Identification of potential on-farm sources of *Listeria monocytogenes* in herds of dairy cattle

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Objective—To elucidate the ecology of *Listeria monocytogenes* on dairy cattle farms by determining the prevalence of the organism in various samples.

Sample Population—Dairy cattle operations in central New York State.

Procedures—A repeated cross-sectional study design was used. Various samples were obtained from cattle (feces, composite udder milk, and udders), their environment (silage, feed bunks, water troughs, and floor bedding), inline milk filters, and bulk tank milk from 50 dairy farms. Samples were tested for *L monocytogenes* by use of a PCR assay with 2 steps of bacterial enrichment. Data were analyzed with mixed-effect logistic regression to control for the potential clustering of *L monocytogenes* on particular farms.

Results—*L monocytogenes* was detected in composite milk, udder swab samples, and fecal samples at prevalences of 13%, 19%, and 43%, respectively. There was no significant clustering of the pathogen by farm. *Listeria monocytogenes* was more common in samples obtained from cattle and the environment during winter and summer versus the fall. The prevalence of *L monocytogenes* was twice as high in samples obtained from feed bunks, water troughs, and bedding, compared with that in samples obtained from silage (65%, 66%, 55%, and 30%, respectively).

Conclusions and Clinical Relevance—*L monocytogenes* was more prevalent in samples obtained from dairy cattle and their environment than in milk samples. Strategies to control the pathogen in dairy operations should focus on cow hygiene and sanitary milk harvesting on the farm. (*Am J Vet Res* 2009;70:383–388)

Listeria spp are ubiquitous in nature, but only *Listeria monocytogenes* poses an important health risk. The facultatively anaerobic, non–spore-forming coccobacillus lives in a plant and soil environment and can be found in vegetables, sewage, genital secretions, and nasal mucus of apparently healthy animals.[1–3] *Listeria monocytogenes* is resistant to drying, can survive up to 2 years in dry soil and feces, and is capable of growing in a wide range of temperatures (ie, 4° to 44°C).[4–6] Infection with the organism can result in a spectrum of clinical conditions, including septicemia, meningitis, meningoencephalitis, abortion, and in some instances, death.[1,7–10]

Listeriosis occurs sporadically in cattle, sheep, and goats and can also occur in pigs, dogs, cats, some wild animals, and humans.[1,10] The incidence of the disease is increasing worldwide and is associated with grave direct economic losses.[11–13] In the United States, these losses have been estimated at $2.3 billion/y.[14] Most reported outbreaks of listeriosis in humans are attributable to the consumption of contaminated products of animal origin.[5,7,11,12,15–19]

Abbreviations

CI	Confidence interval
OR	Odds ratio

Reports[16,19–27] from around the world confirm the existence of *L monocytogenes* on dairy operations. Various strains of the organism have been isolated from clinically infected and clinically normal cows on dairy farms,[18,21–24,26,28] emphasizing the potential role of dairy farms in the transmission of *L monocytogenes* and the importance of controlling the pathogen therein. The development of strategic plans to control *L monocytogenes* on dairy farms relies largely on identifying farms at risk for harboring the pathogen and describing the on-farm ecology of the organism. The purpose of the study reported here was to elucidate the ecology of *L monocytogenes* on dairy farms by determining the prevalence of the organism in various samples obtained from dairy cattle farms in New York State.

Received March 26, 2008.
Accepted June 25, 2008.
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Support by a grant from the USDA Cooperative State Research, Education and Extension Service (proposal No. 2002-35212-12317).
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Materials and Methods

Fifty farms were randomly selected by means of a list of participants in another study[22] of *L monocytogenes* on dairy farms in central New York State. Consent for sampling was obtained from operators of all farms. The study protocol was reviewed and approved by the Animal Care and Use Committee at Cornell University.

To evaluate potential seasonal variation in shedding of *L monocytogenes* by milking cows and in the prevalence of the pathogen in the farm environment, enrolled farms were visited once every season from September 2003 through August 2006. Seasonal intervals were September through December (fall), January through April (winter), and May through August (summer). On each visit to each farm, samples were obtained from cattle (feces, composite udder milk, and teat ends), their environment (corn silage, feed bunks, water troughs, and floor bedding), inline milk filters, and bulk tank milk.

Collection of cattle samples—Cattle were classified as those at high risk of *L monocytogenes* infection (had an ongoing problem with clinical mastitis or a history of listeriosis, abortion, or mastitis) or those at low risk (clinically normal, without history of the aforementioned diseases). A composite milk sample, fecal sample, and teat swab sample were collected from each of 30 cattle on each farm. A 4 × 4-inch piece of sterile gauze was used to swab the teats and then placed in a sterile vial. Approximately 50 mL of milk was collected aseptically into another sterile vial. Approximately 30 g of feces was collected and placed in a sterile vial.

Collection of environmental samples—On each farm, approximately 100 g of fresh silage was obtained from an upright silo. Samples collected from the silo were classified by the apparent status of the silage: unspoiled or spoiled. Silage samples were also collected from silage bunkers (or similar storage spaces) from 5 surface regions of visibly unspoiled silage and 5 surface regions of visibly spoiled silage. All silage samples were transferred into the sterile bags. Approximately 100 g of bedding was collected from 5 locations in each cattle stall and placed into sterile plastic bags. Swab samples were obtained from several locations within feed bunks and from biofilm on the water bunk with 4 × 4-inch gauze pads and were placed in sterile vials.

Collection of milking system samples—Fresh, unsqueezed milk filters were collected and placed into a sterile plastic bag. Samples of bulk tank milk (100 mL) were collected directly from the tank with a sampler that allows mixing of the milk from the bottom up. Each bulk milk sample was transferred into a sterile vial. All samples were refrigerated for transport to the laboratory, stored at 4°C overnight, and processed the next day.

Detection of *L monocytogenes*—All samples were processed by use of an automated, genetic-based system for detecting *Listeria* spp.[a] To do so, samples were enriched with Demi-Fraser broth[b] and incubated for 22 to 26 hours at 30°C. Buffered *Listeria* enrichment broth[c] was used as a selective medium in which samples were again incubated at 35°C for 18 to 24 hours. After enrichment, samples were heated in a lysis reagent solution.[d] The PCR tablets provided by the manufacturer of the PCR assay were hydrated with lysed sample and processed in the automated PCR cycler-detector.[a] Results for detection of *L monocytogenes* were displayed by the PCR assay analysis software as positive or negative symbols.

Statistical analysis—The prevalence of *L monocytogenes* in each type of sample was computed as the proportion of samples that tested positive out of all samples that were examined by means of the PCR system. The probability of detecting *L monocytogenes* in a particular set of samples in comparison with the probability in other sets was evaluated via a mixed-effect logistic regression model,[29] with a random effect introduced to adjust for clustering of the results by farm. Because the primary sampling units (cow or environmental factors) were grouped by farms, it was assumed that this grouping would lead to a correlation in the probability of positive results for *L monocytogenes*. The likelihood ratio test was used to evaluate the significance of farm as a random effect in the mixed model. The effect of each factor on the likelihood of contamination with the organism was quantified by means of the OR, which was computed as the exponent of the respective regression coefficient. Computer software[e] was used for all analyses. A value of $P < 0.05$ was considered significant.

Results

Cattle samples—A total of 4,236 samples were collected from the 50 dairy cattle herds. *Listeria monocytogenes* was isolated from at least 1 cow on each farm. The prevalence of *L monocytogenes* was 13% (184/1,412) in composite milk samples, 19% (268/1,408) in udder swab samples, and 43% (608/1,414) in fecal samples. Statistical analysis revealed a significant association between the type of sample and the probability of detecting *L monocytogenes* (**Table 1**). Specifically, the odds of detecting *L monocytogenes* in teat swab samples were nearly twice as high as the odds of detecting the pathogen in composite milk samples (OR, 1.6; 95% CI, 1.3 to 2.0). On the other hand, the odds of detecting *L monocytogenes* in fecal samples were 5 times as high as the odds of detection in composite milk samples (OR, 5.2; 95% CI, 4.3 to 6.3). No effect of farm on the probability of detecting *L monocytogenes* was detected in the random-effect models.

The variation in the prevalence of *L monocytogenes* in samples collected from cattle by season was graphically summarized (**Figure 1**). The odds of detecting the organism in winter (OR, 4.1; 95% CI, 3.4 to 5.0) and summer (OR, 2.5; 95% CI, 2.1 to 3.1) were higher than the odds of detection in the fall (OR, 1). *Listeria monocytogenes* was significantly more prevalent in all cattle samples obtained in winter versus those obtained in the fall (OR, 4.9; 95% CI, 3.7 to 6.4). Results of multivariate analysis in which potential seasonal variation was controlled indicated the odds of detecting the organism in winter or summer were higher than the odds of detection in the fall (Table 1). Analyses to investigate the potential effect of season on the probability of detecting *L monocytogenes* in various cattle samples

revealed that the odds of detecting *L monocytogenes* in milk samples, udder swab samples, or fecal samples in winter and summer were significantly higher than the respective odds of detection in the fall (**Table 2**). The odds of recovery of *L monocytogenes* from fecal samples collected in winter were 22 times as high as the odds for milk samples collected in the fall. Furthermore, the odds of detecting the organism in fecal samples were higher than that of any other cattle sample, regardless of season.

Environmental samples—*Listeria monocytogenes* was recovered from 66% (160/242) of water trough samples, 65% (158/242) of feed bunk samples, 55% (132/240) of bedding samples, and 30% (72/240) of silage samples. Results of generalized linear mixed models indicated there was no significant clustering of *L monocytogenes* by farm. The odds of detecting the organism in feed bunk and water trough samples were > 4 times as great as the odds of detecting the organism in silage samples (**Table 3**). The odds of detecting *L monocytogenes* in bedding samples were 3 times as great as the odds of detection in silage.

The odds of detecting *L monocytogenes* in various environmental samples increased when controlling for season (Table 3). The organism was 7 times as likely to be detected in samples collected in winter and 4 times as likely to be detected in samples collected in summer, compared with the likelihood of detection in the fall. When season of collection was accounted for in the analyses, a significant interaction was detected (**Table 4**). Specifically, the odds of detecting *L monocytogenes* in environmental samples (silage, feed bunk, water trough, and bedding) collected in the winter and summer were significantly greater than the odds for silage samples collected in the fall.

Milking system samples—*Listeria monocytogenes* was recovered from 45% (62/137) of inline milk filters and 16% (22/137) of bulk milk tank samples, representing a 3-fold difference. Results of univariate analysis in which an adjustment was made for the potential clustering of *L monocytogenes* by farm revealed that

Table 1—Results of mixed-effect logistic regression models of the probability of detecting *Listeria monocytogenes* in various samples collected from dairy cattle in 50 herds in central New York State, with and without season of sample collection taken into account.

Factor	Regression coefficient	SEM	OR	95% CI
Model with cattle alone				
Composite milk	Ref	—	1.0	—
Udder surface	0.459	0.106	1.6	1.3–2.0
Feces	1.653	0.097	5.2	4.3–6.3
Model with cattle and season				
Composite milk	Ref	—	1.0	—
Udder surface	0.482	0.109	1.6	1.3–2.0
Feces	1.769	0.100	5.9	4.8–7.1
Season				
Fall	Ref	—	1.0	—
Winter	1.562	0.103	4.8	3.9–5.8
Summer	1.001	0.107	2.7	2.2–3.4

Ref = Referent category. — = Not applicable.
Farm of origin did not significantly influence results of the model. The OR represents the odds of detecting *L monocytogenes* in the indicated group, compared with the odds in the referent group. Odds ratios for which the 95% CI does not contain 1 are significantly ($P < 0.05$) different from 1 (which indicates no difference in odds).

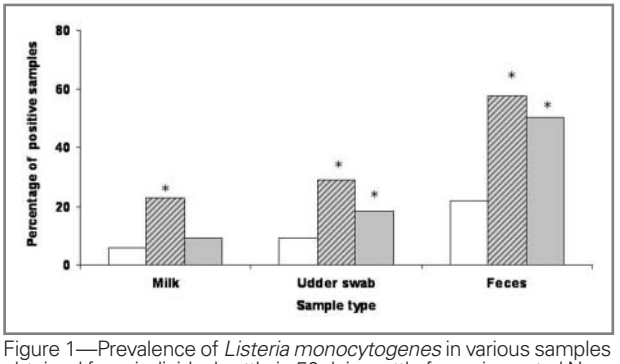

Figure 1—Prevalence of *Listeria monocytogenes* in various samples obtained from individual cattle in 50 dairy cattle farms in central New York State during fall (September through December; white bars), winter (January through April; hatched bars), and summer (May through August; gray bars). *Value is significantly ($P < 0.05$) different from that measured in September through December.

Table 2—Results of logistic regression analysis in which the effect of season on the odds of detecting *L monocytogenes* in various cattle samples from 50 dairy farms was evaluated.

Factor	Regression coefficient	SEM	OR	95% CI
Milk samples in fall	Ref	—	1.0	—
Milk samples in winter	1.554	0.223	4.7	3.1–7.3
Milk samples in summer	0.451	0.257	1.6	0.9–2.6
Udder samples in fall	0.461	0.252	1.6	0.9–2.6
Udder samples in winter	1.865	0.219	6.5	4.2–9.9
Udder samples in summer	1.504	0.223	4.5	2.9–7.0
Fecal samples in fall	1.279	0.231	3.6	2.3–5.6
Fecal samples in winter	3.084	0.215	21.8	14.3–33.3
Fecal samples in summer	2.786	0.217	16.2	10.6–24.8

See Table 1 for key.

Table 3—Results of mixed-effect logistic regression models of the probability of detecting *L monocytogenes* in various samples collected from the environments and milking systems of 50 dairy cattle farms in central New York State, with and without season of sample collection taken into account.

Factor	Regression coefficient	SEM	OR	95% CI
Milking system alone				
Milk bulk tank	Ref	—	1.0	—
Milk filter	1.455	0.289	4.3	2.4–7.6
Environment alone				
Silage	Ref	—	1.0	—
Feed bunk	1.491	0.203	4.4	3.0–6.6
Water trough	1.535	0.204	4.6	3.1–6.9
Bedding	1.089	0.199	3.0	2.0–4.4
Environment and season				
Silage	Ref	—	1.0	—
Feed bunk	1.743	0.222	5.7	3.7–8.8
Water trough	1.827	0.223	6.2	4.1–9.6
Bedding	1.276	0.215	3.6	2.3–5.5
Season				
Fall	Ref	—	1.0	—
Winter	1.910	0.185	6.8	4.7–9.7
Summer	1.429	0.182	4.2	2.9–6.0

See Table 1 for key.

Table 4—Results of logistic regression analysis in which the effect of season on the odds of detecting *L monocytogenes* in various environmental samples from 50 dairy farms was evaluated.

Factor	Regression coefficient	SEM	OR	95% CI
Silage in fall	Ref	—	1.0	—
Bedding in fall	0.474	0.364	1.6	0.8–3.3
Feed bunk in fall	0.890	0.356	2.4	1.2–4.9
Water trough in fall	0.972	0.353	2.6	1.3–5.3
Silage in winter	0.831	0.371	2.3	1.1–4.7
Bedding in winter	2.504	0.385	12.2	5.8–26.0
Feed bunk in winter	3.293	0.440	26.9	11.4–63.7
Water trough in winter	3.236	0.440	25.4	10.7–60.3
Silage in spring	0.637	0.394	1.9	0.9–4.1
Bedding in spring	2.041	0.379	7.7	3.7–16.2
Feed bunk in spring	2.385	0.392	10.9	5.0–23.4
Water trough in spring	2.604	0.405	13.5	6.1–29.9

See Table 1 for key.

farm was not a significant factor. However, the odds of detecting *L monocytogenes* in milk filters were 4 times as great as the odds of detecting the organism in bulk tank milk (Table 3). There was no significant seasonal variation in the odds of detecting the *L monocytogenes* in milk filters versus bulk tank milk, nor were these odds influenced by season of sample collection.

Discussion

The purpose of the study reported here was to elucidate the ecology of *L monocytogenes* on dairy cattle farms to identify on-farm sources of the pathogen. This information is essential for the design of cost-effective strategies for controlling the introduction of the organism into the food supply system.[9] Epidemiologic studies that yield such critical information are lacking, but advances in molecular diagnostic techniques have made it possible to address this need. Other studies[19–21,23,24,26,29] on the prevalence of *L monocytogenes* were carried out in response to an outbreak of listeriosis or focused on 1 component of the farm ecosystem. However, in our study, various samples were collected from clinically normal cattle, the environment, and the milking system.

Forty-three percent of fecal samples collected from cattle in the present study contained *L monocytogenes*, which represented a much higher prevalence than that detected in milk samples in our study and prevalences detected in cross-sectional studies[17,18,20,21,23] in the United States and abroad. In addition to the difference between these studies with respect to target population, the present study differed in that we used a different detection strategy for the organism, which consisted of bacteriologic enrichment and PCR assay. Half of the farms in the present study were previously identified as having harbored *L monocytogenes*.[22] Furthermore, the organism was isolated from at least 1 cow on each of the farms enrolled in the study. In another study[23] in which herds were repeatedly evaluated for the presence of *L monocytogenes*, the organism was detected in 31% of fecal samples. Although the prevalence of *L monocytogenes* varied in the aforementioned studies, the fact remains that the organism is ubiquitous on dairy farms.

Listeria monocytogenes was more commonly detected in fecal samples than in milk samples from each cow in our study. This finding is consistent with those of other studies[20,30–33] in which the prevalence of this foodborne pathogen was evaluated in fecal and milk samples collected from the same cows. One explanation is that for *L monocytogenes* to be excreted in milk, an infection must exist and the organism must cross the digestive track barriers after ingestion. When these results for prevalence are considered in the context of hygiene and sanitary programs, the role of animals as a reservoir or amplifier of *L monocytogenes* in the farm environment cannot be ignored.[32]

In the present study, the probability of detecting *L monocytogenes* in samples collected from cattle varied with time of year. This finding is consistent with those of other studies,[20,34] which revealed *L monocytogenes* was more prevalent during cold or indoor seasons versus other times of the year.[35–38] Several factors could explain the high prevalence of the organism during cold months, including the crowding of cattle in indoor facilities and the difficulty in maintaining excellent hygiene practices in such a circumstance.

Listeria monocytogenes was more common in samples obtained from the environment versus those obtained from cattle in our study. This finding is consistent with the findings in other studies.[20,24,31,32,34,36,39,40] One plausible explanation is that *L monocytogenes* can multiply in the environment when the temperature and humidity are optimal.[20,34,41] *Listeria monocytogenes* was more prevalent in samples collected from the immediate cattle environment (ie, feed bunks, water troughs, and bedding), compared with the prevalence in silage silos and bunkers. Data obtained by means of repeated measurements in other studies involving clinically normal herds is limited. However, most studies[37,38,42] in which outbreaks of listeriosis on dairy farms were investigated focused on spoiled silage. When results of those studies are compared with results of the present study, it appears that cattle are more likely to be exposed to *L monocytogenes* in their immediate environments as opposed to through silage. Although the role of spoiled silage in the risk of listeriosis should not be ignored in dairy animals, attention should also be paid to sanitary measures within the immediate environment. Maintaining cleanliness of water troughs and feed bunks in pens is likely to reduce the risk of exposure of dairy cattle to *L monocytogenes*.

The seasonal variation in prevalence detected in our study corresponds with that detected in other studies[20,24,43] in which samples were collected from cows on dairy farms and their environment. Although the studies differed in design, they all revealed a high likelihood of detecting *L monocytogenes* during winter. The prevalence of the pathogen in cow feces during the same time of the year was also high, which would explain a high prevalence of contamination of water troughs and feed bunks. Coupling the high prevalence of *L monocytogenes* in feces during winter with the potential cumulative effect of poor sanitary measures (eg, less frequent cleaning of feed and water troughs) would explain the higher prevalence of *L monocytogenes* in environmental samples, compared with the prevalence in cow samples.

Although a high percentage of cattle and environmental samples contained *L monocytogenes* in the study reported here, the organism was less prevalent in inline milk filters. The prevalence in milk filter samples (45%) was much higher than has been reported elsewhere[22]; however, our detection method was also different, which might explain the difference in prevalences. The method used in the present study was more sensitive than that used the other studies.[22,44] The higher prevalence of *L monocytogenes* in milk filter samples versus composite milk samples may be attributable to the presence of the organism on the surface of udders or teats. Furthermore, all farms that participated in our study changed the inline milk filter once a day; hence, the high prevalence of *L monocytogenes* may have reflected accumulation of the organism as a result of 2 to 3 milkings/d.

The prevalence of *L monocytogenes* in bulk tank milk samples in the present study was higher than that reported for other studies[18,34,45] in the United States and other parts of the world. This difference may have been attributable to differences in study populations, sampling designs, and detection techniques. Several studies[18,34,36,45,46] revealed a higher prevalence of *L monocytogenes* during warm months, compared with the prevalence at other times. In the present study, the prevalence of the organism was also higher in the summer, compared with the prevalence in the fall. The reason for the higher prevalence in summer is not immediately apparent; however, a plausible explanation is that the rise in temperature during summer might support bacterial growth, resulting in an increased likelihood of detection, compared with the likelihood during other seasons.[47]

Despite the high prevalence of *L monocytogenes* in environmental and cattle samples in the study reported here, the prevalence was considerably lower in bulk tank milk samples. This finding is consistent with those of other reports[18,34] and can be attributed to the filtration process and the dilution factor in the tanks. Again, differences in detection methods may explain differences in prevalences between studies.[44]

The results of our study confirmed that *L monocytogenes* is ubiquitous among dairy cattle farms. What was surprising was the high prevalence of the organism in feces of apparently healthy cattle. This finding emphasizes the role of cattle in amplifying *L monocytogenes* contamination of dairy farm environments. The pathogen appeared to be most prevalent in feed bunks, water troughs, and bedding, each of which is subject to contamination by cattle. Biosecurity and hygiene practices are important for reducing the risk of introduction and perpetuation of *L monocytogenes* on farms. Science-based efforts in preharvest risk reduction should contribute appreciably toward a safe and sustainable food supply system.

a. BAX system PCR assay, DuPont Co, Wilmington, Del.
b. Fisher Scientific International Inc, Hampton, NH.
c. Becton-Dickinson, Rutherford, NJ.
d. DuPont Co, Wilmington, Del.
e. Cytel Statistical Software, Cytel Inc, Cambridge, Mass.

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