05 \).

**RESULTS**

A total of 404 milk filters samples were examined during the study. Based on the descriptive analysis of the information obtained from the farms sampled during the 1-yr period of study, the distribution of data from herd size and BTMSCC level were skewed, where smaller herd and lower level BTMSCC predominated the study population. The median size for dairy herd was 55 cows (range = 19 to 969). The median BTMSCC was 500,000 cells/ml of milk (ranges = 240,000 to 1,900,000 cells/ml). Both variables were later categorized based on quantile values of log-transformed data to comply with normal distribution assumption requirements for statistical analysis. No significant association was found between the likelihood of *L. monocytogenes* and the herd size or BTMSCC in the bivariate analysis.

Fifty-one samples were positive for *L. monocytogenes*, with a prevalence rate of 12.6% (95% CI = 9.4 to 15.8%). We observed a significant difference in the prevalence
of the respective pathogen by region (Figure 1). The likelihood ratio chi-square showed a significant association of region \( (P = 0.016) \). The regional-specific prevalence of \( L. \) monocytogenes ranges from 6.5 (95% CI = 1.7 to 11.3%), 8 (95% CI = 2.7 to 13.3%), and 17% (95% CI = 9.6 to 24.4%) to 19% (95% CI = 11.3 to 26.6%). It was twice as likely to isolate \( L. \) monocytogenes from the central region in comparison with any other region (odds ratio = 1.9 with \( P = 0.03 \)). On the other hand, we were less likely to isolate the organism from northern region than any other region (odds ratio = 0.43 with \( P = 0.047 \)). We were three times more likely to isolate \( L. \) monocytogenes from the central region than the northern. Although there appears to be some variation in the seasonal prevalence, the variation was not found statistically significant. However, the prevalence rate in spring (18%) was higher compared to other seasons of the year (Figure 2). The rate declined by 6 to 8% from spring to winter. It was almost twice more likely to isolate \( L. \) monocytogenes in spring than in other seasons (odds ratio = 1.8). In the final selection test for significance of a variable while controlling for possible confounders, only the region was found significant in explaining the outcome (Table 1).

Six samples were positive for \( Salmonella \) spp. during the study period. The prevalence rate was 1.5% (95% CI = 1.1 to 4.2%). Table 2 shows the serotypes of the \( Salmonella \) spp. isolated. One isolate of \( Salmonella \) spp. was confirmed to be \( Salmonella \) Typhimurium DT 104. A similar statistical analysis described above was performed for \( Salmonella \) spp. The analysis revealed no significant association between the outcome and the different variables that were investigated.

There was a concern about the potential biases that would have resulted from our study populations between the farms that voluntarily enrolled in the QMPS program, as opposed to the farms that were enrolled by mandatory requirement (BTMSCC \( \geq 750,000 \) cells/ml). To reconcile this concern, the study population was categorized accordingly and analyzed for independence to the outcome using chi-square at 1 df. We did not find any significant difference in the farms that were enrolled either voluntarily or mandatory, to the presence or absence of the pathogen of interests (\( P = 0.86 \)).

**DISCUSSION**

The prevalence of \( L. \) monocytogenes and \( Salmonella \) spp. among dairy herds in New York State has not been previously established. Further, no published studies have used the apparent convenience of milk filter samples to establish the prevalence of either \( Salmonella \) 

![Figure 1](image1.png)

**Figure 1.** Region-specific prevalence of \( Listeria \) monocytogenes in dairy farms of New York State (19% dark gray, 17% medium gray, 8% light gray, 6.5% white). The location of each region QMPS laboratory is indicated (♦).

![Figure 2](image2.png)

**Figure 2.** The proportion of positive milk filter for \( Listeria \) monocytogenes from New York State dairy herd by the season of sampling. The vertical bars represent the standard errors.

**Table 1.** Association between central, western, and eastern regions to the probability of a milk filter positive with northern region as reference variable, and the odds ratio estimates with 95% confidence intervals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter coefficient</th>
<th>Standard error (SE)</th>
<th>( P )-value (OR)</th>
<th>Odds ratio (OR)</th>
<th>95% OR Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>0</td>
<td>. . .</td>
<td>. .</td>
<td>1.00</td>
<td>. .</td>
</tr>
<tr>
<td>Central</td>
<td>1.15</td>
<td>0.47</td>
<td>0.01</td>
<td>3.15</td>
<td>2.2–4.1</td>
</tr>
<tr>
<td>Western</td>
<td>1.01</td>
<td>0.47</td>
<td>0.03</td>
<td>2.75</td>
<td>1.8–3.7</td>
</tr>
<tr>
<td>Eastern</td>
<td>0.17</td>
<td>0.54</td>
<td>0.09</td>
<td>1.18</td>
<td>0.1–2.24</td>
</tr>
</tbody>
</table>
Table 2. Serotype of *Salmonella* spp. isolated from milk filters collected from New York State dairy herds for each season and region of study population.

<table>
<thead>
<tr>
<th>Season</th>
<th>Region</th>
<th><em>Salmonella</em> serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>Central</td>
<td>S. Kojovi</td>
</tr>
<tr>
<td>Summer</td>
<td>Central</td>
<td>S. Hartford,</td>
</tr>
<tr>
<td></td>
<td>Eastern</td>
<td>S. typhimurium var. copenhagen</td>
</tr>
<tr>
<td>Fall</td>
<td>Eastern</td>
<td>S. typhimurium var. copenhagen</td>
</tr>
<tr>
<td>Winter</td>
<td>Northern</td>
<td>S. typhimurium DT 104,</td>
</tr>
<tr>
<td></td>
<td>Eastern</td>
<td>S. meleagris</td>
</tr>
</tbody>
</table>

spp. or *L. monocytogenes* in the United States. We are aware of one study in Canada that attempted to determine the prevalence of *Salmonella* spp. in a random sample by screening milk filters (McEwen et al., 1988). This screening method has also been applied in few other *Salmonella* spp. and *Escherichia coli* studies in Canada. These studies suggested the milk filter screening as a potentially sensitive sampling approach for epidemiological surveillance (Clarke et al., 1989; McClure et al., 1989; McEwen et al., 1988).

Milk filters from the study population were frequently covered with milk clots, fibrin, feed, fecal material, and dirt from the farm environment. The filter provides a good composite sample of the farm because it gathers material from various sources into a single channel. The entrapped material may even assist the attachment of higher proportions of bacteria to the filter. Furthermore, the filtration factor of the milk filter would enhance the capture of particles in gallons of milk, making it sensitive for detecting bacteria present in low numbers. This rationale is consistent with the finding of Schultz (1967) who previously reported that *L. monocytogenes* has to reach a concentration of $10^5$ to $10^7$ cfu/ml before the organism can be detected. Indeed, given the dilution factor due to the pooling of milk, it is unlikely to find this concentration of *Listeria* in naturally contaminated milk. In this study, a median of 1351 kg of milk had been filtered through each milk filter.

A milk filter is probably less suitable for sampling if the objective is to detect infected cows because of the difficulty of tracing the source of a pathogen, either from the environment or from what is shed by milking cows. Sanaa et al. (1993) suggested that the principal source of *L. monocytogenes* in milk on dairy farms is environmental, which includes contamination by soil, feed, and sewage. Animals shedding the organism in their feces and milk may also potentially contaminate the milk tank. Fecal excretion of *L. monocytogenes* is rather common in cattle (Skovgaard and Morgen, 1988). As many as 1% of asymptomatic cows may shed the organism in their milk (Ryser and Marth, 1991). The authors are aware of potential misclassification of pathogen status in farms; by the rationale stated above, we believe that there was some likelihood that farms may be falsely diagnosed as negative. However, the misclassification was likely to be nondifferential, as the sensitivity and the specificity of measurement remain constant. Therefore, the bias produced in the study would underestimate the magnitude of association.

The study by McEwen et al. (1988) reported a 2.9% prevalence rate for *Salmonella* spp. in milk filters, which is within our 95% prevalence confidence interval. There are a few other epidemiological investigations with findings very consistent with ours. Adesiyun et al. (1996) detected the pathogen in 1.7% raw milk, while Carosella (1990) reported that 1.7% (36/2151) of raw beef in a statistically based sampling program were positive for *Salmonella* spp.

*Salmonella* spp. Typhimurium DT 104, a contemporary outbreak strain was among the strains isolated in our study. This strain has raised much concern due to its multiple antibiotic resistance properties (Ridley and Trelfall, 1998; Villar et al., 1999) and was recently recognized as the second most isolated in clinical cases of salmonellosis after *S. enteritidis* (CDC, 1997). Our study found that the prevalence of *L. monocytogenes* for New York dairy herds was 12.6%. The prevalence was much higher than reported in raw milk, which ranges from 1.7 to 5.8% (Adesiyun et al., 1996; Odonnell, 1995; Rohrbach et al., 1992) or 6.2% in raw meat (Carosella, 1990). However, consistent with the findings by Hayes et al. (1986) in 14% of milk filters of dairy plants and reported ranges of fecal isolation in 9.2 to 20% dairy cows (Husu, 1990; Van Renterghem et al., 1991).

Although there was insufficient data to base the evaluation of the seasonal effect on the occurrence of *L. monocytogenes*, our data suggests that the prevalence of this organism was highest in spring. Our observation agrees with three other studies that reported higher prevalence in spring, specifically between March and June (Carosella, 1990; Odonnell, 1995; Yoshida et al., 1998). This finding is consistent with the speculation that high humidity in the spring influences the propagation of *L. monocytogenes*. The increased growth of pathogens in the environment would increase the likelihood of more animals being contaminated. The region-specific differences were not explained by the factors that were investigated in this study. Herd size or the BTMSCC for the herds did not explain the differences in the milk filter-positive rate. The significantly higher likelihood of isolating *L. monocytogenes* in the central and western region farms was possibly a function of the differences in the data collected from the study population. It could reflect the differences in farm prac-
tices and management or other determinants that are unknown or that were not investigated in this study. The distance from which the samples originated to the central laboratory has been speculated to play a role in the different rate of isolation.

Also, a certain percentage of bacteria likely was lost due to the freezing of samples. The severity of damage done to the bacterial cells by the length of freezing followed by thawing was difficult to ascertain. However, the milk filters were usually soaked in milk, and milk was documented as a good cryoprotectant (Janssen and Busta, 1973; el-Kest and Marth, 1991). In fact, it probably protects better than glycerol, tryptose broth or phosphate buffer (el-Kest and Marth, 1991). The milk components protect *Salmonella* spp. cells from injury and death from freezing and thawing to a certain degree (Janssen and Busta, 1973). The survival rate of *L. monocytogenes* can be as high as 95% after 7 mo of storage (Papageorgiou et al., 1997), while it was reported to be about 50% for *Salmonella* spp. (Janssen and Busta, 1973).

The authors realized the need to compare the prevalence of pathogens in milk filters to the prevalence in cows. This information would provide an estimate of the sensitivity of the method, rendering it more meaningful for use in other studies. Unfortunately, at this point we do not know the sensitivity of the milk filter for detecting these pathogens. However, we believe that given the time and resources, milk filter sampling is a rapid, inexpensive way for detecting, screening, identifying, and characterizing potentially high-risk herds for these pathogens and could be used as a base for future epidemiological studies. Further, potentially significant implications to public health could be made as pathogen that exists in low numbers that are undetectable in bulk tank milk may be captured along the filter line.

CONCLUSIONS

This study used the convenience of sampling the milk filters from dairy farms to determine the prevalence of two of the most important foodborne pathogens. The prevalence of *L. monocytogenes* was 12.6% and the prevalence of *Salmonella* spp. was 1.5%. There was a regional effect on prevalence of *L. monocytogenes*. The odds of *L. monocytogenes* to be present in the milk filter was approximately 3 times higher if the milk filters originated from a farm in the central region of New York State, and least likely to be positive for the pathogen if the milk filter was from the northern region. The use of milk filter was a convenient, rapid and potentially reliable method for screening pathogens in dairy farms for epidemiological surveillance.

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