Listeria monocytogenes in the Irish Dairy Farm Environment

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ABSTRACT

Listeria monocytogenes is a potentially lethal foodborne pathogen commonly found in the environment. European Union hygiene legislation places responsibility for safety on primary production facilities, including farms, as part of a policy to introduce traceability throughout the food chain. This study aimed to determine the occurrence of L. monocytogenes in the Irish dairy farm environment and in particular the milking facility. Two hundred ninety-eight environmental samples were collected from 16 farms in the southern region of Ireland. A number of farms within the group supply raw milk to the unpasteurized milk cheese industry. The samples taken included cow feces, milk, silage, soil, water, etc. Samples were enriched in Listeria enrichment broth and incubated for 48 h, followed by plating on chromogenic agar Listeria Ottavani & Agosti and further incubation of the plates for 24 to 48 h. Presumptive L. monocytogenes isolates were purified and confirmed by PCR targeting the hly gene. Overall, 19% of the samples (57 of 298) were positive for L. monocytogenes. These were serotyped using conventional and PCR methods; serotypes 1/2a, 1/2b, and 4b made up 78% of the typeable isolates. A correlation was found between the level of hygiene standards on the farm and the occurrence of L. monocytogenes. There was little difference in the occurrence of L. monocytogenes between farms supplying milk to the unpasteurized milk cheese industry and those supplying milk for processing. This study demonstrates the prevalence of L. monocytogenes in the dairy farm environment and the need for good hygiene practices to prevent its entry into the food chain.

Listeria monocytogenes is a gram-positive, rod-shaped microorganism that is ubiquitous in the environment. It is the causative agent of epidemidemic and sporadic listeriosis, and although the disease incidence is low, it has a high mortality rate of about 30% (5, 41). There was a significant increase in the number of cases of listeriosis in Ireland in 2007. The crude incidence rate increased from 0.17 per 100,000 population in 2006 to 0.50 per 100,000 population in 2007 (10), and Ireland is listed as one of six European Union (EU) countries with a statistically significant increase in human listeriosis cases between the years 1999 and 2006 (4). In 2006, there were 1,583 reported cases in the EU (5), while the Centers for Disease Control and Prevention lists the annual number of cases in the United States at nearly 2,500 (33). In England and Wales it was reported that in the year 2000, while listeriosis accounted for <0.1% of foodborne illness, it accounted for 17% of foodborne-illness–related deaths (1).

Although the numbers of reported cases are relatively low, the severity of the disease has resulted in stringent criteria on the presence of this organism in foods, particularly ready-to-eat (RTE) foods able to support the growth of Listeria (7). This includes many farm-produced specialty foods such as cheese and dairy products. EU legislation requires absence of the organism in 25 g before the food has left the immediate control of the food business operator who has produced it and <100 CFU/g on products placed on the market during their shelf life (7).

Listeria spp. have been isolated from a variety of foods including RTE foods, fish, meat, and raw milk, and this has been recently reviewed (25). Their occurrence in raw milk and food processing environments (dairy, fish, pork, etc.) has also been widely reported (23, 24, 38, 39). In most cases, contamination is due to postprocessing environmental cross-contamination of foods, as heat treatment and the effects of processing, such as smoke treatment, kill the organism. Because of the absence of a pasteurization step, raw milk products, especially soft cheeses, are considered high-risk products (27), as are smear cheeses (35).

Listeria spp. have been isolated from foods in Ireland. Sheridan et al. (37) examined 549 varied food samples, including prepackaged cooked meats, frozen meats, frozen fish, and cooked meats packaged at retail level. In prepackaged cooked meats, no samples were found to be positive for L. monocytogenes. Of the retail-packaged cooked meats, 21% were found to be contaminated with Listeria spp. Frozen beef burgers were heavily contaminated (97% of samples were positive for Listeria spp.), as were frozen fish stick–style products, with 95% of samples scoring positive for Listeria spp. In Northern Ireland, Harvey and Gilmour (17) examined 513 food samples of which 35% were positive for Listeria spp. and 18.3% were positive for L. monocytogenes. Madden et al. (28) examined beef carcasses in Northern Ireland and found that only 6 of 200 samples were positive for Listeria spp., none of which were L. monocytogenes. Kells and Gilmore (23) studied and examined two milk processing facilities in Northern Ireland and detected L. monocytogenes from 94.1% of the areas sampled. The
overall incidence of *Listeria* on equipment was 18.8% (6.3% *L. monocytogenes*); in the environment it was 54.7% (40.6% *L. monocytogenes*), and in raw milk it was 44.4% (22.2% *L. monocytogenes*). In a survey of the environmental occurrence in Scotland, Fenlon et al. (9) found that dairy farm environmental, crop, soil, and fecal samples were positive for *L. monocytogenes*.

Most of the published studies on the occurrence of *L. monocytogenes* in the environment refer to the food processing environments, and there is very little information on the occurrence of *L. monocytogenes* in the general dairy farm environment, especially in Ireland. There have been reports from Iceland, Japan, Finland, and the United States (3, 14, 21, 40), but much of this is related to targeted occurrence in listeriosis outbreak situations, where the prevalence may be artificially high. In general, isolation rates from non–listeriosis-associated farms varied, and good hygiene practices were correlated with low incidence (21, 32).

RTE foods are routinely screened for *L. monocytogenes* as part of the official testing requirements of EU regulation EC 2073/2005; however, there is little requirement to examine the prevalence of the disease-causing organism at the primary production stage. There is a gap in data on the occurrence of *L. monocytogenes* on dairy farms. From a point of view of identifying the source of *L. monocytogenes* in processing units, this is particularly important, yet information on the prevalence of this organism in Irish farm environments is lacking.

The purpose of this study was to determine the environmental occurrence of *L. monocytogenes* on Irish dairy farms, determine the serotypes isolated, and compare farms supplying unpasteurized milk for farmhouse cheese production with farms supplying milk for processing.

**MATERIALS AND METHODS**

**Farm samples.** Three sets of samples were obtained from different farms as follows: set A comprised farms supplying milk to the unpasteurized milk cheese industry, set B comprised farms supplying milk for processing, and set C consisted of dairy farms.

For set A, a total of four farms were identified and each farm supplied milk to only one cheese producer. Each farm was visited monthly during the summer for 3 months, and eight samples were taken on each occasion. For set B, three farms were tested and each farm was visited monthly during the summer for 3 months with 10 samples taken on each occasion. Sampling was undertaken during the summer, as Ireland has a seasonal milk production system, with maximum production in the summer months. In the case of set C, nine dairy farms were visited monthly for 2 months (February and March), and three samples were taken from each farm on both occasions. Although these farms were sampled earlier in the year, the temperature difference between this earlier period and summer is small, and since *L. monocytogenes* can grow at refrigerated temperatures, the seasonal difference in sampling is unlikely to affect the result. All samples were packed in a cooler and transported to the laboratory at ~4°C and analyzed on arrival at the laboratory.

The samples for farm sets A and B came from a variety of sources including farm water, cow feces, feed, bedding, and dust from the milking facility as well as unpasteurized milk. For set C, the samples consisted of one farm water sample, one soil sample, and one cow fecal sample.

**Farm hygiene monitoring.** A hygiene inspection was carried out on the farms of set A, i.e., those farms supplying milk for unpasteurized milk cheese, at the same time as the samples were taken. The person taking the samples, who was trained in environmental science, designed and conducted the hygiene checklist and inspection. A checklist for the milking facility was adapted from a combination of two currently existing documents: EC no. 853/2004, which lays down specific hygiene rules for food of animal origin (6), and The British National Dairy Farm Assured Scheme criteria (31). The criteria used were as follows: (i) the facility must be clean and tidy and free from accumulated dung; (ii) the floors and walls must be complete and undamaged; (iii) the ducts, jars, and pipes must be properly maintained; and (iv) the facility must be free from airborne and accumulated dust. Each farm was assessed for the four criteria and awarded a score on a scale from 1 to 5, 5 being very poor.

**Analysis of samples for *L. monocytogenes*.** For analysis of *L. monocytogenes*, the samples were enriched in Listeria enrichment broth (Oxoid, Basingstoke, UK). Twenty-five grams (10 to 25 g for about 10% of samples when the sample amount was insufficient) of sample was added to 225 ml of Listeria enrichment broth, without antibiotic selection, and incubated at 37°C for 2 to 3 h to allow recovery of injured cells. After addition of the antibiotic solution, the Listeria enrichment broth was incubated at 37°C for 45 to 46 h. After this enrichment step, 20 to 100 μl of the culture was spread on an agar Listeria Ottavani & Agosti (ALOA) plate (Lab M Ltd., Lancashire, UK), which was incubated at 37°C for 24 to 48 h. Typical *L. monocytogenes* colonies (green colonies with a surrounding halo) were selected from each plate, grown in tryptic soy broth, purified, and frozen at −20°C. All frozen isolates were confirmed as *L. monocytogenes* by PCR of the hemolysin (*hly*) gene (34).

**Confirmation by PCR.** For confirmation of isolates as *L. monocytogenes*, one colony was suspended in 50 μl of sterile distilled water. This was heated to 90°C for 15 min, and 10 μl was assayed by real-time PCR by using a Roche LightCycler instrument (34). The primers and probe used targeted the *hly* gene and were as follows: forward primer, 5′ CAT GGC ACC ACC AGC ATC T-3′; reverse primer, 5′ ATC CGG GTG TTT CIT TTC GA-3′; and Taqman probe, 5′ FAM-CGC CTG CAA GTC CTA AGA CGC CA-TAMRA-3′.

**PCR on enriched cultures.** In order to determine if *L. monocytogenes* could be detected from enriched cultures before plating on ALOA (and thus shortening the time to result by 2 days), 500 μl of enriched culture was taken from 39 samples (which had been frozen at −20°C) in which *L. monocytogenes* isolates were obtained from ALOA agar plates. This was purified with a QIAamp DNA stool mini kit (Qiagen, West Sussex, UK). The isolated DNA was assayed by PCR as described above.

**Serotyping of isolates.** Serotyping of isolates was achieved using a combination of antisera specific to the *L. monocytogenes* somatic O antigen (Denka Seiken Co., Ltd., Tokyo, Japan), in tandem with a PCR-based serovar determination assay (5).

For the antisera O antigen determination, a cell suspension grown on brain heart infusion agar, in 0.2% (wt/vol) NaCl. This was heated at 100°C for 30 min, followed by centrifugation at 1,000 × g for 20 min. The precipitate was resuspended in 0.2% (wt/vol) NaCl, and 10 μl of the suspension was mixed with a drop of antisera on a glass slide. A reaction was classified as positive if there was strong agglutination within 1 min of tilting.
TABLE 1. Occurrence of L. monocytogenes on dairy farms that supply milk to the unpasteurized-milk cheese industry—farm set A*

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Farm A1 1st visit</th>
<th>Farm A1 2nd visit</th>
<th>Farm A1 3rd visit</th>
<th>Farm A2 1st visit</th>
<th>Farm A2 2nd visit</th>
<th>Farm A2 3rd visit</th>
<th>Farm A3 1st visit</th>
<th>Farm A3 2nd visit</th>
<th>Farm A3 3rd visit</th>
<th>Farm A4 1st visit</th>
<th>Farm A4 2nd visit</th>
<th>Farm A4 3rd visit</th>
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<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<td>ND</td>
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<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td>Total no. of samples</td>
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<td>5</td>
<td>7</td>
<td>7</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>11</td>
<td>5</td>
<td>8</td>
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<tr>
<td>% positive per farm</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>35</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
</tbody>
</table>

* Each + represents a positive sample; each – represents a negative sample. ND, not done.

The mixture back and forth. NaCl (0.2% [wt/vol]) was used as a negative reaction control.

The PCR serotyping assay was set up using the primer sets. reaction setup, and conditions as described by Doumith et al. (5) with the following modifications: the primer concentrations used were 0.4 μM each for lmo0737, 0.4 μM each for ORF2819, 0.4 μM each for ORF2110, 1.4 μM each for lmo1118, and 80 nM each for prs. PPP Master Mix (Top-Bio, Prague, Czech Republic) was used for amplification of target DNA fragments, as per the manufacturer’s instructions. PCR products were visualized by running 10 μl of the completed reaction mixture on a 2% (wt/vol) agarose gel and stained with SYBR safe DNA stain (Invitrogen, Paisley, UK).

Biochemical testing. Isolates from ALOA that were identified as false positives by PCR were further characterized using API Listeria strips (BioMerieux, Basingstoke, Hampshire, UK), performed as per the manufacturer’s instructions. These isolates were also assayed for catalase activity.

RESULTS

Occurrence of L. monocytogenes on farms supplying milk to the unpasteurized milk cheese industry. Four farms that supply milk to the unpasteurized milk cheese industry were sampled for 3 consecutive months. A range of samples were taken from the dairy farm environment and analyzed for the presence of L. monocytogenes. The results are shown in Table 1. L. monocytogenes was detected on all four farms, with occurrence varying from 0 to 35% between the farms and also varying in the sample type in which it was detected. The most common sample to test positive was the surface runoff water from either the general farmyard or the milking facility, with 60% of samples testing positive. Occurrence in fecal matter and dust was about 50%. No L. monocytogenes was detected in milk filters, feed, or silage.

The level of hygiene on each of the farms was recorded by a visual inspection of the milking facility as described in “Materials and Methods.” Three areas were inspected: (i) dairy equipment (including milking cups and milk sock), with verification of availability of hand washing facilities and hot water, etc.; (ii) floors, pipes, walls, etc.; and (iii) housing facilities, bedding, and water troughs. The hygiene score was averaged over the three areas. Farms A1 and A2 were found to have the best hygiene score, and this correlated with the lowest percentage of samples found to be positive for L. monocytogenes (Fig. 1). The lower hygiene scores for farms A3 and A4 corresponded with higher percentages of L. monocytogenes occurrence.

Occurrence of L. monocytogenes on farms supplying milk for processing. Three farms that supply milk for processing were sampled on three consecutive summer months. Various farm samples were taken and analyzed for L. monocytogenes. The results are shown in Table 2. Occurrence varied between 22 and 27%, with feces and water troughs being the most commonly contaminated sample types.

Occurrence of L. monocytogenes in soil, water, and feces. As water and feces were major sources of contamination on the first seven farms tested (sets A and B) and no soil samples had been tested to date, it was decided to survey nine further dairy farms (set C), examining water, soil, and feces only. One sample of each type was taken on each of two independent visits to the farms. As can be seen from the results shown in Table 3, 17% of the water samples (6 of 36) were found to be positive, as were 12% of the fecal samples (4 of 34) and 3% of the soil samples (1 of 35). While the average number of positive samples was similar at both sampling times (11.6 and 9.2%), there was only one farm where positive samples were obtained at both sampling times. On 55% of the farms no positive samples were obtained at any sampling time.

PCR for confirmation. All of the isolates (shown in Tables 1 to 3) were confirmed as L. monocytogenes by PCR amplification of the hly gene, a known pathogenicity gene for L. monocytogenes (34). Isolates that appeared to be positive on ALOA and were subsequently found to be negative
for \textit{hly} gene were discounted from the study and are not included in Tables 1 to 3.

\textbf{Biochemical testing.} During the course of the study, four strains that displayed the typical \textit{L. monocytogenes} phenotype on ALOA but were negative when \textit{hly} PCR was performed were further tested by biochemical tests to eliminate the possibility that they were false negatives by PCR. These four strains were then subjected to API \textit{Listeria} and catalase testing. Serotyping had identified one of these isolates as a possible non-\textit{monocytogenes} \textit{Listeria} species. The strain was also found to be catalase positive, a characteristic associated with \textit{Listeria} spp. It was assigned an \textit{L. welshimeri} “doubtful profile” (ID score, 99%) by API. The other three isolates were not assigned a possible \textit{Listeria} spp. profile by the API system and were all catalase negative.

\textbf{PCR on enriched cultures.} In an effort to shorten the detection time for the presence of \textit{L. monocytogenes} by 2 days, samples of enriched media (that were confirmed as positive on ALOA) were taken and DNA was extracted and amplified by PCR with specific probes for the \textit{hly} gene. Of 39 ALOA-positive samples, only 16 were found to be positive by PCR of enriched samples.

\textbf{Serotyping of isolates.} Results of serotyping are illustrated in Table 4. Fifty-four isolates were successfully serotyped, with five isolates not conclusively typed. Serovar 1/2a was the most prevalent among the \textit{L. monocytogenes} organisms isolated, at 31%, followed by 1/2b at 27%, 4b at 20%, 3a at 8%, 4c at 3%, and finally 3b at 2%. Eight percent of the samples were untypeable.

\section*{DISCUSSION}

Many listeriosis outbreaks caused by \textit{L. monocytogenes}, in which contaminated dairy products, including raw-milk products, were identified as the etiological source of infection, have been documented in the literature,\cite{2, 26}. Many of these studies detail the occurrence of \textit{L. monocytogenes}

\begin{table}[h]
\centering
\caption{Occurrence of \textit{L. monocytogenes} on dairy farms that supply milk to a cooperative—farm set B\textsuperscript{a}}
\begin{tabular}{lccccccccc}
\hline
Sample type & \multicolumn{3}{c}{Farm B1} & \multicolumn{3}{c}{Farm B2} & \multicolumn{3}{c}{Farm B3} \\
               & 1st visit & 2nd visit & 3rd visit & 1st visit & 2nd visit & 3rd visit & 1st visit & 2nd visit & 3rd visit \\
\hline
Milk filter & -         & -         & +         & -         & ND        & -         & ND        & -         & -         \\
Water trough & +         & +         & +         & +         & +         & +         & +         & +         & +         \\
Water from well & -         & -         & ND        & -         & ND        & -         & ND        & ND        & ND         \\
Cow feces & +         & +         & +         & +         & +         & +         & +         & +         & +         \\
Silage     & ND        & ND        & ND        & -         & ND        & -         & ND        & -         & -         \\
Hay        & ND        & ND        & ND        & -         & ND        & -         & ND        & -         & -         \\
Bedding    & -         & -         & -         & ND        & ND        & -         & ND        & -         & -         \\
Feed       & ND        & ND        & ND        & -         & ND        & -         & ND        & -         & -         \\
Grass      & -         & -         & -         & +         & -         & -         & ND        & ND        & -         \\
Hen feces & ND        & ND        & ND        & ND        & ND        & ND        & ND        & ND        & ND         \\
Surface runoff & -           & ND        & ND        & ND        & ND        & ND        & +         & +         & +         \\
Total no. of samples & 10 & 11 & 8 & 11 & 13 & 13 & 9 & 12 & 9 \\
\hline
% positive per farm & 24 & 22 & 27 & & & & & & \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a} Each + represents a positive sample; each − represents a negative sample. ND, not done.
TABLE 3. Occurrence of L. monocytogenes in water, soil, and feces on dairy farms—farm set C

<table>
<thead>
<tr>
<th>Farm</th>
<th>Visit no.</th>
<th>Water</th>
<th>Soil</th>
<th>Cow feces</th>
<th>% positive per farm</th>
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</tbody>
</table>

*Each + represents a positive sample; each – represents a negative sample.

*L. monocytogenes* only in the food products themselves or in the production line linked to an outbreak (2, 29), leaving a gap in the knowledge of the original source of the contamination. This information may be crucial to identifying possible contamination routes and thus controlling the spread of the organism from farm to fork.

This study assessed the environmental occurrence of *L. monocytogenes* on Irish dairy farms, which included farms supplying milk to unpasteurized milk cheese manufacturers and farms supplying milk for processing. Farm set A, supplying milk to the unpasteurized milk cheese industry, comprised a total of 91 samples, taken from four separate farms (Table 1). Of the 91 samples tested, *L. monocytogenes* was found in 17 (19%). Surface runoff and yard dust and/or debris samples were found to contain the highest incidence of *L. monocytogenes*, with 60 and 57% of samples, respectively, testing positive. Fifty percent of fecal samples from cattle were also positive; however, all 13 silage samples tested were negative, suggesting that contamination may be originating from already established strains cross-contaminating the environment, rather than via the cattle as a result of their feed. All milk filter samples were also negative for *L. monocytogenes*, again leading to the hypothesis that contamination on the farms tested was from an environmental source, rather than hygiene of the herd.

Levels in samples where *L. monocytogenes* was detected from farms supplying milk for processing, farm set B, were slightly higher overall than those found on farms supplying the unpasteurized milk cheese industry, with detection ranging from 27 to 33% (Table 2). This farm set consisted of 102 samples in total, from three separate farms. Twenty-five percent of hay for feeding and 23% of cattle bedding samples tested positive for *L. monocytogenes*, and this, together with the data from silage feed and water trough samples, suggests that the herd may be a vector for recontamination of the dairy farm environment. This could be confirmed by pulsed-field gel electrophoresis subtyping of the strains. It has been shown that feeding with contaminated silage is one of the risk factors leading to increased transmission and occurrence of *L. monocytogenes* within a habitat (3, 22). *L. monocytogenes* was detected in cow feces samples taken from farm B1 on all three sampling visits and on two of the three sampling visits in the case of farm B2. As no direct sampling of the animal intestinal content was taken, contamination of the fecal samples by other means while on the dairy farm environment cannot be ruled out. Healthy cattle, however, are known to be carriers of *L. monocytogenes* (18–20), and in the context of these results, it is clear that hygiene standards are very important in relation to the spread of *L. monocytogenes* in the dairy farm environment. Although *L. monocytogenes* was not isolated from the cow fecal samples taken from farm B3 of this farm category, hen fecal samples taken from farm B3 were positive for *L. monocytogenes* on all three samplings, indicating that other farm fauna may represent a potential reservoir for contamination of the dairy farm environment. Fenlon et al. (9) found that hens, ducks, and seagull fecal samples were positive for *L. monocytogenes*. Ho et al. (18) noted that the prevalence of fecal shedders among dairy cattle varied as much as 0 to 100% and that levels of shedders could change dramatically on a day-to-day basis, which indicates that although fecal samples from cows on farm B3 were negative on all three sampling occasions, this...

TABLE 4. Serotyping of *L. monocytogenes* isolates

<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of occurrences on farm set:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1/2a 5 7 2 14</td>
</tr>
<tr>
<td>B</td>
<td>1/2b 3 4 7 14</td>
</tr>
<tr>
<td>C</td>
<td>3a 0 5 0 5</td>
</tr>
<tr>
<td></td>
<td>3b 0 1 0 1</td>
</tr>
<tr>
<td></td>
<td>4b 6 6 0 12</td>
</tr>
<tr>
<td></td>
<td>4c 0 0 2 2</td>
</tr>
<tr>
<td></td>
<td>Unknown 3 0 0 3</td>
</tr>
<tr>
<td>Total</td>
<td>17 23 11 51</td>
</tr>
</tbody>
</table>

* Data for farm sets A, B, and C are given in Tables 1, 2, and 3, respectively.
does not necessarily indicate that the herd were not shedding at other times. *L. monocytogenes* was not detected in milk samples taken from any of the three farms in this grouping; however, a milk filter tested from farm B2 was found to contain the bacterium on the first visit, indicating that sporadic contamination of milk by *L. monocytogenes* may occur.

A third set of farms (set C) were selected for the study of *L. monocytogenes* occurrence, with water trough, soil, and cow fecal samples selected for analysis. Nine farms were sampled on two occasions, and a total of 105 samples were taken. *L. monocytogenes* was isolated from five of the nine farms tested. Only one of these, however, was found to have *L. monocytogenes* on both occasions (farm C7), and this farm was found to contain *L. monocytogenes* in all three sample types. Among all farms tested, trough water was found to be the most common source of *L. monocytogenes* (17% positive), with 12% of fecal samples positive and 3% of soil samples positive. *L. monocytogenes* was isolated from soil on farm C7 only.

In total, 24 milk samples (including milk filters) were taken, with 4% (1 sample) positive. Although this is a relatively small number of samples, the rate of occurrence is similar to already published figures, which usually vary from 1 to 10% (16, 21, 30, 35), although there is one report showing an occurrence of 22% in unpasteurized milk (23).

Despite the fact that the hygiene inspection was not rigorous, the partial correlation between hygiene level and *L. monocytogenes* occurrence not only supports the relevance of the two documents to effective farm hygiene but also indicates the effectiveness of farm maintenance, ensuring, for example, intact pipe and building structures, in combination with good farm cleanliness standards, for the successful control of *L. monocytogenes* on the farm. A correlation between good farm hygiene and reduced occurrence of *L. monocytogenes* has also been reported by Husu et al. (21) and Nightingale et al. (32).

Serotyping provides a useful tool in the epidemiology of *L. monocytogenes* and is important in tracing sources of outbreaks of listeriosis among populations, by facilitating elimination of possible sources of the infection. Thirteen different serotypes of *L. monocytogenes* have been identified by using antisera targeting the somatic (O) and flagellar (H) antigens, and all 13 have been identified as etiological agents of human listeriosis (8). In the case of *L. monocytogenes*—induced human listeriosis, however, strains of serovars 1/2a, 1/2b, and 4b account for 95% of disease incidence (12) and invasive disease is mostly associated with serotype 4b strains (5, 36).

Of the isolates from this study for which a definitive serotype was identified, 78% of these were either 1/2a, 1/2b, or 4b. In previous studies from many different countries (11, 13–15, 42), these serovars have also been most commonly isolated. The occurrence of serotype 4b in small-scale cheese factories was suggested by Wagner et al. (42) to be related to hygiene. In the present study, 73% of serotype 4b isolates were obtained from cow feces or surface runoff water, supporting this theory. In our study, strains of serovars 3a and 3b were found only among isolates from farm set B, which was composed of farms from a geographical location different from that of sets A and C. This indicates that there may be regional variation of persistent serovars in Ireland. Serotype 4c was isolated from only a single farm among farm set 3, indicating that its source may be intrinsic to that particular farm.

*L. monocytogenes* contamination of RTE food products is an important issue in the food industry, and surveillance of its presence in these products is of utmost importance. Contamination of foodstuffs by the bacterium can represent large loss of income, which can be especially severe for smaller manufacturers, such as artisan producers. Control of contamination of products, including unpasteurized milk, by *L. monocytogenes*, as such, can be crucial for production of safe foods. This study provides insight into the presence of *L. monocytogenes* in the Irish dairy farm environment, having found that levels of the bacterium could frequently be detected at levels of up to 35%. It can be concluded that control of the bacterium at the farm level is crucial to minimizing cross-contamination of farm products destined for foodstuff production, such as unpasteurized milk. It further demonstrates that good hygiene practices can contribute to reduction of *L. monocytogenes* at the farm level, therefore reducing the risk of it entering the food chain.

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