Occurrence of gastrointestinal pathogens in soil of potato field treated with liquid dairy manure

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Abstract

In parallel with a study of liquid dairy manure (LDM) effects on potato (Solanum tuberosum) production, an investigation was conducted to monitor the occurrence of three major foodborne pathogens in soil amended with LDM under field conditions. LDM was added prior to planting potatoes in experimental plots (randomized complete block design, 5 replications) in 1999 and in 2000. Soil samples were collected periodically from plots treated with or without LDM and analyzed for the presence of Listeria monocytogenes, Salmonella sp., generic Escherichia coli, and E. coli O157:H7. In the 1999 potato-growing season, L. monocytogenes was consistently detected in LDM-treated, but not in untreated, soil samples during the first six weeks after LDM application. However, L. monocytogenes became undetectable approximately ten weeks after LDM application. A rapid decline in the number of generic E. coli, from 292 cfu g⁻¹ soil in June to 10 cfu g⁻¹ soil in July, was also observed. Salmonella and E. coli O157:H7 were not detected in 120 soil and potato samples analyzed. In the 2000 potato-growing season, no pathogen was detected in 60 soil and potato samples analyzed, although generic E. coli was detected once at a very low level (2 cfu g⁻¹ soil). This study indicates that L. monocytogenes can be present in soils during the first 40 to 70 days after LDM application.

Key words: Listeria monocytogenes, survival, dairy manure, soil, potato contamination.

Introduction

During the past two decades, an increasing number of foodborne outbreaks of human illness have been attributed to the consumption of raw fruits and vegetables contaminated with gastrointestinal pathogens. Although the epidemiological data are currently unavailable, it is generally believed that the sources of pathogens involved in fresh produce contamination may have originated from feces or inadequately treated manure. Thus, the potential risk of introducing fecal pathogens through contaminated manure is an issue of serious concern during the past few years. Several reports have shown that major foodborne pathogens such as Listeria monocytogenes, Salmonella sp., generic Escherichia coli, and E. coli O157:H7 are able to survive in artificially-inoculated soil or manure for a limited period of time under laboratory conditions. Very little is known about the survival of these same pathogens in agricultural soil planted with crops under field conditions. Presently, farmers are urged to apply manure to soil at least two weeks before planting and at least 120 days before harvest to avoid pathogen contamination of crops. This recommendation was largely based on experience and research on survival kinetics of foodborne pathogens in soil and manure conducted under laboratory conditions. Data from field research is needed to validate these observations under field environmental conditions. The aim of this study was to monitor the presence of three major bacterial pathogens, including L. monocytogenes, Salmonella, and E. coli O157:H7, in soil of a potato field treated with liquid dairy manure (LDM) and to assess the potential risk of pathogen contamination on potato tubers grown in LDM-treated soil.

Materials and Methods

Field studies and sample collection: Field studies were conducted at the experimental site of the New England Plant, Water, and Soil Research Laboratory (U.S. Department of Agriculture, ARS) in Newport, Maine, USA. LDM held in the lagoon of a local dairy farm was surface applied to a coarse-loamy, mixed, frigid typic soil on the same day when it was obtained. LDM was applied by hand to ensure even manure application two weeks before planting potato (Solanum tuberosum L. Norwis). Manure was applied at a rate of 42,430 l ha⁻¹ and incorporated with a disk harrow within 4 hr of application. Manure treatment (with or without) constituted the main plots, and the amount of N fertilizer applied constituted the subplots. For the study reported herein, all soil and plant samples were taken from subplots receiving no N fertilizers. The experimental design was a randomized complete block, split plot design with five replications. Soil samples were collected from plots treated with or without LDM at 2-4 week intervals. Soils were randomly sampled within each plot area (3.6 m wide; 12.1 m long) and to a 15 cm depth. Approximately 15 cores (2 cm diameter, 15 cm deep) were taken from each plot, mixed, and mailed overnight in a cooler to the Eastern Regional Research Center (ERRC, USDA, ARS) in Pennsylvania for microbiological analyses. Potato tubers randomly collected at the end of growing season in late September were also analyzed for the presence of the three major pathogens described below.

Sample processing: The soil or potato tuber samples were usually processed on the same date they arrived. If short-term storage was required, the samples were kept in the cooler and placed in a cold room. The procedures for enrichment of L. monocytogenes or Salmonella sp. were based on the methods previously described with slight modification as summarized in Fig. 1. Soil samples in 25-gm units were placed in 125-ml of non-selective broth for enrichment of Listeria or Salmonella. However, when negative results were obtained, a larger proportion of soil sample as compared to enrichment broth (50 gram of soil in combination with 50 ml of enrichment broth) was used.

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For enumeration of *E. coli*, 25-gram unit of soil suspended in 125 ml of phosphate buffered saline (PBS) was usually used. But when the number of *E. coli* in the sample was low, 50 gram of soil suspended in 50 ml of PBS was used. For analysis of pathogen contamination on potato tubers, three tubers (weighing approximately 400 gram) collected at harvest from experimental plots were placed in a Stomacher bag containing 250 ml of PBS and the dirt and soil on the surface of tuber was removed by hand-rubbing. Twenty-five ml of wash fluid was mixed with 225 ml of non-selective enrichment broth for *Salmonella* or *L. monocytogenes*. Following non-selective enrichment, the samples were transferred to the selective enrichment broth or differentiation agar plates for specific pathogens as described below.

**Pathogen identification and enumeration:** Suspected *Listeria* colonies observed on Oxford agar plates were first identified by a commercial ELISA test (Organon Teknik Cop., Durham NC, USA). Differentiation of *Listeria* species was conducted using the biochemical tests including hemolytic activity, nitrate reduction, and acid production from mannitol, rhamnose, and xylose as previously described. The isolates identified as *L. monocytogenes* by biochemical tests were further confirmed by PCR analysis (Qualicon BAX® system for screening of *L. monocytogenes*, Wilmington, DE, USA). Generic *E. coli* was enumerated using the Petrifilm *E. coli* Count Plates (3M Microbiol. Prod., St. Paul, MN, USA). Presence of *E. coli* O157:H7 was determined using the Petrifilm *E. coli* HEC Count Plates (3M Microbiol. Prod., St. Paul, MN, USA). If needed, suspected *Salmonella* colonies observed on the selective medium, xylose-lysine-tergitol 4 (XLT4) agar, were further characterized by the immunodiffusion assay (1-2 Tests, BioControl Lab., Bothell, WA, USA) or PCR analysis (the Qualicon BAX® system for screening of *Salmonella*). All culture media used in the study were obtained from the Difco Lab. (Detroit, MI).

**PCR assays:** The Qualicon BAX® system for screening of *L. monocytogenes* was used to confirm the identity of soil and manure isolates initially characterized by biochemical tests. Ten μl aliquot of bacterial suspension was added into 200-μl of lysis buffer. The samples were then allowed to incubate at 55°C for 60 min and 95°C for 10 min. Following a brief ice quench, 50 μl of the lysate was combined with a freeze-dried tablet containing PCR reagents (provided in the kit) and then subjected to amplification in a GeneAmp Model 9700 Thermocycler (PE Applied Biosystems, Forster City, CA, USA) using the cycling program recommended by the manufacturer. Positive detection of *L. monocytogenes* was indicated by the amplification of 400-bp pathogen-specific DNA fragment and proper PCR was indicated by the amplification of a 200-bp internal control DNA fragments.

**Results**

**Contamination of manure with pathogens:** Four different types of animal manure including cow manure slurry, powdered chicken manure, pig manure slurry, and liquid dairy manure were analyzed for the presence of *L. monocytogenes*, *Salmonella*, generic *E. coli*, and *E. coli* O157:H7 using the methods as summarized in Fig. 1. Results (Table 1) show that generic *E. coli* was detected in all four different types of manure at the concentration of approximately 10-77 cfu g-1 manure. *E. coli* O157:H7 was not detected in any of those 24 samples analyzed. *L. monocytogenes* was detected in 1 to 2 out of 6 cow manure slurry or liquid dairy manure (LDM) samples analyzed. By comparison, *Salmonella* was detected in 2 to 3 out of 6 poultry or cow manure samples examined. These results indicate that contamination of animal manures with fecal pathogens is common although they may be present in extremely low numbers. Complete elimination of the pathogens from manure to be used for fertilizer supplements thus becomes a critical control point to prevent the transmission of pathogen into farmland soil and field crops.

**Occurrence of *L. monocytogenes* in LDM-amended soil:** Soil samples collected periodically from the experimental plots treated with or without LDM were analyzed for the presence of three major pathogens at 2 to 4 week intervals in two potato-growing seasons. Results (Table 2) show that 22 out of 25 soil samples collected from LDM-treated plots in 1999 were found to contain the putative *Listeria* species. However, only 7 out of 25 samples collected from control plots (not treated with LDM) were positive for the presence of *L. monocytogenes*. The identity of *L. monocytogenes* isolates obtained during the study was mutually confirmed by the biochemical tests and PCR assays (Fig. 2). The population of *L. monocytogenes* appeared to decline quite rapidly in soil. *L. monocytogenes* became undetectable in soil approximately 10 weeks after the application of LDM. However, the non-pathogenic strains of *Listeria* sp. continued to be present in the samples collected in August and September. *L. monocytogenes* was not detected in any of those 50 soil samples and 10 potato samples analyzed in 2000, although generic *E. coli* was detected only once and at extremely low number in soil samples collected from a plot treated with LDM. *Listeria* species were also detected occasionally in soil samples collected from control plots receiving no LDM during the first six weeks after LDM application. It is not clear if the *Listeria* isolates including one *L. monocytogenes* isolate found in control samples were naturally present in soil or due to cross contamination from the neighboring LDM-treated plots.

**Population changes of generic *E. coli* in soils amended with LDM:** Generic *E. coli* was consistently detected in soils of experimental plots treated with LDM. However, the numbers of generic *E. coli* in soil rapidly declined from 254 cfu g-1 to 50 cfu g-1 in just two weeks from June 2 to June 15 (Fig. 3). Since only a very low level of generic *E. coli* (2 to 4 cfu g-1 soil) was detected in soil receiving no LDM, the sharp increase in the population of *E. coli* in soil was possibly due to the outgrowth of *E. coli* naturally present in LDM. As noted earlier, presence of generic *E. coli* is common among four different types of manure analyzed (Table 1). Like *L. monocytogenes*, generic *E. coli* also did not appear to survive well in soil under field conditions. Generic *E. coli* also became undetectable approximately ten weeks after LDM application.

**Potato tubers grown in LDM-treated soil are free of pathogens:** *E. coli* O157:H7 and *Salmonella* were not detected in 100 soil samples collected in two potato-growing seasons in 1999 and 2000. Furthermore, 20 potato tuber samples collected from plots treated with or without LDM appeared to be free of *L. monocytogenes, E. coli* O157:H7, and *Salmonella*. In spite of the finding that LDM-treated soil was contaminated with *L. monocytogenes* at the early potato-growing season, the transmis-
Table 1. Detection of *Listeria monocytogenes*, *Salmonella*, generic *E. coli*, *E. coli* O157:H7 in various types of manure.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Liquid dairy</th>
<th>Pig</th>
<th>Chicken</th>
<th>Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>2/6</td>
<td>0/6</td>
<td>0/6</td>
<td>1/6</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>0/6</td>
<td>0/6</td>
<td>2/6</td>
<td>3/6</td>
</tr>
<tr>
<td>Generic <em>E. coli</em></td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

Conducted according to the procedures as summarised in Figure 1.

Table 2. Detection of *Listeria* spp. and *L. monocytogenes* in soil sprayed with liquid dairy manure during the potato growing season of year 1999.

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Samples from LDM-treated plots (n=5)</th>
<th>Samples from control (without LDM) (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. sample containing Listeria spp.</td>
<td>No. sample containing <em>L. monocytogenes</em></td>
</tr>
<tr>
<td>June 2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>June 15</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>July 19</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>August 19</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>September 22</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1. Procedures for isolation of *Listeria monocytogenes*, *Salmonella* spp., generic *E. coli* and *E. coli* O157:H7 from manure and soil samples.
Figure 2. Identification of *Listeria monocytogenes* by PCR analysis (Qualicon BAX system for screening of *L. monocytogenes*). (Panel A) Amplification of *L. monocytogenes*-specific 400-bp fragment from DNA samples prepared from a soil isolate (lane 3) and from strain Scott A (lane 4). No targeted DNA fragment was amplified from DNA samples prepared from *Salmonella* Chester (lane 2), *E. coli* O157:H7 (lane 5) & *Pseudomonas fluorescens* (lane 6). (Panel B).

Figure 3. Changes in the populations of generic *E. coli* in soil sprayed with liquid dairy manure (LDM) during the potato growing season of year 1999. The soils collected from experimental plots receiving no LDM were used as controls.

Discussion
A total of 100 soil samples collected periodically from the experimental plots treated with or without LDM in two potato-growing seasons were analyzed for the presence of three major pathogens including *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7. *L. monocytogenes* was detected in 10 out of those 100 soil samples analyzed. Nine out of the 10 samples testing positive originated from LDM-treated plots during the first 6-10 weeks after LDM application. Only one out of the 10 samples testing positive originated from the control plot receiving no LDM. Occurrence of *L. monocytogenes* in the field is therefore linked to the application of LDM. It is not clear however if the *L. monocytogenes* strains isolated originated from those naturally present in LDM or in soil. Contamination of animal manure with fecal pathogens has been demonstrated before and confirmed here as summarized in Table 1. Presence of *L. monocytogenes* in agricultural soils and sewage, has been previously reported. Therefore, the possibility that LDM application may activate the biological activity of resting *L. monocytogenes* cells in soil cannot be ruled out. Although *E. coli* O157:H7 and *Salmonella* had been shown to survive in soil or manure for several weeks, these two organisms were not detected in any of those 120 soil and potato samples collected over a two-year period. It has been reported before that *E. coli* O157:H7 and *Salmonella* generally survive more poorly in soil...
than *L. monocytogenes*. Failure to detect these two organisms could be due to their absence in LDM or in soil or due to unfavorable environmental conditions for their survival or growth under field conditions. It has been previously reported that persistence of fecal pathogens in soil or manure is greatly affected by soil type, soil and manure moisture content, temperature, and plant vegetation. Occurrence of *L. monocytogenes* on potato tubers collected at retail markets has been previously reported. It was not known however if the contamination occurred in the field or after harvest. In this study, all of 20 potato tuber samples collected at harvest from experimental plots treated with or without LDM tested negative for the presence of *L. monocytogenes*, *E. coli O157:H7*, and *Salmonella*. There is no indication that emergence of *L. monocytogenes* in LDM-treated soil at the early stage of the potato-growing season as revealed in this study would automatically lead to transmission of the pathogen to growing tubers. Like generic *E. coli*, the population of *L. monocytogenes* in LDM-treated soil possibly declined very sharply during the first 10 weeks and became undetectable approaching the end of the potato-growing season. There is no indication that *L. monocytogenes* or other pathogens were transmitted to potato tubers from contaminated soil. Van Renterghem et al. reported that radishes sown in soil inoculated with *L. monocytogenes* were found to be contaminated with the pathogen. Al-Ghazali et al. showed that alfalfa plants grown on farmland soil treated with *L. monocytogenes*-containing sewage sludge cake were contaminated with this pathogen. The transmission of *L. monocytogenes* to crops grown in contaminated soil as reported by Van Renterghem et al. and Al-Ghazali and Al-Azawi may be due to the presence of relatively high levels of *L. monocytogenes* in soil or sewage cake used in their studies as compared to the low level of pathogen detected in this study.

**Conclusions**

This study shows that *L. monocytogenes* can be present in soil treated with LDM for 6 to 10 weeks and becomes undetectable in soil by the end of potato-growing season. Potato tubers harvested from LDM-treated plots appear to be free of pathogen contamination.

**Acknowledgement**

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**References**